

## KEYNOTE LECTURE

### AORTIC ANEURYSMS: FROM GENE TO THERAPY

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Aortic aneurysms are an important cause of mortality in the western world. Monogenic disorders such as the Marfan syndrome (MFS) and the vascular type of Ehlers-Danlos syndrome (EDS) are good models for the study of the pathogenesis of aortic aneurysm. In the MFS, progressive dilatation of the aortic root leads to aortic aneurysm and dissection, often associated with precocious death. Early pathogenetic models for MFS focused upon structural weakness of the tissues imposed by microfibrillar deficiency. However, recent studies of transgenic mouse models have challenged this model and demonstrated a central role for the upregulation of the TGFbeta signaling pathway. The discovery of a new aortic aneurysm syndrome, the Loeys-Dietz syndrome (LDS), confirmed the importance of the cytokine transforming growth factor (TGFbeta) in aneurysm pathogenesis. The main distinguishing features between LDS and MFS include the presence of hypertelorism, cleft palate/bifid uvula and arterial tortuosity/widespread aneurysms. LDS is caused by mutations in the genes encoding the receptors for TGFbeta (TGFB1/2). Timely recognition of LDS is important in view of the different management strategies needed in this disorder. We have recently also looked into the involvement of TGFbeta signaling in other non-syndromic causes of aortic aneurysms, including the genes encoding the smooth muscle contractile apparatus proteins alpha-actin and myosin heavy chain 11 encoded by the ACTA-2 and MYH-11 gene. In analogy to LDS, we demonstrated an upregulation of TGFbeta in arterial tortuosity syndrome. Finally, all these insights have also lead to new therapeutic insights. In transgenic mouse models it was shown that losartan, an angiotensin II type 1 receptor with known inhibiting effects on TGFbeta, rescues the aortic phenotype. If these promising results are confirmed in human trials, losartan might have beneficial effects in the treatment of more common non-hereditary aortic aneurysms.

## SYMPOSIUM

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### S1-03

#### **What makes us human: Insights from sequencing extinct hominin genomes.**

*Johannes Krause*

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A genetic comparison between modern humans and their extinct relatives could both address the relationship between us and them and offer the possibility to identify genetic changes that happened specifically on the human lineage. Furthermore it may allow identifying and understanding the evolutionary history of genes and positions in the modern human genome that experienced recent positive selection after divergence of modern humans and their extinct relatives. Using a combination of high-throughput DNA sequencing technologies and multiple improvements in ancient DNA retrieval, library construction and targeted library enrichments, the Leipzig laboratory has recently, in collaboration with several groups, completed a first version of the Neandertal genome as well as a genome sequence of an extinct hominin discovered in the Altai mountains in southern Siberia named Denisovan. The analysis of both the Neandertal and Denisovan genome revealed evidence of gene flow between certain modern human populations and both extinct hominins. From the analysis of the data we were furthermore able to draw conclusion about diversity within and among the extinct hominins and by scanning the human genome for regions of positive selection using the Neandertal and Denisovan genome, we identified several strong candidate genes involved in diet, cognitive traits, and skeletal morphology that were potentially selected on the modern human lineage.

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**S2-01****The Rasopathies: distinct disorders related by dysregulation of the Ras/MAPK signalling pathway***Karen W. Gripp, MD*

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The rasopathies are a group of genetic conditions for which our understanding changed dramatically through the delineation of pathogenic mutations. Noonan syndrome was first recognized as the combination of cardiac defects, short stature, cryptorchidism, characteristic facial features and normal karyotype. Cardio-facio-cutaneous syndrome (CFC) presents with similar cardiac defects, facial features that coarsen with age, and skin findings including eczema and nevi. In contrast to Noonan syndrome, individuals with CFC typically have intellectual disabilities. Costello syndrome shares cardiac and facial features with Noonan and CFC, and entails a striking predisposition to malignancies, in particular rhabdomyosarcoma and bladder cancer. Noonan syndrome is often familial, allowing for positional cloning of the first Noonan syndrome gene, PTPN11. Its gene product, SHP2, is a component of the mitogen activated protein kinase (MAPK) signaling pathway. Subsequently, mutations in other Ras/MAPK genes were discovered, with HRAS mutations in Costello and BRAF or MEK1/2 changes in CFC syndrome, respectively. Dysregulation of the Ras/MAPK pathway was extensively studied because of its prominent role in malignancies. Increased signal transduction occurs either by gain-of-function in abnormal gene products or through loss-of-function of negative regulators, and the transforming potential of specific mutations has been evaluated. Such point mutations in the proto-oncogene HRAS decrease intrinsic hydrolytic activity of the abnormal protein product, which remains active and promotes increased signal transduction through the Ras/MAPK pathway. The most common somatic HRAS alteration in sporadic tumors encodes a p.G12V amino acid change with very high transforming potential. When this mutation arises de novo in the germline, the affected child has severe, neonatal lethal Costello syndrome. The majority of Costello syndrome individuals share a less strongly activating change, p.G12S, which is more compatible with survival, albeit resulting in the 15% tumor risk. The phenotype associated with HRAS p.G13C is milder, but encompasses distinctive ectodermal findings including loose anagen hair and unusually long eye lashes. Due to HRAS' central role within the Ras/MAPK pathway, this ongoing genotype phenotype correlation in Costello syndrome serves as a paradigm for other Ras/MAPK pathway disorders.

We now view Noonan, CFC and Costello syndrome, as well as neurofibromatosis type 1, Noonan syndrome with multiple lentigines (previously known as LEOPARD), Noonan syndrome with loose anagen hair and related conditions as a family of disorders resulting from germline Ras/MAPK pathway changes, collectively referred to as rasopathies. While some of these disorders are rare individually, combined they are common and patients benefit from multidisciplinary "rasopathy clinics" by having specialists become familiar with these conditions.

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**S2-02****Old men and selfish spermatogonia: how much do they contribute to the mutation burden?***Andrew OM Wilkie<sup>1</sup>, Jasmine Lim<sup>1</sup>, Gil McVean<sup>2</sup>, Gareth Turner<sup>3</sup>, Grete Krag Jacobsen<sup>4</sup>, Ewa Rajpert-DeMeyts<sup>5</sup>, Anne Goriely<sup>1</sup>*

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Gain-of-function mutations in 5 genes (RET, FGFR2, FGFR3, PTPN11 and HRAS) that cause congenital malformations exhibit the collective properties of very high apparent rates of germline base substitution, near-exclusive paternal origin, and increased average age of the father from whom the mutation has arisen (paternal age effect, PAE). Somatic mutations in these same genes have been described in various human cancers.

We have developed methods to quantify several of these mutations in human sperm and have also identified FGFR3 and HRAS mutations in spermatocytic seminoma, a rare type of testicular tumour. The combined evidence suggests that these mutations, although occurring rarely, provide a selective growth advantage to the mutant spermatogonial cell, resulting in clonal expansion over time, accounting for the PAE. To date, all examples of these "selfish" spermatogonial mutations locate within a single signalling pathway, the growth factor receptor-RAS pathway, which is a key determinant of spermatogonial stem cell proliferation and renewal. Our recent immunohistochemical analysis of normal testes shows features

consistent with the occurrence of mutational microclones; a variety of patterns of antigen positivity are observed, consistent with different underlying driver mutations.

Regulation of cell turnover is important in many disease contexts, for example neurogenesis and neoplasia, so the consequences of mutations that hijack this process within the testis are potentially far reaching. Depending on the spectrum of average PAE mutations levels, they may contribute significantly to the 'dark matter' in human heritability, currently speculated to be explained by uncommon alleles of moderate effect. Hence this mechanism is likely to be important in the origins of common complex diseases such as certain cancers and psychiatric disorders, as well as in congenital malformations.

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## S2-03

### Clinical Genetics in the Era of Next Generation Sequencing

*Han G. Brunner*

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Now that massively parallel (or next generation; NGS) sequencing has become a reality, it is time to ask the question whether this technology will change the way we practice genetics and Dysmorphology in the clinic. I believe that it will do just that, and in several ways.

Next Generation Sequencing technology will allow all relevant genes for a clinical question or problem to be analysed in depth in a single experiment. The current projected cost of consumables for sequencing up to 100 genes in a single experiment is about 1000 Euro using Roche 454 sequencing.

While true costs that include personnel, bioinformatics support and equipment will be considerably higher, we may expect that such costs should be reduced by at least 50% over the next 2 years. All of this means that it will soon be realistic to test panels of 50-150 genes for many clinical situations (muscular dystrophy; neurodegenerative disorders; blindness, deafness, cardiomyopathy; long QT syndrome; Noonan and related conditions etc). This will largely replace the testing of single genes, or of several genes in succession as is now customary.

The projected cost of consumables for sequencing all 22000 human genes (the exome) using the SOLID platform is 3500 Euros. This could be used to test for instance to detect de novo changes in mental retardation patients. Turnaround time will need to go down, but 3-4 weeks is probably not unrealistic in the mid-term.

The impact of this will be manifold:

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

This means that clinical geneticists should themselves aim to be involved in the diagnostic process from the start. This argues for multidisciplinary diagnostic clinics

Clinical diagnostic skills (in dysmorphology, or other) will become less important. Because the question which gene is to be tested is no longer paramount.

Understanding genes and their functions in terms of the human organism becomes a critical skill for clinical geneticists.

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

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

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

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## S3-01

### The Evolution of Transcriptional Regulation in Mammals

*Duncan Odom*

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Our laboratory investigates the complex relationships among tissue-specific transcription, genetic sequence, and evolution using high-throughput methodologies. We have demonstrated the remarkable divergence in tissue-specific transcriptional regulation that has occurred among mammals. We showed that

a set of liver-specific transcription factors whose function, amino acid sequence, and targeted binding motif are highly conserved throughout mammals changes both their potentially targeted genes and also their precise binding locations globally. An outstanding mechanistic question arose from the discovery of the rapid divergence of transcription factor binding: what directs these transcription factor binding changes? We showed that an aneuploid mouse line carrying a human chromosome could isolate genetic sequence as an independent variable in directing transcriptional regulation. Remarkably, mouse-encoded transcription factors bound the human chromosome in mouse liver almost precisely as do human-genome-encoded transcription factors in human liver. We also found that the mouse nucleus could also accurately re-create the epigenetic landscape and even mRNA regulation across the entirety of this human chromosome. Thus, genetic sequence variation is primarily responsible for interspecies differences in transcriptional regulation.

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### S3-02

#### **Disruption of long-range cis-regulatory elements as a cause of developmental genetic disease in humans**

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There is an increasing awareness of the role of cis-regulatory mutations (C-RM) in human developmental disorders. C-RMs can be classified in several ways but almost always involve gain or loss of non-coding genomic elements with a high degree of evolutionary conservation, called enhancers. Most loci with confirmed C-RMs were first identified via molecular characterization of chromosomal rearrangements. Structural genomic mutations – deletions, translocations and inversions - at some C-RM loci, such as the region 3' to PAX6, result in a consistent phenocopy of intragenic loss-of-function mutations in PAX6 itself (Fantes et al., 1995, Hum Mol Genet, 4, 415-22). Similar mutations at other loci appear to be analogous to tissue specific conditional knock-outs in mice. For example, heterozygous, intragenic, loss-of-function mutations in SOX9 cause a severe developmental disorder called campomelic dysplasia. A specific type of cleft palate, termed Pierre Robin Sequence (PRS) is a common feature of campomelic dysplasia. Mutational disruption of specific developmentally-critical, tissue-specific cis-regulatory elements 5' of SOX9 have been shown to cause PRS as an isolated disorder (Benko et al., 2009, Nat Genet, 41, 359-64; Kurth et al., 2009, Nat Genet, 41, 862-3). Interestingly duplication of the entire 5' regulatory region of SOX9 give a completely different distal limb-specific phenotype (Kurth et al., 2009, Nat Genet, 41, 862-3). Mutations resulting in gain of function of a cis-regulatory element resulting in miss-expression of SHH have been described as a cause of preaxial polydactyly (Lettice et al., 2002, Proc Natl Acad Sci U S A, 99, 7548-53; Sharpe et al., 1999, Curr Biol, 9, 97-100). New genetic technologies have resulted in a large number of non-coding genomic mutations being identified in many different conditions. Our ability to identify these mutations as genuinely altering cis-regulatory function of a specific developmental gene is currently hampered by our primitive understanding of regulatory domains required for complex gene activation. Human genetic analysis has a major role in elucidating these fascinating genetic mechanisms.

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### S4-01

#### **Genetics of Bone disease**

*Stuart H Ralston MD FRCP.*

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Over the past 10 years, tremendous advances have been made in understanding the role that genetic factors play in the regulation of bone metabolism and bone disease. Most bone diseases have a strong genetic component. Osteoporosis is the most common bone disease and is characterised by reduced bone mineral density (BMD) and increased fracture risk. The heritability of BMD is about 70% and fractures also have a heritable component although this decreases markedly with age as environmental factors assume a more important role. Multiple genes have been identified by genome wide association studies that regulate BMD and fracture risk but these have small effect sizes and so far explain only 5-6% of the genetic variance. Studies are in progress to determine if larger effect alleles also exist. Several rare bone diseases exist in which BMD levels are unusually high or low including osteopetrosis (due to mutations in genes that regulate osteoclast function) and sclerosing bone dysplasias (due to mutations in genes that regulate bone formation). Genetic analysis of families with these diseases has resulted in the identification of several key molecules that regulate bone turnover such as Lipoprotein-receptor related protein 5 (LRP5), Sclerostin (SOST) and Cathepsin K. Paget's disease of bone (PDB) has a frequency of about 1-2% and has a strong genetic component. Current evidence suggests that PDB is caused by a combination of rare alleles of large effect that cause Mendelian inheritance of the disease and commoner alleles of medium effect that increase disease risk by 30-50%. Causal genes include SQSTM1, TNFRSF11A, TNFRSF11B, CSF1, OPTN and

TM7SF4 which are involved in regulating osteoclast differentiation and function. Advances in genetics have not only advanced our knowledge of the pathophysiology of bone disease but have also identified several molecules that are being targeted for the next generation of drug treatments for bone disease.

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## **S4-02**

### **Genetics of psoriasis**

*Francesca Capon*

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Psoriasis is an immune-mediated, disfiguring skin disorder, which affects approximately 2% of the general population. Familial clustering of the disease being well established, psoriasis has long been regarded as a common and complex trait, resulting from gene-gene and gene-environment interactions.

Searches for disease susceptibility genes were initially undertaken by means of genome-wide linkage analysis. These studies, carried out by us and others on a wide range of Caucasian populations, provided overwhelming evidence for the existence of a major psoriasis susceptibility locus (PSORS1), lying within the class I region of the Major Histocompatibility Complex (MHC). Despite the robustness of these findings, the identification of the PSORS1 gene proved considerably more challenging, owing to the conservation of linkage disequilibrium across the MHC. To overcome this difficulty, we have carried out highly powered analyses of dense SNP maps and deep re-sequencing data. Although the results of these studies point to HLA-C as the most likely PSORS1 candidate, they cannot conclusively identify the underlying susceptibility allele. With genetic studies having now reached their maximal resolution, the identification of the PSORS1 variant will have to rely on the functional analysis of candidate SNPs.

Outside of the MHC, the performance of linkage studies has been poor, mostly owing to lack of statistical power. Conversely, the advent of genome-wide association scans (GWAS) has had a profound effect on the field, resulting in a dramatic acceleration in the pace of gene discovery. As the number of validated susceptibility genes continues to increase, important insights into disease pathogenesis have been emerging. Association results obtained by us and others have highlighted the critical involvement of IL23/IL17 and NF- $\kappa$ B signalling. Moreover, the follow-up of GWAS hits, which we have pursued by integrating the use of functional experiments with that of bioinformatic tools, has demonstrated an important role for innate immune pathways. Finally, our studies have documented a significant genetic overlap between psoriasis and other immune-mediated conditions, such as Crohn's disease. In particular, we have observed a complex pattern of allelic heterogeneity, with independent variants conferring risk to clinically distinct disorders.

The next few years are expected to witness even more progress in our understanding of psoriasis genetics, as computational advances (e.g. the development of methods for SNP imputation, which allow the meta-analysis of GWAS carried out on different genotyping platforms) and technological breakthroughs (e.g. the advent of next-generation sequencing, which can be used for the systematic identification of rare penetrant mutations and structural variants) are exploited by researchers.

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## **S4-03**

### **Architecture of inherited susceptibility of common cancer**

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The past decade has seen a shift from models of cancer predisposition based on single-gene causative mutations to multigenic models. These models suggest that a high proportion of cancers may arise in a genetically susceptible minority as a consequence of the combined effects of common low-penetrance alleles and rare disease-causing variants that confer moderate cancer risks. As well as providing direct evidence for common susceptibility to cancer the recent genome-wide association studies are providing insights into the biological basis of disease susceptibility and the allelic architecture of inherited cancer risk.

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**S5-03****miRNAs in Cancer***Joanne Weidhaas*

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It is well known that personal genetic variation is an important factor placing some individuals at a greater risk for developing cancer than the general population. To interpret how cancer-associated variations function, most published studies have focused on DNA alterations within protein coding regions of genes from tumor tissue [1-3]. However, our group has shown that cells (normal and/or tumor) from cancer patients also contain genetic variations within miRNAs and the 3'UTR of cancer genes, and in some cases the variations specifically alter miRNA binding sites [4]. MiRNAs are small non-coding RNAs that are known to target important cancer genes by binding to 3'UTRs and are responsible for mRNA stability and translation. Alterations in miRNAs have been demonstrated to play a crucial role in affecting molecular and cellular processes of the cancer state [5]. Despite the critical importance of miRNAs and their binding sites in cancer gene regulation and the availabilities of large volumes of data from the next generation sequencing platforms, no studies have been developed to systematically investigate cancer-associated variations in these regions and construct networks among susceptibility miRNAs and target genes. We have been trying to fill this critical gap by studying 3'UTRs to pinpoint single nucleotide polymorphisms (SNPs) associated with cancer. Several of these variants will be presented and their association with cancer discussed.

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2. Meyerson, M., S. Gabriel, and G. Getz, Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet*, 2010. 11(10): p. 685-96.
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4. Chin, L.J., et al., A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. *Cancer Res*, 2008. 68(20): p. 8535-40.
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**S6-01****Mouse Models of Genetic Eye Diseases***Bo Chang*

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The number of known serious or disabling eye diseases in humans is large and affects millions of people each year. Yet research on these diseases frequently is limited by the obvious restrictions on studying pathophysiologic processes in the human eye. Likewise, many human eye diseases are genetic in origin, but appropriate or available families often are not easy for genetic studies. Mouse models of genetic eye disease provide powerful tools for quick genetic analysis and characterization. The mouse eye is remarkably similar in structure to the human eye, and many developmental or invasive experiments can be done in mice that are impossible in human beings. Comparative mapping and sequencing between human and mouse genomes shows that knowing the gene location in either man or mouse allows for the same gene to be found more quickly in the other. Finally, the use of inbred strains, where all mice in the strain are alike genetically except for the mutation being studied, is a powerful tool for linkage analysis, and assures phenotypic reproducibility of any model found in a strain. The virtual identity of mice in an inbred strain also allows for fewer numbers of mice to be studied clinically. The Jackson Laboratory, having the world's largest collection of mouse mutant stocks and genetically diverse inbred strains, is an ideal place to discover genetically determined eye variations and disorders. While screening mouse strains and stocks at The Jackson Laboratory (TJL) for genetic mouse models of human eye diseases, we have identified numerous spontaneous or naturally occurring mutants. We characterized these mutants using serial biomicroscope (slit lamp) and indirect ophthalmoscopy, fundus photography, electroretinography (ERG) and histology, and performed genetic analysis including linkage studies and gene identification. To date we have discovered over 100 new diseases affecting all aspects of the eye including the eye lid, cornea, iris, lens and retina, resulting in corneal diseases, glaucoma, cataracts and retinal degenerations. We have established a program for discovering genetic eye diseases in mice and characterizing their genetics and phenotypes

sufficiently to make them valuable to investigators in the eye research field. The purpose of this lecture is to (a) describe how new mouse models of genetic eye diseases are identified and characterized and (b) to delineate some mouse models that we discovered at TJL.

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## **S6-02**

### **Finding Genes and Mutations Causing Retinitis Pigmentosa**

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Finding genes and mutations causing retinitis pigmentosa (RP) in patients and families can be extremely challenging. Although RP is believed to be monogenic in each affected individual and in his or her family (with exceptions), it is highly heterogeneous in populations and there are no reliable clinical features indicating the likely gene in most cases. For example, mutations in 21 different genes are known to cause autosomal dominant RP, 35 genes cause recessive RP, 6 genes cause X-linked RP, and many additional genes account for syndromic and systemic forms of RP. Further, there are many known mutations at each locus; different mutations in the same gene may cause distinctly different diseases; and the same mutation in different individuals may have different clinical consequences, even within the same family. Finally, genetic testing of patients with most types of RP reveals the cause in less than 50% of cases, suggesting that many genes and mutations remain to be discovered.

Our research is focused on autosomal dominant RP (adRP). In addition to routine screening of RP cases, we have established a cohort of over 230 adRP families for more aggressive testing. Using a combination of sequencing known and candidate genes, deletion screening using MLPA, and linkage mapping, we have identified the disease-causing gene and mutation in 65% of the cohort, leaving 80 families without mutations in known genes. The remaining families are the subject of whole-genome linkage mapping using Affymetrix 6.0 SNP/CNV microarrays, and whole-exome capture and next generation sequencing using 454 and Illumina sequencing platforms. To date, using these approaches, we have identified the disease-causing gene and mutation in a further 5% of the cohort families and have mapped the disease locus in an additional 5 families.

Next-generation methods, eventually leading to whole-genome sequencing, are powerful tools for gene discovery and mutation detection for inherited retinal diseases. However, they are accompanied by significant problems in data management, sequence assembly and mapping, variant filtering and annotation, and validation. These problems will get worse as the amount of genome data increases, but it is a reasonable hope that identification of the underlying gene and mutation in most RP patients will be a routine part of clinical care within 5 years. Individual genotype information will be essential for directing patients into the many gene-specific and mutation-specific treatments currently or soon-to-be in clinical trials.

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## **S6-03**

### **Clinical trial of gene therapy for early onset severe retinal dystrophy resulting from defects in RPE65**

*Bainbridge JWB, Smith AJ, Fitzke FW, Holder GE, Stockman A, Bhattacharya SS, Yzer Z1, van den Born I1, Rubin GS, Moore AT, Ali RR and the Moorfields Eye Hospital / UCL Institute of Ophthalmology Gene Therapy Study Group*

UCL Institute of Ophthalmology and Moorfields Eye Hospital Biomedical Research Centre for Ophthalmology, 1Rotterdam Eye Hospital

Early-onset severe retinal dystrophy caused by defects in the gene encoding the retinal isomerase RPE65 is associated with poor vision at birth and complete loss of vision in early adulthood. In a phase I/II dose-escalation trial, we have delivered subretinally recombinant adeno-associated virus (rAAV) vector expressing RPE65 under the control of an RPE65 promoter in 9 human subjects with early onset severe retinal dystrophy associated with mutations in RPE65. We have examined systemic vector dissemination and immune responses following vector delivery, assessed visual function pre- and post- vector delivery using a range of psychophysical techniques, and performed detailed electrophysiology and retinal imaging studies.

There have been no serious adverse effects of surgical delivery of vector in the subjects enrolled to date. We have detected no systemic dissemination of vector genome. Although we have detected an increase in systemic neutralising antibodies to AAV capsid in two subjects but there have been no evidence of immune responses to RPE65 protein. We have measured significant improvements in retinal sensitivity by

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microperimetry and dark-adapted perimetry, and improved performance in a test of visually-guided mobility. The outcomes in the first 9 subjects to date suggest that subretinal delivery of rAAV vector can be safe in humans in the short term and can improve retinal sensitivity. These findings support further clinical studies in subjects with RPE65 deficiency and the development of gene therapy for other inherited retinal disorders.

## SEL SELECTED PRESENTATIONS

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### SEL-01

#### **Loss of Cav1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness**

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Hearing impairment is the most common sensory disorder, occurring in approximately 1 out of 500 newborns. In developed countries, the majority of cases with congenital deafness is of genetic origin. Deafness is genetically very heterogeneous and part of several syndromes. Because they may be life-threatening, deafness syndromes with impaired cardiac function are of particular importance. Delayed rectifier potassium channels have been linked to human deafness associated with QT-prolongation and ventricular arrhythmia in Jervell and Lange-Nielsen syndrome (JLNS). JLNS is associated with a high risk of sudden cardiac death in childhood, which may be the first indication of a syndromic background. By positional cloning, we identified a homozygous founder mutation in the alternatively spliced exon 8B of CACNA1D, the gene encoding the pore-forming alpha 1-subunit of voltage-gated Cav1.3 L-type calcium (Ca<sup>2+</sup>) channels, in two consanguineous deafness families. The mutation results in an insertion of a glycine residue in a highly conserved alternatively spliced region near the channel pore. Cav1.3 channels tightly control Ca<sup>2+</sup>-dependent glutamate release at cochlear inner hair cell (IHC) ribbon synapses in response to sound and play an important role in controlling the diastolic depolarization in sinoatrial node (SAN) pacemaker cells. Targeted deletion of Cav1.3 channels in mice causes deafness and pronounced, non-fatal SAN arrhythmia and bradycardia. Strikingly, all patients also had pronounced SAN dysfunction with SAN arrhythmia, bradycardia and junctional escape rhythms. SAN arrest and/or exit block were also observed. As in Cav1.3<sup>-/-</sup> mice, QRS and QTc intervals did not differ from those of healthy people, indicating that Cav1.3 channels do not significantly impact intraventricular conduction and ventricular repolarization. We have termed the condition SANDD syndrome (sinoatrial node dysfunction and deafness). To determine the functional consequences of the SANDD-causing mutation, we expressed wildtype and mutant Cav1.3 channel complexes in tsA-201 cells and analyzed their biophysical properties using whole-cell patch-clamp recordings. Our electrophysiological findings predict that homozygous individuals lack significant L-type Ca<sup>2+</sup> currents from exon 8B-containing Cav1.3 channels. The SANDD phenotype and mouse mRNA expression studies strongly suggest that the exon 8B splice variant is predominant in human IHC and SAN pacemaker cells. In summary, we describe a novel human channelopathy with a cardiac and auditory phenotype, and the underlying CACNA1D mutation results in non-conducting calcium channels and altered voltage-dependent gating. Our results constitute the first evidence that Cav1.3 channels are involved in human cardiac pacemaking, implying them as a potential drug target for controlling heart rate in disease conditions. Regular cardiological follow-up of SANDD patients seems appropriate to avoid cardiovascular complications.

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**SEL-02****CEP152 is a genome maintenance protein disrupted in Seckel syndrome**

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Seckel syndrome is an autosomal recessive disorder mainly characterized by proportionate short stature, severe microcephaly associated with structural brain anomalies and mental retardation, and a typical “bird-head” facial appearance. So far the only reported genetic alteration found in patients with classical Seckel syndrome is a hypomorphic mutation in Ataxia-Telangiectasia and Rad3-related protein (ATR), which plays an important role in the activation of signaling pathways regulating the response to DNA-damage.

Using a genome-wide SNP homozygosity mapping approach we identified a homozygous splice-site founder mutation in CEP152 in six Turkish families with Seckel syndrome. Independently, we also identified CEP152 as causative gene in an additional Seckel patient born to consanguineous parents using an exome sequencing strategy. We could demonstrate that mutation identification through exome sequencing together with simultaneous analysis of homozygous stretches of exome variants can be used as an efficient approach to gene identification in autosomal recessive disorders. Additional screening identified two other patients carrying compound heterozygous mutations in CEP152.

Functional analysis of CEP152 showed that CEP152 deficient fibroblasts exhibit severe defects in mitosis and spindle organization resulting in cells with multiple centrosoms and abnormal nuclei pattern. Moreover, Seckel cells showed an overall increased sensitivity to oxidative stress and responded with increased apoptosis. Cell cycle analysis in CEP152 knockdown cells using shRNA suggested that CEP152-deficiency delays S-phase entry resulting in fewer Seckel cells progressing to the G2/M phase while an increased proportion of cells stayed in G0/G1. In a yeast-two-hybrid screen using CEP152 as bait, the CDK2-interacting protein (CINP) was identified as an interaction partner of CEP152. We confirmed the CEP152-CINP interaction using complementary immunoprecipitation approaches and showed that similar to CINP-deficiency impaired CEP152 function leads to genomic instability via increased H2AX phosphorylation due to enhanced activation of ATM signaling pathways. These findings provide evidence for an important role of CEP152 in DNA damage response and genome maintenance.

In summary, our data indicate a dual function for CEP152, which is essential for centrosomal function and mitotic processes and additionally plays an important role genome maintenance and DNA damage response.

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**SEL-03****Syndactyly and Craniosynostosis phenotypes caused by Copy Number Variations (CNVs) involving the IHH locus on chromosome 2q**

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Indian hedgehog (IHH) is a secreted signaling molecule of the hedgehog family which is known to play important roles in the regulation of chondrocyte differentiation, cortical bone formation, and the development of joints. Mutations in IHH are associated with brachydactyly type A1 (MIM 112500), an autosomal dominant disorder, and acrocapitofemoral dysplasia (MIM 607778), an autosomal recessive skeletal dysplasia. We investigated families with syndactyly type 1 (SD1, MIM 185900) and craniosynostosis Philadelphia type (MIM

601222), a rare form of premature fusion of cranial sutures in combination with syndactyly. These conditions were previously mapped by linkage analysis to a region on chromosome 2q35 encompassing the IHH locus. By array CGH we detected microduplications at the IHH locus of 59 kb, 48 kb and 52 kb, respectively. These microduplications overlap in a ~9.1 kb region 40 kb 5' of IHH which contains conserved non-coding elements (CNEs). The duplication observed in the SD1 family included the complete IHH and most of the gene's regulatory region and is therefore likely to result in an increase in IHH expression. Since the limb phenotypes are highly similar in the three families, we infer that the two duplications which do not involve the IHH coding region likewise lead to an increased IHH expression. To proof this hypothesis we generated a transgenic mouse model containing the orthologous mouse sequence cloned into a LacZ reporter gene vector and tested for lacZ expression pattern at different embryonic stages. By these experiments we were able to show that the CNEs are able to drive lacZ expression specifically in the limbs and the skull. The lacZ expression pattern resembles the endogenous wildtype *Ihh* expression. Thus, these CNEs are most likely IHH long-range enhancers which are located within an intron of the neighboring gene *NHEJ1*. Interestingly this scenario is reminiscent of the situation for *SHH* where the limb control region ZRS is also located in an intron of a flanking gene (*LMBR1*) and ZRS microduplications result in ectopic *SHH* expression and limb defects. We postulate that the observed duplications in syndactyly type 1 and craniosynostosis Philadelphia type lead to a misexpression and/or overexpression of IHH and by this affect the complex regulatory signaling network during digit and skull development. In summary these data underscore the importance of long range regulation in development and the relevance of CNVs in non-coding regions for developmental defects.

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#### SEL-04

### **Hyperphosphatasia mental retardation (HPMR) syndrome is caused by mutations in the PIGV gene**

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HPMR, also known as Mabry syndrome (MIM%239300), was initially described as an autosomal recessive syndrome characterized by mental retardation and greatly elevated alkaline phosphatase levels. Within a group of individuals with this rare syndrome, a previous study delineated a specific clinical entity characterized by a distinct facial gestalt including hypertelorism, long palpebral fissures, a broad nasal bridge and tip, and a mouth with downturned corners and a thin upper lip, as well as brachytelephalangy. More variable neurological features included seizures and muscular hypotonia. We performed whole-exome sequencing in three siblings of a nonconsanguineous union with HPMR and established PIGV as the disease gene. Additionally we identified several further missense mutations in other unrelated HPMR cases. PIGV encodes a member of the GPI-anchor biosynthesis pathway: The highly conserved backbone structure of the GPI anchor is synthesized in the endoplasmic reticulum through at least nine sequential reaction steps mediated by at least 18 proteins. GPI-anchored proteins comprise functionally divergent classes including hydrolytic enzymes, receptors, adhesion molecules and proteins with roles in the immune system. Although over 100 mammalian proteins are modified by a glycosylphosphatidylinositol (GPI) anchor at their C terminus, little is known to date about the phenotypic consequences of mutations in genes of this synthesis pathway. PIGV constitutes the second mannosyltransferase in this molecular pathway and was of particular interest for HPMR syndrome because alkaline phosphatase is a GPI-anchored protein. We showed that the identified missense mutations negatively influence GPI anchor synthesis: We showed a substantial reduction of GPI-anchor synthesis. The surface expression of GPI-anchored marker proteins as well as alkaline phosphatase is markedly reduced in PIGV mutant cells. In summary we identified HPMR syndrome as a molecular pathway disease of the GPI-anchor synthesis.

## W1 MONOGENIC DISEASE I

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### W1-01

#### **Autosomal recessive posterior microphthalmos/nanophthalmos is caused by loss-of-function mutations in LOC646960, a novel gene encoding a trypsin-like serine protease**

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Posterior microphthalmos (MCOP)/nanophthalmos (NNO) is a rare isolated developmental anomaly of the eye. In adults, autosomal recessive MCOP (arMCOP/MCOP6) is clinically characterized by an essentially normal anterior segment, extreme hyperopia (from +7.5 to +21 diopters) due to short axial length (14-20 mm compared to normal >21 mm), steep corneal curvatures, shallow anterior chamber, thick lenses, thickened scleral wall, and occasionally microcornea. The population of the Faroe Islands shows a high prevalence of arMCOP. Based on previous linkage data obtained in consanguineous Tunisian families with arMCOP/NNO, we have refined now the position of the disease locus (MCOP6) in an interval of 250 kb in chromosome 2q37.1 in two large Faroese families using microsatellite and single nucleotide polymorphisms. Subsequently, we detected three different disease-causing mutations in a novel gene, LOC646960: Patients of the Faroese families were either homozygous (n=22) for c.926G>C (p.Trp309Ser) or compound heterozygous (n=6) for c.926G>C and c.526C>G (p.Arg176Gly), whereas a homozygous 1 bp duplication (c.1066dupC) was identified in 5 patients with arNNO from a consanguineous Tunisian family. In one patient with MCOP from the Faroe Islands and another one from Turkey, no LOC646960 mutation was detected suggesting non-allelic heterogeneity of the trait.

LOC646960 is expressed in the human adult retina and retinal pigment epithelium. The expression of the mouse homologue in the eye can be first detected at E17 and is highest in adults. The predicted LOC646960 protein is a 603 amino acid long secreted trypsin-like serine peptidase. c.1066dupC is likely to result in a functional null allele whereas for the two point mutations the replacement of evolutionary conserved and functionally important residues is predicted. Molecular modelling of the p.Trp309Ser mutant suggests that both affinity and reactivity of the enzyme towards *in vivo* protein substrates are likely to be substantially reduced.

Postnatal development of the ocular refractive components is a tightly regulated adaptive process by which optical defocus leads to changes in axial length moving the retina towards the image plane. The postnatal growth of the eye is mainly due to an elongation of the posterior segment from 10-11 mm at birth to 15-16 mm at the age of 13 years. This emmetropization process involves a retinal feed-back mechanism. However, very little is known about the molecular pathways involved in this remodeling. In line with the expression pattern of LOC646960, the pathophysiology involved in MCOP may theoretically be explained by a postnatal growth retardation of the posterior segment (axial length of the vitreous). We propose that the proteolytic activity provided by the protein encoded by LOC646960 is essential for this process during development. It is tempting to anticipate the involvement of secreted peptidases in catalytic processes in the vitreous and/or the sclera.

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**W1-02****Loss-of-Function Mutations in the DFNB42 gene Cause Autosomal-Recessive Hearing Impairment**

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Using homozygosity mapping in a consanguineous Pakistani family we detected linkage of nonsyndromic hearing impairment to a 7.6 Mb region on chromosome 3q13.31-q21.1 within the previously reported DFNB42 locus. Subsequent candidate gene sequencing identified a homozygous nonsense mutation in a gene of unknown function (the DFNB42 gene) as the cause of hearing impairment. By analyzing additional consanguineous families with homozygosity at this locus, we detected DFNB42 mutations in the affected individuals of 10 more families from Pakistan and Iran. The identified variants include missense, nonsense, frameshift and splice-site mutations as well as a start codon mutation in the family that originally defined the DFNB42 locus. The DFNB42 gene encodes a putative transmembrane receptor of unknown function. In situ hybridization detected expression of the murine ortholog of this gene early in development both in the vestibule and in hair cells and supporting cells of the cochlea. Expression in hair cell-containing neurosensory organs is conserved in the zebrafish ortholog, which is prominently expressed in the ear and neuromasts of the lateral line. These data identify the DFNB42 gene as a novel gene for nonsyndromic prelingual sensorineural hearing impairment with a conserved expression pattern pointing to a key role in hearing in vertebrates.

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**W1-03****Novel insights into the genetics of autosomal recessive hearing loss**

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Sensorineural hearing impairment is the most common sensory disorder affecting approximately 1 in 2000 newborns. Nonsyndromic hearing loss (NSHL) is the most frequent form of inherited deafness and in 80% of the cases, the mode of inheritance is autosomal recessive. To date, more than 32 genes and over 80 DFNB loci have been identified in families with autosomal recessive nonsyndromic hearing loss (ARNSHL). In an ongoing study, we aim to identify additional DFNB loci and novel genes, and to gain more insights into the pathogenesis of ARNSHL. Using SNP-arrays in a large consanguineous family from Morocco, we mapped the disease to the DFNB79 locus on chromosome 9q34. Sequencing of 62 positional candidate genes located within the critical region identified a causative homozygous 11 bp deletion (c.42\_52del) in exon 1 of the TPRN gene. The mutation co-segregating with the disease in all seven affected individuals, was heterozygous in parents, and was not found in 140 healthy control individuals from Morocco. Furthermore, we identified a homozygous 1 bp deletion (c.1347delG) in exon 1 of TPRN in all affected members of a Dutch family with a progressive type of hearing impairment. The function of the encoded protein, Taperin, is yet unknown, but it shows partial homology to the actin capping protein phostensin suggesting a putative role in actin dynamics.

Moreover, we mapped a novel DFNB locus (DFNB69) in a consanguineous Moroccan family to chromosome 3p14.3-p14.2. The critical region of this locus is 6.7 Mb in size and flanked by the SNPs rs1379725 and rs978879. Sequencing of positional candidate genes is currently performed to identify the disease causing gene in this family. In a consanguineous family from Pakistan we found linkage to the DFNB39 locus. Very recently, noncoding hot-spot mutations in HGF have been described in DFNB39 linked families from Pakistan. Interestingly, sequencing of all coding and noncoding exons as well as conserved regions of the HGF gene did not reveal any causative mutation in our family, suggesting further genetic heterogeneity at this locus.

Future identification of DFNB69- and DFNB39-linked genes and subsequent functional characterization of encoded proteins in the inner ear will give further insights into the pathogenic mechanisms of congenital hearing loss.

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## W1-04

### Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes

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N-methyl-D-aspartate (NMDA) receptors are neurotransmitter-gated ion channels involved in regulation of synaptic function in the central nervous system. A unique property of these highly calcium-permeable cation channels is their voltage-dependent activation as a result of a channel block by extracellular magnesium. NMDA receptors are heterotetrameric assemblies of two glycine-binding NR1 and two glutamate-binding NR2 subunits and the identity of the specific NR2 subunits (A, B, C or D) determine many of their physiological and pharmacological properties. Inappropriate activation of NMDA receptors has been implicated in Alzheimer's disease and schizophrenia; however, single gene mutations in subunits of NMDA receptors have not yet been reported in Mendelian disorders.

In two patients with mental retardation (MR), behavioral anomalies, and abnormal electroencephalogram (EEG) we identified de novo chromosome translocations and a common breakpoint in 12p13.1, directly disrupting the GRIN2B gene which encodes the NR2B subunit of NMDA receptors. Sequencing of GRIN2B in 468 patients with mild to severe MR revealed four heterozygous de novo mutations: a frameshift (c.803\_804delCA), a missense (c.2044C>T), and two splice-site mutations (c.411+1G>A and c.2360-2A>G). The c.2044C>T transition predicts the amino acid substitution p.R682C, affecting a highly conserved arginine within the NR2B glutamate-binding domain (LBD). In silico protein structure analysis predicted loss of two hydrogen bonds that may cause destabilization of the tertiary structure of the NR2B LBD. RNA analysis in the two patients with splice-site mutations indicates monoallelic expression of the GRIN2B wild-type allele due to nonsense-mediated mRNA decay (NMD) of aberrantly spliced GRIN2B transcripts.

Another translocation, t(16;17)(p13.2;q11.2), that co-segregates with epilepsy and variable degree of cognitive impairment in a family was found to interrupt the GRIN2A gene encoding the NR2A subunit. We sequenced GRIN2A in a cohort of 127 patients with idiopathic epilepsy and/or abnormal EEG and variable degree of MR and identified a heterozygous nonsense mutation (c.652C>T/p.Q218X) in a mildly mentally retarded boy. RNA analysis revealed monoallelic expression of the wild-type allele suggesting that GRIN2A transcripts with the premature stop codon were efficiently degraded by NMD. We detected the same mutation in the patient's mother and maternal grandmother, both of which had a history of seizures during childhood. Finally, we found a heterozygous de novo missense mutation in GRIN2A (c.1845C>A/p.N615K) in a girl with early-onset epileptic encephalopathy, abnormal EEG, and severe developmental delay. Analysis of NR1-NR2A N615K (NR2A subunit with the p.N615K alteration) receptor currents revealed a decrease in calcium permeability and a loss of the magnesium block. We conclude that loss-of-function mutations in GRIN2A, such as gene disruption and the nonsense mutation are associated with a relatively mild phenotype, while the severe phenotype in the patient with p.N615K can be explained by a dominant negative effect on NMDA receptor function. In summary, our findings suggest that disturbances in the neuronal electrophysiological balance during development result in variable neurological phenotypes, depending on which NR2 subunit of NMDA receptors is affected.

**Manitoba-oculo-tricho-anal (MOTA) Syndrome is caused by mutations in *FREM1***

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Manitoba-Oculo-Tricho-Anal (MOTA; OMIM 248450) syndrome is a rare condition comprising eye defects with eyelid colobomas and – less frequently – cryptophthalmos and anophthalmia, an aberrant anterior scalp hairline, hypertelorism, a bifid nasal tip and gastrointestinal anomalies including omphalocele and anal stenosis. The condition has considered to be autosomal recessive because of consanguinity in the affected Oji-Cre population of Manitoba, but so far no chromosomal aberration or locus have been identified.

We report on four previously published families (Fryns, 2001; Li et al., 2007; Yeung et al., 2009) and a new family with MOTA syndrome. The new family includes three affected daughters and their affected male second cousin who had variable expression of MOTA phenotypes with bifid nasal tip, aberrant anterior scalp hairline and ocular anomalies, but no anal malformations. All individuals had normal intelligence.

We used the Affymetrix Genome-Wide Human SNP Array 6.0 to look for linkage to a shared ancestral allele as well as for copy number variants in a Manitoban family with two affected offsprings (Li et al., 2007) and the new family who was not known to be related but originated from the same population. We identified an intragenic deletion of approximately 60 kb in size in *FREM1* that removes exons 7-22 of this 37 exon gene. The two affected children from the previously published Manitoban family and the male cousin from the new family were homozygous for the deletion, whereas the three affected sisters were heterozygous for the deletion which was inherited from their clinically normal father. Sequencing of *FREM1* in the three sisters revealed a maternally inherited, synonymous single nucleotide substitution in exon 30 that occurred one base pair prior to the last coding nucleotide of this exon (c.6349A>G) and was predicted and experimental confirmed to abolish the donor splice site. In a patient reported previously with an unclassified syndrome comprising micro-ablepharon of the upper eyelids and vaginal atresia (Fryns, 2001) we identified a homozygous 4bp deletion in exon 11, c.2097\_2100del, which leads to a frameshift and a premature stop codon. In a Dutch family described by Li et al. (2007) we detected two missense mutations in exon 21 and 35. In the two affected patients from another previously published family (Yeung et al., 2009) no *FREM1* mutations could be detected and segregation analysis indicated that the affected children did not share a *FREM1* allele.

We conclude that mutations in *FREM1* are responsible for MOTA syndrome, but there is evidence for the existence of at least one further causative gene. Homozygous, loss-of-function mutations in *FREM1* have previously been described in two families with bifid nose, renal agenesis and anorectal malformations (BNAR) syndrome, thus making MOTA syndrome and BNAR allelic conditions. Although eye defects have not been reported in BNAR, it seems reasonable to consider both MOTA and BNAR as part of a phenotypic spectrum. Moreover, these findings relate these two conditions clinically and pathogenetically with the more severe Fraser syndrome to constitute a group of "FRAS-FREM complex diseases". Interestingly in both mouse and zebrafish, the phenotypes resulting from *Frem1* loss-of-function have also been milder than those of *Fras1* and *Frem2* loss-of-function associated with human Fraser syndrome.

**Disruption of the histone acetyltransferase MYST4 leads to a Noonan syndrome-like phenotype and hyperactivated MAPK signaling**

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Epigenetic regulation of gene expression through covalent modification of histones is a key process controlling growth and development. Accordingly, the transcription factors regulating these processes are important targets of genetic diseases. However, surprisingly little is known about the relationship between aberrant epigenetic states, the cellular process affected and their phenotypic consequences. By breakpoint mapping of a balanced de novo chromosomal translocation t(10;13)(q22.3;q34) in an individual with a Noonan syndrome-like phenotype encompassing short stature, blepharoptosis and attention deficit/hyperactivity disorder, we identified haploinsufficiency of the MYST4 gene, as the underlying cause. MYST4 belongs to the family of MYST histone acetyltransferases (HAT) and specifically acetylates H3k14. We not only observed significantly reduced H3 acetylation and unchanged H4 acetylation in our affected individual, but also a correlation of MYST4 levels with H3 acetylation in HEK293 and HeLa cell lines after siRNA knock down in 3 independent experiments (Rank Correlation coefficient  $p < 0.02793$ ). In Mice homozygous for a hypomorphic gene trap allele of *Myst4* [Querkopf (Qkfgt/gt)] we found a failure to thrive phenotype with facial dysmorphism and high expression in the primordial of the cartilage and the neuronal stem cells. Genome wide transcriptome profiles in cell lines of the affected individual, the HEK293 and the HeLa siRNA cell lines, and Qkfgt/gt adult dorsal cortex and dorsal telencephalon cells to uncover involved pathways of MYST4 H3 acetylation, showed an enrichment of significantly differentially expressed genes in 10 KEGG pathways. To distinguish between primary and secondary targets we performed ChIP-CHIP in two cell lines and found enrichment only for the MAPK signaling pathway. As hyperactivation of the RAS-MAPK transduction pathway is the main underlying pathomechanism in Noonan syndrome we also explored the phosphorylation levels of MEK1/2, ERK1/2, and AKT in our affected individual. This confirmed a hyperactivation of the MAPK pathway by hyperphosphorylation of key proteins by MYST4. In addition, we were able to reverse this effect by MYST4 overexpression in MYST4 deficient cell lines.

This further elucidates the complex role of histone modifications in mammalian development and adds a new pathomechanism to the phenotypes resulting from misregulation of the RAS signaling pathway. Such epigenetic effects should therefore be considered in genetically heterogeneous conditions like Noonan syndrome and may represent a future target for novel therapeutic strategies.

## W2 COMPLEX DISEASES

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### W2-01

#### Classical candidate association study identifies a new RPE/retina-specific AMD susceptibility gene

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**Background:** Age-related macular degeneration (AMD) is a complex disorder of the retina/choroid and the most common cause of legal blindness in industrialized countries. Two major susceptibility loci have been identified including CFH on 1q32 and ARMS2/HTRA1 on 10q26. Furthermore, three additional complement gene loci (C2/CFB, C3 and CFI) have also been shown to play a role in AMD pathogenesis. These gene loci are detectable even in genome-wide association studies (GWAS) with moderate sample sizes. Other susceptibility genes with moderate to minor contributions to AMD risk are likely but difficult to detect in GWAS. However, a candidate gene approach which typically analyses a restricted number of SNPs within a preselected candidate locus in a large case-control sample can provide reasonable statistical power to detect relatively rare and weak risk effects.

**Methods:** 25 AMD candidate genes were selected based on functional implications in phenotypically related retinopathies, known AMD pathomechanisms and/or an RPE/retina-specific gene expression. These genes were analysed following a haplotype-tagging SNP approach (N = 109) in a German discovery sample consisting of 794 AMD patients and 612 controls. Replication of positive hits was done in a second independent German sample of 868 AMD patients and 548 controls which could be extended by two additional independent Caucasian replication studies from the US and the UK resulting in a pooled replication sample of 2,025 AMD patients and 1,099 controls.

**Results:** After quality control and analysis of the 109 SNPs we detected three relatively weak association signals in three independent gene loci ( $0.014 \leq P \leq 0.032$ ). One of these signals remained statistically significant in the first replication sample ( $P = 0.0023$ ) strongly suggesting the corresponding RPE/retina-specific gene to be involved in AMD predisposition. Follow-up of this SNP in the US and the UK sample revealed consistent association signals ( $P = 0.012$  and  $P = 0.031$ ; respectively) and resulted in a highly significant association in the pooled replication sample ( $P = 2.6 \times 10^{-5}$ ). The observed differences in effect allele frequency in all four studies were similar (13.3–14.3 % in the AMD cases; 10.3–10.9 % in the controls) revealing odds ratios from 1.34 to 1.56. Further fine-mapping of the gene locus narrowed the candidate region to approximately 150 kb that exclusively encompassed the candidate gene in question and so far indicated 15 correlated variants whose functional consequences remain to be elucidated.

**Conclusions:** The current candidate gene approach successfully indicated a novel AMD susceptibility gene with an RPE/retinal-specific expression revealing a consistent association with AMD in four independent Caucasian case-control-studies. The observed effect allele frequencies and odds ratios classified the locus to be a moderate AMD risk modifier. Ongoing functional analysis of the new AMD susceptibility gene might uncover a functional variant. Further knowledge of the function of the novel gene may point to a new mechanism underlying AMD pathology.

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**W2-02****Investigation and functional characterization of rare genetic variants in the adipose triglyceride lipase in a large healthy working population.**

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Background: Although GWAS have been tremendously successful, there is still a considerable gap between the variance explained by common SNPs and traditional heritability estimates. Rare variants, which are not targeted by conventional GWAS, were repeatedly used for explaining the "missing heritability". Indeed, several studies detected an accumulation of rare variant in the extremes of a trait distribution. However, few data is available from general populations. Since ATGL catalyzes a crucial step of the lipolysis, it represents a candidate gene to evaluate the impact of rare mutations on the lipid metabolism.

Objective: To capture the entire genetic variation in the ATGL gene locus, investigate the effects of the detected protein variations in-vitro and assess their association with plasma free fatty acid levels.

Methods: The entire ATGL was screened for genetic variants in 1473 individuals of the healthy working population SAPHIR from Salzburg, Austria. All identified non-synonymous variants were expressed in COS7 cells and both intracellular localization and hydrolase activity were investigated. To assess the impact of rare ATGL variants on FFA levels in a general population, we performed genetic association studies and compared the proportion of rare variant carriers between the upper and lower 10 % quantiles of the FFA distribution.

Results: Besides several known SNPs, we detected 55 mostly rare or even private variants not yet listed in the databases, including 11 novel rare amino acid exchanges. Indeed, 7.7 % of the individuals carried a rare variant, showing that rare variants are collectively frequent even in a healthy population. The investigation of the triglyceride hydrolase activity revealed a wide spectrum in the residual catalytic activities of the protein variants, ranging from total inactivity to wild type activity and all but one were still able to bind to the lipid droplets. Association studies showed a moderate shift of rare variants carrier towards lower FFA levels, respectively an accumulation of rare variants in the lower 10 % quantile of the FFA distribution.

Conclusion: Our screening revealed large allelic heterogeneity in a healthy population, confirming previous assumptions of the "common disease - rare variant hypothesis". However, although these protein variants showed a large variability in their in-vitro activity, they exerted only a minor impact on the FFA levels in the population. This suggests that most of the naturally occurring rare ATGL variants may be only mildly deleterious and exert only a minor effect on FFA levels in the general population, probably due to the heterozygous nature of these variants.

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**W2-03****Soluble transferrin receptor (sTfR) is associated with the proprotein convertase PCSK7 gene locus.**

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Body iron storage and the erythropoietic need for iron are indicated by the serum levels of ferritin and soluble transferrin receptor (sTfR), respectively. A meta-analysis of five genome-wide association studies (GWAS) on sTfR and ferritin revealed novel association to the PCSK7 and TMPRSS6 loci for sTfR and the HFE locus for both parameters. The PCSK7 association was the most significant (rs236918,  $p = 1.1 \times 10^{-27}$ ) suggesting that proprotein convertase 7, the gene product of PCSK7, may be involved in sTfR generation and/or iron homeostasis. Conditioning the sTfR analyses on transferrin saturation abolished the HFE signal and substantially diminished the TMPRSS6 signal while the PCSK7 association was unaffected,

suggesting that the former may be mediated by transferrin saturation whereas the PCSK7-associated effect on sTfR generation appears to be more direct.

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## W2-04

### Systematic identification of SNPs influencing human hippocampal gene expression (eQTL analyses)

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Recent studies have demonstrated that human phenotypic diversity is strongly influenced by cis- and trans-acting influences on gene expression. In addition, these studies found that allele-specific expression is relatively common among non-imprinted autosomal genes. Studies aiming at the identification of the underlying genetic variation (expressed Quantitative Trait Loci - eQTLs) have been performed in peripheral tissues and in post-mortem brain. At this point, no data are available from pre-mortem brain samples, although these data would be very valuable for brain-related phenotypes. It is clear that the main reason for a lack of such studies is the limited availability of pre-mortem human brain tissue. In the present study, we had the unique chance to use a large biobank of fresh-frozen human hippocampus samples from surgery of treatment-resistant epilepsy patients for such an eQTL study. We isolated genomic DNA and RNA of n=138 hippocampus samples and subjected the DNA samples to genome-wide, array-based genotyping using > 600,000 SNPs. In parallel, individual gene expression levels for more than 99.9% of all known human genes (~25,000 annotated RefSeq and UniGene genes with > 48,000 probes) were interrogated with microarrays. Gene expression levels were then systematically correlated with individual genotype information. We used 530,777 quality-controlled SNPs (SNP call rate >98%, MAF >0.01, HWE 1x10<sup>-5</sup>; probe detection p-value >0.01) and 15,426 probes for eQTL analyses. Bonferroni correction (p-value threshold <e-12) yielded 75 significant trans associations. For cis-regulating elements, we performed permutation based analyses which resulted in 1192 significant cis eQTLs (genome-wide Westfall-Young correction). We are currently interpreting the resulting cis- and trans-eQTLs in depth, including the genome-wide distribution and overlap with published eQTLs from other tissues and post-mortem brain tissue. Apart from that, an important application of our findings will be the interpretation of genome-wide association (GWA) study signals obtained for brain phenotypes, in particular neuropsychiatric disorders.

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## W2-05

### Suggestive evidence for an association of copy number variants in four chromosomal regions in major depressive disorder

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Major depressive disorder is the third leading cause of global burden of disease world-wide. Several studies implicated copy number variants (CNVs) in the etiology of neuropsychiatric disorders, such as schizophrenia, bipolar disorder and autism. To our knowledge no genome-wide screening for CNVs has been performed for major depressive disorder, so far. We performed a systematic genome-wide survey for CNVs in a German sample of 575 patients with a DSM-IV diagnosis of major depression and 1,618 controls. All individuals were genotyped either on Illumina's HumanHap550 or Human610 arrays, with a consensus

set of approximately 550,000 markers. To identify potential CNVs, each individual's SNP-chip information was analyzed with QuantiSNP and PennCNV. Both algorithms use Hidden-Markov models to predict CNVs from SNP intensity and allele frequency data. To minimize the number of false-positive CNV calls, we developed a stringent quality protocol, requiring CNVs to have a minimum of 30 consecutive SNPs and a log Bayes Factor (QuantiSNP) or confidence value (PennCNV) of at least 30. The latter quality measures indicate the confidence of each predicted CNV, with higher values pointing towards higher statistical reliability. The two datasets generated by QuantiSNP and PennCNV were analyzed separately. Only those regions for which both programs discovered nominal significance were included in further analyses. Using PLINK, we performed permutation-based tests for association of CNVs in specific chromosomal regions with major depressive disorder.

We discovered a significant overrepresentation of CNVs in four different chromosomal regions (7p21, 15q26, 16p11, 18p11) in patients compared to controls. To our knowledge, CNVs in 7p21, 15q26, and 18p11 have not been described in MDD or other psychiatric disorders so far. The finding on chromosome 16p11 is of particular interest since microdeletions and microduplications in this region have been reported with autism (Weiss et al. 2008) and schizophrenia (McCarthy et al. 2010). In our sample we observed three rare CNVs in 16p11 (one microdeletion, two microduplications) in patients and none in controls ( $P = 0.0178$ ).

This is the first study implicating specific CNVs in the etiology of major depressive disorder, and providing evidence for an extension of the neuropsychiatric phenotypes associated with CNVs in chromosomal region 16p11.

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## W2-06

### Association study and differential gene expression of Dupuytren's disease

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Dupuytren's disease is a multifactorial fibromatosis that causes progressive and permanent contracture of the palmar fascia with subsequent flexion contracture of the fingers. A strong genetic predisposition exists but few is known about the epidemiology and the molecular etiology and pathogenesis of the disease. It is the most frequent genetic disorder of connective tissue with a prevalence of about 3% in Germany. We have embarked on a comprehensive study to unravel the genetic factors involved in Dupuytren's disease. Clinical and epidemiological data were collected using a standardized questionnaire. DNA was extracted from blood samples, RNA and fibroblasts were isolated from fresh tissue samples. Until now we have recruited 600 patients from German and Swiss origin. 19% of the patients were females; 40% had a family background for Dupuytren's contractures. The age at first surgical treatment was between 22 and 86 years of age, the mean age was  $58 \pm 10$  years in male and  $60 \pm 9$  years in female patients. In cases with positive family history both hands were affected in 64% as compared to 45% with no known family history. 10% of the patients also had diabetes, 3% rheumatoid arthritis, and 1% epilepsy, pointing to a potential association with diabetes. We did not observe a significant effect of factors such as smoking or alcohol consumption. Cultured patient cells exhibited an increased synthesis of stress fibers and myofibroblast markers such as alpha-smooth muscle actin before and after stimulation with transforming growth factor beta, which is in agreement with the excessive extracellular matrix deposition in Dupuytren's disease. A first genome wide association study of 186 patients and 1521 controls using Affymetrix SNP array 6.0 has been completed. Cluster analysis identified eight candidate regions on chromosomes 1, 2, 6, 7, 9, 11, 16, and X, with at least one marker with significant association ( $p < 5E-8$ ) in four of these. A study of differential gene expression with RNA samples from affected tissues compared to RNA from fascia of control persons using Illumina gene expression arrays identified changes in cell/matrix interaction and matrix homeostasis, which is maintained by matrix metalloproteinases and their inhibitors. More generally, alterations in signalling pathways and response to oxidative stress were seen, which point to underlying defects in basic cellular processes and might contribute in particular to the aging association of the phenotype. An integrative analysis of differential gene expression and GWAS results and replication studies with Dutch and English cases of Dupuytren's disease are currently under way.

## W3 EPIGENETICS

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### W3-01

#### **Ultra-high resolution tiling arrays reveal species-specific methylation patterns in the language gene CNTNAP2 in human and chimpanzee cortices**

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Accelerated brain development is a unique feature of the human species. Not only the size but also morphology, in particular the connections between frontal cortex and basal ganglia distinguish the human brain from great apes and other primates. Recent findings suggest that structural features which may be important for language acquisition are influenced by FOXP2, key regulator of CNTNAP2. CNTNAP2 is one of the largest genes in the human genome, encompassing 2.3 Mb. It encodes a neurexin with essential roles in the vertebrate nervous system. The aim of our study was to compare the methylation patterns of CNTNAP2 in human and chimpanzee brains, assuming that epigenetic regulation is essential for human brain development and language abilities. To this end, we designed NimbleGen tiling arrays for both the human and the chimpanzee CNTNAP2, each covering the entire gene plus 0.1 Mb up- and downstream flanking sequence with an average resolution of 13 bp. Methylated DNAs of six human and five chimpanzee brains (frontal cortex, area A10) were immunoprecipitated by MeDIP and hybridized to the human and chimpanzee arrays, respectively. Bioinformatic analyses revealed 834 distinct regions that were methylated in at least one species. Differential methylation between species was assessed by setting up region scores ranging from -1 to +1, with high absolute scores indicating major differences between methylation signals. Empirical p-values reflecting the probability of a particular score arising by chance were determined by a permutation procedure and assigned to each region. This resulted in five regions reaching a p<0.05; of those, four regions displayed selective methylation in human cortices, whereas only one region was exclusively methylated in chimpanzee cortices. Given the impact of DNA methylation on gene expression and the role of CNTNAP2 in brain development, we suggest that morphological and functional features exclusive to the human brain are at least in part a consequence of sophisticated epigenetic control mechanisms regulating the concerted expression of CNTNAP2 and other genes implicated in human language acquisition.

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### W3-02

#### **Hypomethylation of the imprinted MEST gene in offspring of mothers with gestational diabetes suggests a role for epigenetics in fetal programming of adult disease**

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Epidemiological studies in humans and experiments in animal models suggest that the origins of obesity, diabetes and other adult metabolic disorders lie not only in genes and environmental risk factors, such as unbalanced diet and physical inactivity, but also in early (intrauterine) life conditions. The offspring of obese women and/or women with diabetes are at greater risk for developing metabolic disorders themselves later in life. Epigenetic processes such as DNA methylation are primary candidates when searching for mechanisms that can stably modulate gene expression and metabolic pathways, depending on the particular intrauterine conditions. To test the effects of a maternal hypernutritional status on the epigenome of the next generation, we have analyzed the methylation profiles of representative genes in newborns from mothers with insulin-dependent gestational diabetes (GDM), dietary-treated GDM, and healthy controls (without GDM and overweight). Quantitative bisulfite pyrosequencing was used to determine the methylation levels of 7 imprinted genes (H19, LIT1, MEG3, MEST, NESPAS, PEG3 and SNRPN), 4 genes involved in energy metabolism (NDUFB6, NR3C1, LEP and PPARA), one proinflammatory gene (IL10), and 2 pluripotency markers (NANOG and OCT4) in two different tissues (chorionic villi and umbilical cord blood) of 20-50 newborns from each group. Various data evaluation strategies (corrected for multiple testing) revealed a highly significant (p<0.001) and very robust methylation difference in the MEST imprinting control region between newborns from GDM mothers and controls. Both insulin-dependent and dietary-treated GDM were associated with a 5-7 percentage point decrease in MEST methylation, compared to controls. Our results suggest that an intrauterine environment over-rich in nutrients leads to methylation changes in MEST and

other genes, which may contribute to fetal programming of adult disease. In animal models, upregulation of the imprinted Mest gene has been linked to adipocyte hypertrophy.

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### W3-03

#### **Parental-allele specific methylation analysis of developmentally important genes in individual two-cell embryos from in vitro and in vivo matured mouse oocytes**

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To date, human oocytes used for ART are typically matured in vivo by various hormonal treatments. In vitro maturation (IVM) would greatly enhance the pool of available oocytes for ART procedures, while simultaneously minimizing undesirable side effects of conventional IVF protocols associated with the intensive hormonal treatment. To assess possible epigenetic effects of IVM on the epigenome of the next generation, we have analyzed functionally important methylation patterns in two-cell mouse embryos from IVM oocytes. In early embryos quantitative methylation analysis is usually accomplished by investigating pools of >100 cells by either cloning or pyrosequencing of the amplification products following bisulfite conversion. However, bisulfite treatment leads to degradation of the DNA, which then exhibits a degenerated sequence composition and low complexity. Therefore, amplification bias favoring single alleles cannot be excluded when a larger pool of cells is analyzed. Moreover, single aberrantly methylated alleles can easily be masked by an excess of normally methylated alleles. Here, we analyzed for the first time methylation profiles of single alleles of three imprinted genes (H19, Igf2r and Snrpn) as well as the pluripotency gene Oct4. By using a limiting dilution approach and bisulfite pyrosequencing, we were able to compare the methylation profiles of individual two-cell embryos from in vitro grown and matured oocytes after 13-day preantral follicle culture and in vivo matured control oocytes. IVF or natural fertilization of *Mus musculus* oocytes with *M. castaneus* sperm allowed us to delineate parental allele specific methylation patterns, as well as the parental origin of methylation errors. Altogether, we found only one abnormally methylated maternal H19 allele (imprinting mutation) in the IVM group; all control embryos (after IVF of in vivo matured oocytes or spontaneous conception) displayed normal methylation patterns in the four analyzed genes. The IVM group displayed a higher rate (7.2%) of stochastic methylation errors (at single CpGs sites within larger amplicons), compared to the controls (2.9% in embryos from in vivo matured oocytes and 0% in naturally conceived embryos). We conclude that at least in the mouse model in vitro growth and maturation does not appear to dramatically increase in number of imprinting mutations, which might interfere with normal development, but may be associated with a higher number of stochastic methylation errors and epigenetic variability.

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### W3-04

#### **A familial disorder with genome-wide disturbance of DNA methylation at both paternally and maternally imprinted loci**

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A yet unknown number of human genes show parent-of-origin specific DNA methylation and expression. Defined defects in this process called 'genomic imprinting' lead to well-recognized syndromes associated with abnormal development, growth or behavior. We present three offspring of a healthy couple with prenatal onset of severe growth retardation and dysmorphism.

Child 1: Omphalocele and shortened femora were observed at 21 weeks of gestation. A detailed ultrasound scan performed at 31 weeks showed that all long bones were shortened and that the thorax was very narrow incompatible with a normal lung function. Therefore a feticide was done. The female fetus had a

relatively large cranium, a small triangular face with microretrogenia and suborbital skin folds. Weight and length were below the 3rd centile. On pathologic examination pancreas anulare, polynesia and macronesia of Langerhans islets and gallbladder agenesis were diagnosed. The kidneys were asymmetrically enlarged with signs of hydronephrosis. Bone age was retarded. Placenta histology was normal.

Child 2: At 11 weeks the fetus had short limbs and an omphalocele. The placenta was thick and molar. The free  $\beta$ -HCG in maternal blood was 10 MoM. Subsequently the mother developed ovarian cysts similar to those found in ovarian overstimulation. There was asymmetrical growth retardation. Due to a pathologic CTG heart rate pattern a boy was delivered at 32 weeks with a weight of 1150g (<3rd centile), a length of 38cm (<3rd centile), and a head circumference of 29.5cm (10th centile). Mesenchymal dysplasia of the placenta was diagnosed. The boy was hyperexcitable, and had a stenosis of the hypopharynx. A persistent Ductus arteriosus required surgery. There were umbilical hernia, dolichocephaly, frontal naevus flammeus, microretrogenia, telekanthus, deep set pinnae and coarse facial features. At the age of 13 months he could roll over, and speak syllables. During the first two years proportions harmonized. At this age substantial developmental delay was noticed.

Child 3: At 11 weeks of gestation free  $\beta$ -HCG in the maternal blood was massively elevated. The placenta was thick, molar and contained lacunae. Biparietal diameter was on the 50th centile for gestational age, femur length was on the 5th centile. CVS was performed at that time. Fetal demise was diagnosed one week later. Again mesenchymal dysplasia was observed in the placenta.

The second child was tested for Russell-Silver syndrome. This revealed hypomethylation not only of the H19 locus but also of the KCNQ1OT1 locus. Subsequent analysis of DNA of fibroblasts and blood showed hypomethylation of the maternally imprinted loci SNRPN, NDN, PLAGL1, MEST, PEG3 and GNAS and of the paternally imprinted locus MEG3. Analysis of muscle tissue of the first child showed hypomethylation at the maternally imprinted loci PLAGL1, MEST, PEG3, KCNQ1OT1 and GNAS. Methylation was normal at the SNRPN and NDN locus as well as at H19. In the third pregnancy methylation analysis in chorionic villi showed marked hypomethylation at KCNQ1OT1, PEG3, and NESPAS, a slight reduction at PLAGL1 and MEST and normal methylation at H19. Investigations to further characterize the pattern of hypomethylation and to discover the underlying cause of this widespread defect are underway.

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### W3-05

#### Retrotransposition and imprinting

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Based on genome-wide DNA methylation analysis in a patient with a generalized imprinting defect we have found that the human retinoblastoma (RB1) gene on chromosome 13 is imprinted. Skewed expression in favour of the maternal allele is linked to a differentially methylated CpG island in intron 2 of the RB1 gene. This CpG island (CpG 85) is part of a truncated processed pseudogene (KIAA0649P), which is derived from the KIAA0649 gene on chromosome 9, and serves as a promoter for an alternative RB1 transcript. To examine the evolutionary origin of the imprint we compared the sequences and methylation patterns of the ancestral gene and its pseudogenes in different primates (human, chimp, rhesus, orangutan and marmoset). Our results reveal that apart from the rhesus all primates studied have a small CpG island in exon 4 of the ancestral gene, which is fully methylated, and indicate that the pseudogene copies in the RB1 gene are differentially methylated. We also analysed KIAA0649 pseudogene copies on other chromosomes. In humans and chimps, there are additional copies on chromosome 22, which must have been derived from independent retrotransposition events. These copies do not have CpG islands, and the CpG dinucleotides are fully methylated. Interestingly, only the chimp has another copy on chromosome 8. This copy contains a large CpG island, which is completely methylated. The rhesus and orangutan genomes do not contain pseudogene copies outside of the RB1 gene. The marmoset has an additional copy whose neighbouring sequences map to human chromosome 6. This copy seems to be differentially methylated. Our findings support the hypothesis that imprinting builds on host defence mechanisms by which the genome protects itself against foreign DNA elements and shows that the evolutionary fate of the cDNA copies is distinct depending on the site of integration.

Currently we are investigating the presence of alternative transcripts in rhesus and marmoset and will check for the imprinting status if such transcripts are observed.

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## W3-06

### Eight novel patients with uniparental disomy, epimutations or a deletion of the imprinted 14q32 region

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Human chromosome 14q32 harbours a cluster of paternally expressed protein-coding genes such as DLK1 and RTL1 and non-coding maternally expressed genes such as MEG3 (alias GTL2), RTL1 antisense, MEG8, and a cluster of snoRNA and microRNA genes. Imprinting at 14q32 is regulated by two differentially methylated regions (DMRs), the DLK1-MEG3 intergenic DMR (IG-DMR) and the MEG3-DMR. Both DMRs are methylated on the paternal chromosome 14 and unmethylated on the maternal chromosome 14. Consistent with these findings, both maternal and paternal uniparental disomies for chromosome 14 (upd(14)mat and upd(14)pat) cause distinct phenotypes. Upd(14)mat (Temple syndrome) is associated with a recognizable phenotype that includes pre- and postnatal growth retardation, hypotonia, feeding problems and precocious puberty. The phenotypic spectrum ranges from a nearly normal phenotype to a severe phenotype reminiscent of Prader-Willi syndrome. Upd(14)pat results in a unique phenotype characterized by facial abnormalities, small bell-shaped thorax, abdominal wall defects, placentomegaly, and polyhydramnios. Recently, we and others have described rare nondisomic cases with a upd(14)mat-like phenotype and nondisomic patients with a upd(14)pat-like phenotype. The underlying molecular defect in these patients is either an epimutation affecting both DMRs or a deletion affecting the maternally or paternally derived 14q32 imprinted region, respectively. Here we report on six novel cases with a upd(14)mat-like phenotype and two with a upd(14)pat-like phenotype. One patient was found to have upd(14)mat for all informative markers along chromosome 14 and a marker chromosome containing heterochromatic material for the centromeric region of chromosome 14. Another patient has a segmental upd(14)mat affecting only the terminal part of the long arm of chromosome 14 (14q24>qter). In four of the cases the underlying molecular defect is an epimutation. One of these patients was found to have a small amount of methylated alleles indicating that she is a somatic mosaic for the epimutation.

One of the patients with a upd(14)pat phenotype has a paternal isodisomy for all informative markers along chromosome 14. By Affymetrix Genome Wide Human SNP Array 6.0 analysis we found that the other patient with phenotypic features of upd(14)pat has a maternal deletion of about 165 kb with both deletion breakpoints inside the imprinted region in 14q32. From the array data the proximal breakpoint could roughly be mapped between the IG-DMR and MEG3 and the distal breakpoint between the snoRNA and the microRNA gene clusters. Fine mapping of the deletion breakpoints and extended methylation analysis of the IG-DMR may help to get more information about regulation of imprinting at the 14q32 imprinted region and the development of the upd(14)pat phenotype.

## W4 INTELLECTUAL DISABILITY

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### W4-01

#### Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits

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Mental retardation affects 2-3% of the population and shows a high heritability. Neurodevelopmental disorders that include pronounced impairment in language and speech skills occur less frequently. For most cases, the molecular basis of mental retardation with or without speech and language disorder is unknown due to the heterogeneity of underlying genetic factors. We have used molecular karyotyping on 1523 patients with mental retardation to detect copy number variations (CNVs) including deletions or duplications. These studies revealed three heterozygous overlapping deletions solely affecting the forkhead box P1

(FOXP1) gene. All three patients had moderate mental retardation and significant language and speech deficits. The additional findings of obesity and mild craniofacial anomalies (prominent forehead and frontal hair upsweep) in two patients might be part of the clinical spectrum associated with FOXP1 deletions. Since our results are consistent with a de novo occurrence of these deletions, we considered them as causal although we detected a single large deletion including FOXP1 and additional genes in 4104 ancestrally matched controls. FOXP1 belongs to a functionally diverse family of forkhead box (FOX) transcription factors that are all characterized by a highly conserved FOX domain. FOX genes have been shown to play important roles in diverse cellular functions including metabolic and developmental processes. Currently, four FOX genes are listed in the Online Mendelian Inheritance in Man database that have been found to be causative for human diseases. Our findings are of interest with regard to the structural and functional relationship between FOXP1 and FOXP2. Mutations in FOXP2 have been previously related to monogenic cases of developmental verbal dyspraxia. Both FOXP1 and FOXP2 are expressed in songbird and human brain regions that are important for the developmental processes that culminate in speech and language.

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#### W4-02

### **Systematic mutation search in families with X-linked intellectual disability by next-generation sequencing**

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X-linked intellectual disability (XLID) affects 1-2/1,000 males and accounts for 10% of all forms of intellectual disability. Previous studies of the European MRX Consortium have indicated that mutations in known XLID genes account for at least 42% of XLID families (de Brouwer et al, 2007). Recent mutation screening of X-linked genes has revealed pathogenetic mutations in only 25% of the 208 families studied (Tarpey et al, 2009). To resolve this discrepancy and to shed more light on the molecular causes of XLID, we have combined genome partitioning techniques and Next Generation Sequencing (NGS) to find the causative gene defect in another 245 families from the European MRX Consortium. In this study group, about 18% of the families carry apparently pathogenic mutations in known XLID genes, including nonsense and missense mutations. About 6% of the families carry deleterious mutations in novel XLID genes and 8% carry truncating or convincing missense/in frame deletion mutations in novel candidate XLID genes. Many other families carry non-synonymous, possibly pathogenic changes in candidate XLID genes. Thus, the majority of genes whose loss gives rise to non-syndromic XLID may already be known. In about 40% of the families the causative gene defect is not detected yet. There are several plausible explanations for this, including that the fundamental defect may not reside in coding regions of the X-chromosome or missed upon enrichment and NGS.

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#### W4-03

### **Mutations in genes encoding adaptor protein complex cause autosomal recessive intellectual disability**

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A substantial part of Intellectual Disability (ID) cases are of autosomal recessive inheritance (ARID), but those have not been extensively studied, so far. We applied systematic homozygosity mapping in consanguineous families with children with ID. In one family we identified significant linkage to a locus at 14q11-q12 (~9Mb, 90 RefSeq genes), and in a second family at 1p13.3-p12 (~ 12.3Mb, 120 RefSeq genes). We applied genome partitioning using SureSelect (Agilent) followed by whole exome sequencing on the Solexa system (Illumina) in the first family and identified a nonsense mutation in the gene for adaptor protein complex 4 subunit S1 (AP4S1, c.124C>T;p.R42X). In the second family we applied Sanger sequencing after

prioritizing candidate genes based on their expression and function and identified a 3 bp insertion within exon 5 of the gene encoding adaptor protein complex 4 subunit B1 (AP4B1, c.487-488insTAT;p.E163X). Segregation was confirmed in both families with Sanger sequencing.

Adaptor proteins (AP) form a major component of the vesicle coat machinery and mediate trafficking between the Golgi network, endosomes and the plasma membrane. Four different protein complexes, AP1 to AP4, have been described, each with a typical heterotetrameric structure consisting of four subunits. Recently, mutations in two of the subunits of the AP4 complex, AP4M1 and AP4E1, have been described as causing ARID in one patient, each. Based on this and on the fact that the variants we identified are probably deleterious, we consider these as causative mutations. Patients with mutations in any of the four subunits of AP4 have severe ID, normal head circumference or mild microcephaly, are hypotonic in the neonatal period, but have hypertonia later in life. They do not become ambulant, show stereotypic movements including laughters and drooling, and have neither epilepsy, nor hearing or vision impairment. Our findings indicate that defects in intracellular trafficking can cause severe ID.

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#### W4-04

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

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Cornelia de Lange Syndrome (CdLS) is a disorder, characterized by distinctive dysmorphic features, impairment of growth and cognitive development, limb malformations and additional features with variable expressivity. Mutations in the genes NIPBL, SMC1A and SMC3 were identified as a cause for CdLS.

During the last four years samples of 91 unrelated patients with a suspected diagnosis of CdLS were referred to us for molecular analysis of the genes NIPBL and SMC1A.

In 64/91 patients we found no mutation in the genes NIPBL and SMC1A. In 30 of 64 patients we were able to confirm the clinical diagnosis of CdLS, in 13/64 patients we suggest another condition different from CdLS. In 22/64 patients we have insufficient clinical information.

In 27/91 (13 female and 14 male patients at ages between 20 weeks of gestation and 25 years) we found mutations (25 mutations in NIPBL and two mutations in SMC1A), that are, according to further analysis probably pathogenic. 21/27 mutations are novel mutations. Thirteen of these 27 patients have a mild CdLS phenotype, three patients have an intermediate phenotype whereas six patients have a severe phenotype. In 5/27 patients there were no clinical data available.

95% of the patients with molecularly proven diagnosis have typical facial features like synophrys, long eye lashes, short and anteverted nose, thin lips with down-turned corners of the mouth. Mental retardation (MR) of different severity is present in all patients (mild MR 59%, moderate MR 18% and severe MR 23%). Furthermore 18% of the patients with mutations have severe malformations of the upper limbs and another 36% patients have typical X-ray findings such as short first metacarpal or short fifth middle phalanx. For 72% of these patients intrauterine growth retardation was reported and 67% had short stature at time of assessment. 75% of these patients were dystrophic and 94% had microcephaly. Further clinical signs are feeding difficulties (76%), cleft palate (24%), congenital heart defects (40%), kidney anomalies (12%), gastroesophageal reflux and hearing loss.

It is of note that one mutation in the SMC1A gene is familial. The mother of the patient also shows short stature, microcephaly and mild MR.

In addition we report a case of gonadal mosaicism describing two affected sibs with parents not carrying the disease causing mutation in the NIPBL gene.

We discuss the clinical and molecular findings of our patients and compare them with the literature. Our study contributes to the understanding of the CdLS phenotype and possible genotype-phenotype correlation.

**Phenotypic spectrum associated with CASK loss-of-function mutations**

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Since 2008, we and others have shown that mutations in the CASK gene in Xp11.4 are associated both with a severe and distinct brain malformation phenotype in females including disproportionate pontine and cerebellar hypoplasia and a milder phenotype in males comprising mental retardation (MR), nystagmus, and microcephaly. Preliminary data show that the severe phenotype is caused by loss-of-function mutations of CASK, whereas the milder one is due to hypomorphic missense mutations. However, data on both groups are limited so far due to the small number of patients.

In order to expand the knowledge on the clinical and neuroradiologic features associated with CASK mutations and to establish a possible genotype-phenotype correlation, we identified 14 new patients with a CASK alteration and reviewed clinical and molecular data of a total of 18 patients, four of whom have been published previously. All patients were female and have CASK mutations that most likely lead to a null allele: six submicroscopic deletions of ~350 kb to 4.5 Mb including (part of) CASK, two intragenic deletions, two intragenic duplications, three nonsense mutations, one 1-bp deletion, three splice-site mutations, and one chromosomal inversion disrupting CASK. Brain imaging consistently showed pontocerebellar hypoplasia with dilated 4th ventricle, but of remarkably variable degree; corpus callosum was normal in all but one patient examined. The core phenotype comprised severe developmental delay/MR and significant microcephaly ( $\leq 3SD$ ) of prenatal or early postnatal onset, often associated with growth retardation, (axial) hypotonia with or without hypertonia of extremities, optic nerve hypoplasia and/or other eye abnormalities. A recognisable facial phenotype emerged including prominent and broad nasal bridge and tip, epicanthi, hypertelorism, a long and flat philtrum, and/or large ears. We could not establish a correlation between the genotype and specific clinical features or the degree of brain anomalies. In particular, the phenotype of patients with larger deletions including multiple other genes than CASK was indistinguishable of that caused by mutations affecting CASK only.

In conclusion, the combination of developmental and brain imaging features, comprising disproportionate pontocerebellar hypoplasia of variable degree, postnatal microcephaly, severe MR, hypo-/hypertonia, short stature, and mild facial dysmorphism, observed in individuals with CASK mutations is highly sufficient to allow clinical recognition of this disorder and subsequent testing of the CASK gene.

**Mate-pair deep sequencing as a tool for chromosome breakpoint analysis in a mentally retarded patient with balanced translocations**

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We have investigated a possible genetic cause of disease in a male patient with consanguineous parents that presented at the age of 4 years with mild psychomotor delay. While array-CGH using high-resolution SNP-chips revealed unremarkable hybridization patterns, routine chromosome analysis confirmed by fluorescence in-situ hybridization identified a complex chromosomal rearrangement involving a balanced translocation between chromosomes 2p and 7q, as well as structural rearrangements on chromosome 2 [46,XY,inv(2)(p27;q31)t(2;7)(2p25.1;q32)]. In order to map the exact chromosome break points we applied 5kb mate-pair library deep sequencing. A single sequencing lane allowed us to establish 44x theoretical spanning coverage of the genome resulting in the identification of at least four chromosomal breakpoints.

PCR confirmed that two of these lead to the disruption of annotated genes and give rise to an abnormal mRNA. This mRNA was found to be expressed in lymphoblasts and fibroblasts of the patient and is likely to encode for a fusion protein that may interfere with nucleo-cytoplasmic transport. Our study highlights the capability of new generation sequencing to increase the resolution limit at sites of major genomic rearrangements down to a single base level and to identify candidate genes implicated in mental retardation.

## W5 DISEASE MECHANISMS

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### W5-01

#### **Hypofunctional CHD1L mutations in patients with congenital anomalies of the kidneys and urinary tract (CAKUT)**

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Recently, we identified a microduplication in chromosomal band 1q21.1 encompassing the CHD1L/ALC1 gene encoding a chromatin remodeling enzyme in a CAKUT-patient. Here, CHD1L was analyzed for mutations in 85 CAKUT-patients. We identified three novel heterozygous missense variants in three patients, two of which were not found in more than 200 control chromosomes. All variants lead to amino acid substitutions in or near the CHD1L macro-domain, a poly-ADP-ribose(PAR) binding module interacting with PAR polymerase 1 (PARP1), and showed decreased interaction with PARP1 by pull-down assay of transfected cell lysates. Quantitative mRNA analysis demonstrated high CHD1L expression in human fetal kidneys, and levels were four-times higher than in adult kidney. In the human embryo at 7-11 weeks gestation, CHD1L immunolocalized in the early ureteric bud and the S- and comma-shaped bodies, critical stages of kidney development. In normal postnatal sections, CHD1L was expressed in the cytoplasm of tubular cells in all tubule segments. CHD1L expression appeared higher in the hydronephrotic kidney of one patient with a hypofunctional CHD1L variant than in normal kidneys, recapitulating high fetal levels. Our data suggest that CHD1L plays a role in kidney development and that hypofunctional CHD1L mutations may cause deficits in nephron differentiation leading to CAKUT.

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### W5-02

#### **The XLID protein PQBP1 is a novel regulator of RNA metabolism**

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The polyglutamine binding protein 1 (PQBP1) gene plays an important role in X-linked intellectual disability (XLID). To date, about 25 families with a mutation in this gene have been identified. Common clinical features of affected males include intellectual disability, microcephaly, and short stature. Most of the mutations cause frameshifts, which result in premature stop codons. Recently, we have shown that in patients who carry a PQBP1 mutation, mutant mRNA is only partially degraded via nonsense mediated mRNA decay and truncated protein exists (Musante et al., 2010).

Based on co-localization and interaction of PQBP1 with splicing factors, it has been suggested that it plays a role in the regulation of pre-mRNA processing. This hypothesis is corroborated by the fact that PQBP1 is a component of the human presplicesomal A and B complexes. To gain more insight into the functional role of PQBP1 in splicing and to unravel the pathomechanism of the disease, we have searched for novel PQBP1 interactors. Among these we have found several splicing factors, which belong to the SR protein family. SR proteins play significant roles in constitutive pre-mRNA splicing and are also important regulators of alternative splicing. In addition they participate in post-splicing activities, such as mRNA nuclear export, nonsense-mediated mRNA decay and mRNA translation. One of the newly found PQBP1 interactors, SRp20, is highly expressed in brain. In RNA-protein immunoprecipitation experiments we could show that, in addition to SRp20 protein, the PQBP1 protein complex contains SRp20 mRNA. Splicing is an essential cellular process, which it is very tightly regulated. As other SR proteins, SRp20 is able to modulate its own protein level, by regulating alternative splicing of its RNA. To test whether PQBP1 is involved in this

feedback mechanism, we have knocked-down PQBP1 and overexpressed a minigene containing the alternatively spliced exon of SRp20. RT-PCR experiments followed by quantification of the transcripts indicated that knock-down of PQBP1 significantly increased skipping of the alternatively spliced exon. Real time RT-PCR amplifications on endogenous SRp20 mRNA confirmed these results. Interestingly, rescue experiments performed with constructs containing PQBP1 mutations revealed deficient splicing activity. Additional experiments aiming to identify other PQBP1 regulated target RNAs are in progress. Taken together, we provide the first evidence that the XLID PQBP1 protein plays a key role in nuclear RNA metabolism, by modulating alternative splicing of the SRp20 target gene.

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## W5-03

### Pathophysiology of REEP1-related hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a genetically heterogeneous disorder characterized by a progressively spastic gait caused by degeneration of upper motor neuron axons. In autosomal dominant HSP loss-of-function mutations in REEP1 are a comparatively common finding. To characterize the role of REEP1 in the pathogenesis of HSP, we generated a Reep1-knockout mouse model. Knockout mice show a progressive, dose-dependent movement phenotype which is consistent with the presence of spastic paraplegia. REEP1 is a member of the DP1/Yop1p family of proteins which has been proposed to be involved in membrane-shaping. By subcellular fractionation we confirm that endogenous Reep1 localises to the endoplasmic reticulum (ER). Using purified brain lipids we show that REEP1 binds and actively bends membranes in vitro. By targeted mutation/deletion we also define the protein domain mediating such shaping activity. As REEP1 expression is particularly strong in upper motor neurons, we are currently investigating the ER in these cells at the ultrastructural level in our knockout mice.

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## W5-04

### The imprinted C15orf2 gene encodes a nuclear envelope associated protein

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The chromosomal region 15q11q13 is subject to genomic imprinting and harbours a cluster of genes which are expressed from the paternal or maternal chromosome only. The loss of function of paternally expressed genes leads to Prader-Willi syndrome, whereas loss of function of the maternally expressed gene UBE3A results in Angelman syndrome.

One of the imprinted genes is C15orf2, which is expressed from the paternal chromosome only, at least in fetal brain. In contrast to all other paternally expressed genes in 15q11q13, there is no orthologous gene in the mouse. However, one interesting finding is that it appears to be under strong positive selection in primates. C15orf2 encodes an 1156 aa protein of unknown function. As expected from the presence of six nuclear localisation sequences in C15orf2, we detected the protein in the nucleus after transient overexpression of C15orf2 in HeLa and HEK293 cells.

To find out more about the function of C15orf2 we performed sequence threading against a structural database using the Phyre software and a pattern and profile search using the InterProScan software. With both tools we found a highly significant similarity of the C-terminal part of C15orf2 to a family of nuclear pore complex (NPC) proteins and to the subfamily of the NPC transmembrane protein Pom121. By immunohistochemistry in C15orf2-transfected HEK293 cells using an anti-C15orf2 antibody and an antibody against NPC proteins we showed that in the grand majority of cells C15orf2 is localised at the nuclear envelope. Using a colocalisation software we found that 17-19 % of the overexpressed C15orf2 colocalises with the NPC. The colocalisation experiments suggest that C15orf2 is part of the NPC or its associated molecular networks. To the best of our knowledge these are the first findings suggesting a role of C15orf2 in nuclear transport or other nuclear envelope-associated functions such as transcriptional regulation, RNA-biogenesis and chromatin organisation.

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**W5-05****A mouse model for the ubiquitous overexpression of Dnmt1**

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One of the epigenetic mechanisms by which gene expression is regulated is the methylation of DNA. Erroneous DNA methylation can cause a vast spectrum of diseases. A mouse model was created in this study to investigate whether the overexpression of the somatic form of Dnmt1, Dnmt1s, can predispose to erroneous methylation and disease.

A CAG promoter-driven transgene containing the eGFP marker protein and the Dnmt1s DNA methyltransferase was constructed. Transgene functionality was tested in cell culture experiments. Because the ubiquitous overexpression of Dnmt1 has been reported to be embryonic lethal, a conditional transgene was created using the Cre-LoxP system. This technique allows the initial expression of the eGFP marker protein only. Upon cross-ins with Cre-recombinant mouse lines, the eGFP sequence is cut out and degraded. This leads to the expression of Dnmt1s under control of the CAG promoter. To easily distinguish between endogenous and transgenic methyltransferase, a RGS-His-tag was added to the N-terminal end of the Dnmt1s transgene.

Pronucleus injections with the Dnmt1s transgene resulted in six founder lines verified by PCR, Southern blot and eGFP fluorescence. Cross-ins of these founders with CMV-Cre mouse lines yielded viable offspring. The ubiquitous overexpression of Dnmt1s was investigated in cross-ins that carry the recombined version of the Dnmt1s transgene and express RGS-His-tagged Dnmt1 as verified by RT-PCR. TaqMan analyses of total Dnmt1 expression of the line with the strongest overexpression revealed a 1.6-fold overexpression in the bone marrow and in the thymus of transgenic mice in comparison to sibling wild type controls. A ~3-9-fold overexpression could be detected in brain, kidney, liver, lung, lymph nodes, spleen, testis and uterus. The strongest overexpression showed heart (~55-fold) and skeletal muscle tissue (~228-fold).

Upcoming analyses will test the transgenic protein expression and the phenotypic consequences of Dnmt1s overexpression.

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**W5-06****Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect**

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Neuromuscular junctions (NMJ) are synapses that transmit impulses from motor neurons to skeletal muscle fibers leading to muscle contraction. Fundamental mechanisms influencing development and function of the NMJ can be revealed by studying inherited disorders of neuromuscular transmission known as congenital myasthenic syndromes (CMS). Here we show a link between neuromuscular transmission and the hexosamine biosynthetic pathway which yields the amino sugar UDP-N-acetylglucosamine (UDP-GlcNAc), an essential substrate for carbohydrate modifications of proteins and lipids. Using genetic linkage, we find 18 different biallelic mutations in the gene for the key enzyme of the hexosamine pathway, glutamine:fructose-6-phosphate amidotransferase 1 (GFAT1), in 13 unrelated CMS families. Moreover, GFAT1 downregulation in zebrafish embryos altered muscle fiber morphology and impaired normal NMJ development. We reveal a so-far unrecognised involvement of GFAT1 and the hexosamine pathway in neuromuscular transmission - which adds to existing concepts of the glycobiology of the NMJ. As the vertebrate NMJ is a classic model synapse, further investigation of the role of GFAT1 for NMJ function is likely to also contribute to our understanding of synapse development and organization in general. GFAT1 and the hexosamine pathways have been implicated in signalling pathways that may become deregulated in diseases of the immune system, diabetes mellitus, cancer, cardiovascular disease, and neurodegenerative diseases. Our findings give additional impetus for investigating the role of GFAT1 in human health and disease.

## W6 HEART DISEASE

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### W6-01

#### **HSPB7 gene variants lead to allele-specific expression pattern and are associated with idiopathic dilated cardiomyopathy**

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#### Background

Dilated cardiomyopathy (DCM) is a structural heart disease with strong genetic background and a prevalence of 1:2,500 in the general population. Monogenic forms of DCM are observed in families with mutations located mostly in genes encoding structural and sarcomeric proteins. However, strong evidence suggests that genetic factors also affect the susceptibility to idiopathic DCM (iDCM). Identification of genetic disease markers in persons-at-risk could play an important role in preventive health care. No comprehensive genetic analysis was performed to date on iDCM.

#### Methods

The cardiovascular gene-centric 50K single nucleotide polymorphism (SNP) ITMAT-Broad-CARe array represents an established compromise between genome-wide association studies and hypothesis-driven candidate gene approach by analyzing polymorphisms in more than 2,000 genes known or predicted to be involved in cardiovascular phenotypes. Using this array, we conducted a screening with three independent replication studies to reveal insight in genetic contribution to iDCM. The four samples from Germany and France included 1,910 iDCM cases and 3,630 healthy control individuals. Allele-specific expression analysis using TaqMan technology was conducted in human skeletal muscle cells.

#### Results

Our study identified a polymorphism (rs1739843, minor allele frequency = 39%) in intron 2 of the HSPB7 gene being strongly associated with susceptibility to iDCM in a German case-control sample (n=664 cases, n=1,874 controls; p=1.06\*10<sup>-6</sup>, OR=0.67 [95% CI 0.57-0.79]). Combined analysis with three replication steps from Germany and France (including a total of n=1,910 iDCM cases und n=3,630 controls) revealed consistent association (p=5.28\*10<sup>-13</sup>, OR=0.72 [95% CI 0.65-0.78]).

HapMap data of HSPB7 coding region showed a number of polymorphisms in perfect linkage disequilibrium with rs1739843. First analysis employing human skeletal muscle cells being heterozygous for the coding SNPs gave a hint for allele-specific HSPB7 expression pattern. The risk allele leads to a twofold to fivefold reduction of HSPB7 transcript level. HSPB7 codes for a small cardiovascular heat shock protein (cvHSP) being expressed in heart muscle and other insulin-sensitive tissues.

#### Summary

Using a comprehensive gene-centric association study, we identified a common intronic variant in HSPB7, encoding a cardiovascular small heat shock protein, to be associated with iDCM. This variant leads to a about 40% higher risk for iDCM. The biological effect may be driven by an allele-specific expression pattern, resulting in a lower dose of cellular cvHSP.

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### W6-02

#### **Targeting of GxxPG Fragments as a Novel Therapeutic Strategy For Thoracic Aortic Aneurysm in a Mouse Model of Marfan Syndrome**

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#### Background:

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder that predominantly affects the skeletal, cardiovascular, and ocular systems. The primary cause of premature death in untreated patients is related to thoracic aortic aneurysms and dissections (TAA). MFS is caused by mutations in the gene for fibrillin-1. Multiple factors such as haploinsufficiency, fibrillin-1 proteolysis, abnormal TGF- $\beta$  signalling, increased matrix metalloproteinase (MMP) expression, and changes in cell-matrix interaction contribute to the complex pathogenesis of this disorder. In previous work, we showed that aortic extracts from the fibrillin-1 underexpressing mouse model of MFS (mgR/mgR) could induce macrophage chemotaxis, and that the chemotactic response was mediated by elastin-binding protein (EBP); additionally, increased numbers of CD68+ macrophages were found in the aortic wall of MFS patients (Guo et al., *Circulation* 2006, 114:1855-62).

#### Results:

In the initial part of this study, we investigated whether extracts from the aorta of human MFS patients can also stimulate macrophage chemotaxis. We identified a statistically significant increase in the chemotactic index (CI) for the MFS aortic samples as compared to control aortic samples. Additionally, the Marfan aortic extracts displayed a statistically significant increase in reactivity to the monoclonal antibody BA4, which recognizes GxxPG containing peptides or fragments of elastin and fibrillin-1.

We additionally performed a study on the mgR/mgR mouse to test the effects of treatment with BA4 in an attempt to antagonize the secondary deleterious effects of elastin and fibrillin fragmentation. We investigated whether BA4 or treatment with the anti-inflammatory medication indomethacin could decrease the development of TAA in the mgR/mgR mouse model. Both treatments were started at 3 weeks of age and continued for 8 weeks. The mgR/mgR mice received BA4 through intraperitoneal injection once a week. In a separate group, indomethacin was added to drinking water continuously for 8 weeks. Here we show that both BA4 and indomethacin treatment significantly improves elastin integrity in aortic wall of mgR/mgR mice. Treatment with BA4 significantly decreased MMP2, MMP9, MMP12 expression and pSMAD2 activity. Compared to the untreated group, there were significantly less macrophages in the aortic wall after BA4 treatment. Indomethacin treatment led to similar results.

#### Conclusions:

The results of this study further confirm the hypothesis that abnormal secondary cellular events caused by GxxPG containing fragments contribute to the development of TAA in MFS. Efficient anti-inflammatory treatment and chemotactic activity observed in Marfan aortic extracts suggest an important role of matrix-fragment induced inflammatory activity in the aortic wall in the pathogenesis of TAA. Furthermore, this study might provide new impact on therapeutic approaches and could contribute to new strategies for the long-term management of aortic aneurysm in MFS.

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## W6-03

### Functional mutations in IGFBP2 and IGF-I in bicuspid aortic valve (BAV) disease

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Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation. About 1-2% of the population have BAV, although the condition is at least twice as common in males. There is a high incidence of associated valvular lesions and aortic stenosis due to intrinsic restriction and accelerated senile calcification. Although a high heritability of BAV is reported, the molecular mechanisms and responsible genes are rather unknown. However mutations in the transmembrane receptor gene NOTCH1 and the vascular smooth muscle cell alpha actin gene ACTA2 have been identified in a minority of patients with BAV.

To ascertain additional genes or gene loci we initially conducted a genome-wide association study (GWAS). Using a whole human genome array (the GeneChip Human Mapping 500k Array set, Affymetrix) we successfully genotyped 154 subjects. For replication, we evaluated 16 SNPs (out of 160 SNPs) most significantly associated with BAV in additional 466 patients and 800 controls. The two SNPs rs9341130 and rs9341145 located in the IGFBP2 gene were significantly associated with the phenotype. The analysis of around 500 patients and over 1000 controls revealed for both SNPs odds ratios of 1.879 ( $p < 10^{-6}$ ).

Sequencing analysis of the IGFBP2 gene in 200 patients with BAV identified two base substitutions within the core promoter region which directly influence the IGFBP2 expression in reporter gene assays. In addition, we detected one missense mutation (R104L) within the highly conserved IGF-binding domain of IGFBP2 which directly affects the interaction of IGFBP2 with IGF-I and -II in protein-binding experiments as well as protein-structural modelling assays. Additional sequencing analysis of IGF-I and IGF-II in 100 patients revealed one missense mutation (C15W) in IGF-I which alters processing of the IGF-I protein.

To further examine the role of IGFBP2 in aortic valve organogenesis, we performed surgical inspections and morphometric measurements of the aorta and aortic cusps in an *Igfbp2* knock-out mice. By this, we could reveal a smaller aortic circumference in *Igfbp2*<sup>-/-</sup> mice as compared to wildtype and heterozygous mice. Correspondingly, *Igfbp2*<sup>-/-</sup> mice displayed a tendency to inhomogeneous enlargement of the left anterior cusp of the aortic valve whose incidence was clearly related to gender.

In summary, the identification and functionally characterization of mutations in IGFBP2 and the functionally associated IGF-I gene as well as our mouse model data strongly indicate the involvement of IGFBP2 in aortic growth or morphogenesis.

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## W6-04

### Thirteen novel genetic loci affecting risk of coronary artery disease - results from CARDIoGRAM

*Erdmann J.*

on behalf, of, CARDIoGRAM

#### BACKGROUND

Coronary artery disease has a significant heritability that is incompletely characterized.

#### METHODS

We combined data from 14 genomewide association studies and compared allele frequencies of more than 2 million single nucleotide polymorphisms in 22,233 cases with coronary disease and 64,762 controls, all of European ancestry. Promising variants were followed-up in up to 49,343 additional individuals. Novel loci confirmed to be associated with risk of coronary disease were further investigated for correlation with traditional cardiovascular risk factors in population-based samples, with gene expression in multiple tissues, and for co-localization with other traits.

#### RESULTS

We established firm association ( $P < 5 \times 10^{-8}$ ) with coronary disease for 23 chromosomal loci, 13 of which are novel. At the 13 novel loci, risk allele frequencies range from 13% – 91% and the odds ratios for coronary disease range from 1.06 – 1.17 per copy. Only three of these loci display significant association with traditional risk factors. Risk alleles at four of these loci are strongly associated with transcript levels of nearby genes including TCF21 (6q23.2), RASD1, SMCR3 and PEMT (17p11.2), SMG6 (17p13.3), and UBE2Z, GIP, ATP5G1, and SNF8 (17q21.32). Risk alleles at five novel loci are, in addition to coronary disease, strongly associated with various other human disease traits.

#### CONCLUSIONS

The genetic predisposition for coronary disease is mediated in part by multiple common genetic variants of small to moderate effect size. Many appear to act via biological mechanisms that are independent of traditional risk factors.

**Mutations in OLFML2B within the QT interval associated region 1q23.3 Disturb Cardiac Repolarization and Predispose to Sudden Infant Death**

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The strongest quantitative trait locus (QTL) modifying electrocardiographic QT interval has been mapped to human chromosome 1q23.3 in several genome-wide association studies (GWAS). We investigated two genes from the region, NOS1AP (CAPON) and OLFML2B for their influence on cardiac repolarization and disease predisposition in patients with sudden infant and sudden cardiac death syndrome (SIDS and SCD). Both genes are expressed in the human heart. Their knockdown in zebrafish induced cardiac dilatation and arrhythmia. In 513 SIDS and 239 SCD cases three children affected with SIDS carried heterozygous OLFML2B missense mutations P504L, G515E and Y557H. No mutations were observed in NOS1AP. All three mutations suppressed cellular export of the secreted OLFML2B glycoprotein. Expression of wildtype OLFML2B in *Xenopus* oocytes reduced current density of the voltage gated IKr potassium channel (Kv11.1) encoded by KCNH2 by 7.7±4.7% while mutations led to larger reductions (P504L; 33.0±3.2%, G515E; 49.9±11.4% and Y557H; 17.6±6.4%). This suggests these mutations predispose to SIDS by decreasing myocardial repolarization reserve. Our findings uncover a novel monogenic disease gene and pathomechanism contributing to SIDS, illuminate novel functional properties of the olfactomedin class of proteins and provide an example how genes from different pathways may independently contribute to a regional QTL.

**Nexilin deficient neonatal mice display dilated cardiomyopathy and systolic dysfunction**

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Background: Dilated cardiomyopathy (DCM) is a leading cause of heart failure. Recently, we identified nexilin (Nex) gene mutations in patients with DCM and showed that loss of Nex in zebrafish leads to impaired cardiac contractile function with instability of the Z-disk and subsequent cardiac dilatation.

Aim: To investigate the role of Nex in the heart with a targeted Nex knockout mouse model.

Material and Methods: A constitutive Nex knockout (KO) mice was produced by gene targeting strategy. Cardiac phenotype of neonatal hearts was studied by echocardiography as well as histology, electron microscopy and heart-to-body weight ratio (HW/BW).

Results: Heterozygous (Het) adult mice displayed no apparent phenotype. Homozygous KO mice were produced by mating Het mice. At birth, the ratio of KO: Het: wild-type (WT) mice approximated the expected Mendelian ratios of 1:2:1. After postnatal day 6, survival of KO mice decreased dramatically, and only two out of 250 KO mice remained viable after one month of age. Western blot exhibited the absence of Nex and lower Nex amount in KO and Het mice, respectively. After postnatal day 4, HW/BW was >2.3-fold higher in KO than in WT mice. Between days 4 and 6, KO mice developed a rapidly progressive cardiomyopathy with left ventricular (LV) dilation and wall thinning as determined histologically. Interestingly, echocardiography revealed a LV phenotype in both KO and Het mice characterized by thinning of the walls (lower AWT<sub>hd</sub> and PWT<sub>hd</sub> than in WT littermates) and dilation (higher LVID<sub>d</sub> and LVID<sub>s</sub> than in WT). This resulted in lower ejection fraction in both Het and KO mice. Total LV protein content did not differ between KO and WT mice, suggesting that the higher HW/BW is due to oedema in KO mice. Electron microscopy showed a blurring of the boundary between the I and A bands within the cardiac myocytes.

Conclusion: Both heterozygous and homozygous Nexilin knockout mice developed DCM and systolic dysfunction after birth. The complete absence of nexilin resulted in neonatal lethality, whereas a lower level of nexilin was sufficient to maintain the viability in heterozygous mice.

## W7 TECHNOLOGY / BIOINFORMATICS

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### W7-01

#### Targeted next generation sequencing as a powerful diagnostic tool for RP

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Next generation sequencing (NGS) opens the way to high throughput analysis of either targeted genomic regions or even the whole exome or genome. Recent publications show the great advantages of NGS in genetic research, e.g. in gene discovery in rare inherited syndromes. However, for the usage of NGS in diagnostics compared to application in research several additional performance parameters have to be met: high quality, high throughput but simultaneously low costs. Here we describe the design and validation of an NGS approach for the diagnostics of inherited dystrophy. We developed a customized 12-plex Nimblegen sequence capture array to enrich all exons, splice junctions and UTRs of 111 known retinal dystrophy genes. This array was then used to enrich the determined gene package in 100 previously unsolved patients with isolated or autosomal recessive RP. Molecular barcoding enabled the enrichment and subsequent sequencing of 12 samples at a time. DNAs of 12 retinal dystrophy patients carrying 24 known disease causing variants served as controls. All 112 samples were sequenced on a Roche 454 GS FLX Titanium. Stringent bioinformatic data analysis and variant detection on average resulted in 4 variants per sample which were validated using conventional Sanger sequencing and subsequently tested for segregation within the family. In 100 patients, a total of 397 variants were selected to be potentially pathogenic and were validated by Sanger sequencing. Up to now, 82% of the so far validated variants could be confirmed. Additionally to variants at basepair-level, we could also identify larger homozygous and heterozygous pathogenic deletions. Preliminary data on the confirmation and segregation analysis in the corresponding families revealed the likely pathogenic mutations in at least 35 probands. These results show that our approach is operational, e.g. that the coverage obtained allows for reliable identification of the disease-causing variations. Targeted next-generation sequencing combined with stringent bio-informatic data analysis is a test that holds the promise to optimize cost-effectiveness in molecular diagnostics for genetically heterogeneous disorders like RP, and we are convinced that this approach is an important step towards the successful implementation of NGS in diagnostics within one year.

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**W7-02****Identity-By-Descent Filtering of Exome Sequence data for Disease-Gene Identification in Autosomal Recessive and X-Linked Disorders**

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Next-Generation Sequencing (NGS) and exome-capture technologies are currently revolutionizing the way geneticists screen for

disease-causing mutations in rare Mendelian disorders. However, the identification of causal mutations is challenging due to the sheer number of variants that are identified in individual exomes.

Sequenced individuals typically have on the order of five to ten

thousand variant calls representing either non-synonymous substitutions in protein coding sequences, small indels, or

alterations of the canonical splice-site dinucleotides (NS/SS/I). Even after filtering out common variants using data from dbSNP, the HapMap project and related resources such as the 1000 Genomes project, the number of potentially disease-causing NS/SS/I variants can remain high if the exome of a single patient is considered in isolation. Current analysis strategies involve intersection filtering, restriction of candidates to sequence variants predicted to be deleterious, searching for de novo mutations in trios, and using linkage analysis to filter

out exome sequences from chromosomal regions that do not demonstrate linkage.

None of the above mentioned analysis strategies are well suited for identification of disease genes in small families segregating

autosomal recessive (AR) or X-linked (XL) diseases. In AR diseases, all of the affected siblings must share the same maternal and paternal haplotypes surrounding the disease gene, that is, the disease gene must be located in a region that is identical by descent (IBD=2) in all affecteds. Similarly, in XL diseases, all affected males must share the same haplotype surrounding the disease gene (IBD=1).

Here, we present an algorithm that can be used to narrow down the

candidate regions in exome sequences of affected siblings of

consanguineous or non-consanguineous parents in AR disorders. Our

algorithm uses a non-homogeneous hidden Markov model (HMM) that

employs local recombination rates to identify chromosomal regions that are identical by descent (IBD=2) in children of consanguineous or non-consanguineous parents solely based on genotype data of siblings derived from high-throughput sequencing platforms. Using simulated and real exome sequence data, we show that our algorithm is able to reduce the search space for the causative disease gene to a fifth or a tenth of the entire exome. We have used our algorithm successively on real data to identify disease genes including the PIGV gene as the cause of Mabry syndrome (Krawitz et al., Nat Genet. 2010,42:827-9). Here, we will present the algorithm as well as a recent modification of the algorithm for XL diseases.

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**W7-03****Analysis Pipeline for Exome Sequencing Data**

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Enrichment techniques for targeted sequencing of coding regions are currently applied to identify rare variants. We developed a pipeline to analyze exome sequencing data. The pipeline is a collection of Perl scripts which start with the sequence files generated by the Illumina software. It calculates quality metrics and performs read alignment to the reference sequence, variant calling, variant annotation and selection of candidate variants according to the genetic model. Variants are stored in a database, which allows user queries through a web interface and enables pedigree or gene based searches. Exomes deposited in the database can be used as controls.

Alignment and variant calling is performed with BWA, the GATK and SAMtools. First, quality metrics are calculated which include base quality, % mapped reads, % duplicates, % reads overlapping the target regions and read depth on a single base level. As a second task, variants are further filtered and annotated. Annotation includes presence in dbSNP, type of mutation and - if applicable - amino acid change. In addition, the frequency of the variants in our exome samples is determined. This information is then used, in conjunction with optional information such as inheritance model, affected siblings or a linkage region, to identify putative causative variants.

The pipeline has a modular composition and subsets of components may be run in an arbitrary combination. For manual inspection of the results, bed- and html-files are provided.

We applied the analysis pipeline to approximately 90 exomes. From an average of ~6 GB of aligned sequence, the pipeline calls ~16,000 coding variants. Approximately 7,500 of these are non-synonymous variants of which ~700 along with ~30 splice site variants and ~60 indels are not present in dbSNP (version 130). Depending on the number of affected individuals and the underlying inheritance model, we are able to confine this list to 1-10 putatively disease causing variants.

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#### W7-04

### **Non-invasive prenatal detection of chromosome aneuploidies using next-generation sequencing: First steps towards clinical application**

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**Introduction:** There is a general interest in the development of non-invasive prenatal diagnosis (NIPD) of fetal genetic disease by analysing fetal DNA present in maternal plasma. With the rapid development of high-throughput sequencing technologies allowing massively parallel sequencing of tens of millions of short sequencing tags, the possibility of detecting the presence of aneuploidies analysing circulating cell free fetal DNA (ccffDNA) in maternal plasma samples has recently been explored. Here we describe the development and the proof-of-concept study of a non-invasive next-generation sequencing test system for the diagnosis of fetal chromosomal aneuploidies.

**Subjects and Methods:** In our study, maternal blood samples were selected from 50 singleton pregnancies. Cell free DNA was prepared and analysed using Solexa/Illumina sequencing platform and work flows. To reduce sequencing costs and increase throughput, samples were bar coded and pooled permitting multiplexed sequencing. To compare the sequence data with the "gold standard", conventional cytogenetic analysis was performed by chorionic villous sampling or amniocentesis to get the fetal karyotype.

**Results:** In all (n=50) sequenced samples, all (n=8) trisomy 21 samples were correctly identified and confirmed by conventional cytogenetics. Furthermore, one sample with a monosomy X was also confirmed by karyotyping. To best of our knowledge, this is the first case diagnosing monosomy X by a NIPD test system.

**Conclusions:** Our results suggest, that the technical requirements for non-invasive aneuploidy tests based on ccff DNA in maternal plasma are fulfilled for the detection of trisomy 21. Further studies are required to improve the quality of this new test system for the detection of all fetal aneuploidies. Recently we have started a large-scale clinical cohort trial to validate our non-invasive prenatal diagnostic test system towards clinical application.

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#### W7-05

### **Preimplantation genetic diagnosis for monogenic disorders on polar bodies and trophectoderm cells**

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We report here our results of 61 ICSI cycles with polar body diagnosis (PBD) for monogenic disorders as well as first results of the parallel testing of trophectoderm cells from day 5 embryos in 5 ICSI cycles.

Polar body diagnosis (PBD) so far in Germany appeared to be the only legal form of preimplantation genetic diagnosis (PID). In July 2010 the German Supreme Court in addition legalized PID of pluripotent embryonal cells obtained by trophectoderm biopsy (TEB) for families at high risk for severe genetic disorders. However, so far most PID centers worldwide perform embryo biopsy of 8 cell embryos, which most authors consider to be totipotent. In Regensburg we chose to further offer PBD for all suitable families and in parallel, after informed consent, to reevaluate day 5 embryos with identified maternal mutation or without genetic diagnosis after PBD in order to optimize and validate trophectoderm biopsy and genetic testing.

So far we obtained polar bodies from 564 out of 611 oocytes after ICSI (mean 9,2 oocytes per cycle; mean maternal age 34,4 years). Based on PBD results 321 oocytes could be genetically diagnosed (56,9 %), resulting in the transfer of 104 embryos (77,1 % transfer cycles), 13 clinical pregnancies with 1 abortion (27,7 % pregnancy rate per transfer cycle) and the birth of 12 children including 2 twin pairs; 2 pregnancies are still ongoing.

For optimization of PID after trophectoderm biopsy so far 17 blastocysts were subjected to mechanical TEB. The obtained 22 cell samples contained 1-11 trophectoderm cells, which were reevaluated with the family specific PBD mutation assay. Trophectoderm samples were also tested by array CGH (24sure BlueGnome) with concordant results regarding amplification efficiency. For 10 samples PCR amplification could be achieved and the mutation assay confirmed the expected mutation status based on the PBD results. For one sample diagnosed by PBD to carry a Dystrophin deletion spanning exons 3-16 the diagnosis could be confirmed by the PID mutation assay as well as array CGH. In contrast, amplification failed for partially lysed cells or samples derived from embryos arrested in development, suggesting biopsy induced problems and/or degradation effects. Our preliminary data indicate that the mode of TEB and the cellular integrity of the sample critically determine success rates of subsequent DNA amplification and genetic testing.

In conclusion, we currently continue to offer PBD for all those families with maternally determined disease status, which overall yields good results and in our experience covers the majority of PID requests for monogenic disorders. However, we would like to apply PID after TEB for undiagnosed pronuclei after PBD as well as for those families with paternally determined disease status. Larger series of ICSI cycles with PID after TEB will be necessary to assess viability of biopsied blastocysts and resulting pregnancy rates and compare them with the available results after PBD as well as PID on 8 cell embryos.

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## W7-06

### **The complete individual genome of a female Crohn's disease patient - what can you learn?**

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Background: Crohn disease is a polygenic disorder with more than 71 disease loci that have been confirmed through a recent meta-analysis (Franke et al., Nat Genet). However, even this deep analysis of the genetic architecture explains less than 25% of the cumulative genetic variance of the disease. We propose that individual, full analysis of the genome will give important additional insights that can prompt individual therapeutic decisions.

Methods: A 47 year old woman with a history of Crohn's disease since 1985 admitted to the IBD outpatient clinic of the University Hospital Schleswig-Holstein, Campus Kiel in 2002 because of chronic active disease. She had failed standard therapies including anti-TNF and had undergone multiple bowel resections. Standard test for tuberculosis (i.e. PPD skin tests, chest X-ray and quantiferon gold test) were negative. The patient had to be intermittently nourished parentally, because she constantly lost weight. Due to her desolate situation, we considered individual whole-genome sequencing to provide additional hypothetical insights for experimental therapeutic decisions.

The patient was thoroughly counselled according to the German Gene Diagnostic Law by the treating clinicians and a human geneticist. She consented to whole-genome sequencing and the full disclosure of her identity, including public release of the sequence data.

Eight different sequencing libraries (fragment, mate-pair with different insert sizes, exome) were created and 18 slides were run on different SOLiD instrument versions available throughout the course of the experiment.

Results: More than 4.58 billion reads uniquely mapped to the reference genome, yielding an average depth of coverage of 58X. More than 90% of the genome had a coverage of 25X or higher. In total, 3308456 SNPs and more than 300000 IndDels were identified. 309216 SNPs were novel and less than 10000 affected the coding sequence. We estimated a false-positive SNP rate of 0.02% and a false-negative rate of 2.1%. Multiple "hits" concentrated in the autophagy pathway. Therefore, the patient was subjected to in depth mycobacterial diagnostics by the German National Mycobacterial reference center, which eventually proved chronic active infection with mycobacterium avium intracellular in peripheral blood samples.

Conclusions: A full genome analysis of an individual patient creates important hypothetical insights into disease etiology. These hypotheses can be used in an interdisciplinary decision making environment (e.g. the comprehensive center for inflammation medicine that has been set up in Kiel and that comprises 7 different medical specialties). Individual full genome sequencing can direct cross-indication prescriptions or other innovative therapeutic concepts on an individual level.

## W8 BASIC MOLECULAR MECHANISMS

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### W8-01

#### Single Base-pair Substitutions in Translational Initiation Codons of Human Genes Causing Inherited Disease

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The 'scanning model' of translational initiation implies that the 40S ribosomal subunit, after recognizing the mRNA cap, scans the mRNA for the nearest AUG downstream. Usually, this AUG is then adopted as the translation initiation codon (TIC). However, a TIC is only recognized efficiently if it is embedded within an appropriate recognition sequence, GCCRCCAUGG, also known as the Kozak consensus sequence. Early in vitro studies suggested that positions -3 and +4 flanking the TIC are particularly important for maximizing translation efficiency. In addition, naturally occurring mutations that either alter the TIC itself or change the flanking sequence have been reported to perturb the initiation step of translation and reduce translational efficiency. Since recognition of a TIC is thus clearly dependent upon its sequence context, mutational disruption of this context may render the TIC unrecognizable, thereby causing translation to fail altogether or to initiate at an alternative AUG downstream. In the latter case, and depending upon the structure of the gene product in question, the functional consequences of a TIC mutation may well be ameliorated to such an extent that the mutation would lack any serious (i.e. pathological) phenotype.

As of 27 August 2010, 405 of the 55,803 disease-causing single base-pair substitutions logged in the Human Gene Mutation Database (HGMD) were located within a TIC. We performed a meta-analysis of these data to obtain new insights into the molecular mechanisms underlying the respective genotype-phenotype relationships. TIC mutations in HGMD were found to be evenly distributed over the three component nucleotides. Mutations at positions -5 to -1 and at +4 flanking the TIC have been reported before as a cause of human inherited disease, but in HGMD, they were much less frequent than changes of the actual TIC itself. This notwithstanding, and under the assumption that mutation prevalence in HGMD is related to the functional importance of the mutated nucleotide, it would appear that nucleotides -3 and +4 are indeed the most relevant for TIC recognition consistent with the aforementioned in vitro studies. Moreover, it turned out that TIC mutations located within less than optimal sequence contexts were more likely to come to clinical attention (i.e. to be included in HGMD) than TIC mutations occurring in better matches to the Kozak sequence. This result is potentially explicable in terms of an optimal Kozak sequence being capable of compensating to some extent for the apparent loss of the TIC consensus sequence. Finally, we were able to demonstrate a statistically significant trend for the distance between a TIC and the nearest downstream in-frame ATG codon to be greater in genes that harbour TIC mutations in HGMD than in human genes in general. This result suggests that at least some TIC mutations may not have come to clinical attention because an alternative downstream ATG codon in close proximity to the mutated TIC substituted for it, thereby ensuring that translation of the protein was not unduly compromised.

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### W8-02

#### Truncating mutations in ROR2 cause brachydactyly type B1 (BDB1) by dysbalancing WNT/beta-catenin and BMP/p-SMAD signalling during digit elongation.

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Mutations in a single gene, ROR2, cause two distinct congenital syndromes: dominant brachydactyly type B1 (BDB1; MIM113000) characterized by hypoplasia or aplasia of distal phalanges, and recessive Robinow syndrome (RRS; MIM268310) characterized by craniofacial, vertebral and other malformations. To first investigate the discrepancy in phenotypic outcome we analyzed ROR2 protein stability and distribution in stably transfected cell lines expressing exact copies of several human RRS and BDB1 mutations. RRS mutant proteins were less abundant and retained intracellularly, while BDB1 mutants were stable and predominantly located at the cell membrane. Mutations causing intermediate phenotypes in patients also showed intermediate behavior in this assay. This suggests that RRS is caused by a loss-of-function mechanism, while mutations leading to BDB1 confer a gain-of-function to the protein, which was corroborated genetically by crossing mouse models for RRS and BDB1. To further analyze the pathomechanism of BDB1, we analyzed a knock-in mouse model carrying a human mutation (ROR2

p.W749X). The mutant showed a failure in digit elongation caused by a breakdown of cell commitment to the chondrogenic lineage. Impaired cartilage condensation was caused by a drastic decrease in BMP/p-SMAD signaling in an organizer-like structure anterior to the growing digit condensation, the phalanx-forming region. Crossing the ROR2 mutant to a canonical WNT reporter mouse line revealed elevated WNT/beta-catenin signaling in the distal limb bud. Reporter assays confirmed that ROR2, but not truncated BDB1 forms, can inhibit canonical WNT signaling. Altogether this indicates a model in which elevated levels of WNT/beta-catenin signaling downregulate BMP/p-SMAD signaling ultimately leading to BDB1. We further show that a similar BMP-based mechanism accounts for digit shortening in a mouse model for the closely related condition BDA1 (IHH p.E95K). Interestingly, several other members of the brachydactyly disease family are caused by mutations in BMP pathway components, namely BDA2 (BMPRI1B), BDB2 (NOGGIN) and BDC (GDF5). This suggests that mammalian digit elongation is controlled by a molecular network with the BMP pathway in a central position, alterations of which cause brachydactyly syndromes.

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## W8-03

### Exome sequencing and cellular complementation aid gene identification in mitochondrial complex I deficiency

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Mitochondrial complex I deficiency is a frequent biochemical condition, accounting for about one third of respiratory chain disorders. Impairment of complex I activity compromises the transfer of electrons derived from carbohydrate and, partly, from fatty acid oxidative catabolism to the downstream protein complexes of the respiratory chain. The situation with a defined biochemical defect in mitochondria, also present in patient-derived cell line obtained during routine diagnosis, is well suited for an exome sequencing approach. Since exome sequencing results in a long list of novel DNA variants, additional filtering and experimental confirmation is necessary to identify the disease causing variants.

We started a whole exome sequencing project for a total of 50 index patients with mitochondrial disorders. Filtering algorithm included a screen for genes coding for mitochondrial proteins. This reduced the list of candidate genes for each patient (currently n=10) to less than five. Taking advantage of patient-derived fibroblast cell lines, we performed lentiviral complementation experiments to substantiate the pathogenic role of newly identified DNA variants in known and novel candidate genes. The complementation experiments were successful in three cases. Mutations in one gene, ACAD9, provided a new function for the Acyl-CoA-Dehydrogenase 9 in complex I assembly. Mutation screening of ACAD9 in our patient cohort (n=150), revealed additional alleles (Haack et al. Nat Genet 2010).

Whole exome sequencing of patients with complex I-deficiency provides a paradigm for molecular diagnostics and fundamental new insights into complex I function.

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## W8-04

### Molecular characterization identifies COH1 as RAB6 effector protein crucially influencing neuritogenesis

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Mutations in COH1 (VPS13B) cause the autosomal recessive Cohen syndrome, mainly characterized by mental retardation, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. Although the genetic basis of Cohen syndrome is well established the function of the protein COH1 remains uncharacterized. Partial homology to yeast Vps13p suggests that COH1 regulates trans-Golgi network (TGN) to endosome sorting of membrane proteins. Consequently, cloning of the ubiquitously expressed COH1 transcript, encoding full length COH1 (3997aa), and subsequent immunofluorescence analysis identified COH1 as Golgi localized protein. Upon Brefeldin A treatment, which redistributes Golgi proteins towards the ER, COH1 showed a high degree of co-localization at ER exit sites with the cis-Golgi matrix protein GM130. Hence, depletion of COH1 using RNAi induced fragmentation of the Golgi ribbon confirmed its role as Golgi matrix protein. Short Brefeldin A treatment was used to analyze membrane carrier formation.

COH1 depletion induces diminished tubulation activity at the Golgi apparatus. The hitherto predicted function as scaffold protein was further strengthened by protein interaction of COH1 with RAB6 and the RAB6 specific guanine nucleotide exchange factor (GEF) complex RIC1/RGP1. Functional analysis establishes COH1 finally as RAB6 effector protein important for neuronal development. In vitro Coh1 depletion by RNAi in primary rat (E18) hippocampal neurons reduced outgrowth of the longest neurite (stage 3 of neuron differentiation) significantly. In summary, this study proposes COH1 as an integrator of RAB6 dependent trafficking during neuritogenesis. In accordance to this, recent findings confirmed the significance of RAB6 in neurite extension and thus finally establish impaired neuritogenesis as underlying cause for postnatal microcephaly in Cohen syndrome patients. To elucidate the pathomechanism of Cohen syndrome in more detail, it remains to be analyzed whether COH1, RAB6, and RIC1/RGP1 operate also in synaptogenesis, which is important for normal brain function.

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## W8-05

### **FGFR3 is a target of the homeobox transcription factor SHOX in limb development**

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The short stature homeobox gene SHOX encodes a transcription factor which is important for normal limb development. In humans, SHOX deficiency has been associated with various short stature syndromes including Leri-Weill dyschondrosteosis, Langer mesomelic dysplasia and Turner syndrome as well as non-syndromic idiopathic short stature. A common feature of these syndromes is a disproportionate short stature with a particular shortening of the distal long bones (ulna/ radius and tibia/fibula). In our studies employing microarray analyses and cell culture experiments, we revealed a strong positive effect of SHOX on the expression of the fibroblast growth factor receptor gene FGFR3, another well-known factor for limb development. Luciferase reporter gene assays show that SHOX activates the extended FGFR3 promoter, and results from ChIP-Seq, ChIP and EMSA experiments suggest a direct binding of SHOX to multiple upstream sequences of FGFR3. To further investigate these regulations in a cellular system for limb development, the effect of viral overexpression of Shox in limb bud derived chicken micromass cultures was tested. We found that Fgfr3 was negatively regulated by Shox, as demonstrated by qRT-PCR and in situ hybridization. This repressive effect might explain the almost mutually exclusive expression patterns of Fgfr3 and Shox in embryonic chicken limbs. A negative regulation that occurs mainly in the mesomelic limb segments, a region where SHOX is known to be strongly expressed, offers a possible explanation for the phenotypes seen in patients with FGFR3 (e.g. achondroplasia) and SHOX defects (e.g. Leri-Weill dyschondrosteosis). In summary, these data present a link between two frequent short stature phenotypes.

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## W8-06

### **The X-linked mental retardation protein alphaPIX (ARHGEF6) is a novel trafficking factor that balances degradation and recycling of the epidermal growth factor receptor**

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X-linked non-specific mental retardation (XLMR) is a very heterogeneous disorder. Mutations in >80 genes encoding proteins with a variety of functions have yet been identified. Some of these proteins, such as GDI1 and RAB39B, are implicated in the regulation of vesicular trafficking suggesting a critical role of intracellular transport mechanisms for the development of human cognitive abilities. The XLMR gene ARHGEF6 encodes the guanine nucleotide exchange factor alphaPIX which modulates the activity of Rho GTPases by catalyzing the exchange of GDP for GTP within particular spatio-temporal contexts. We report here for the first time that alphaPIX is a potent dual-function regulator of epidermal growth factor (EGF) receptor trafficking.

By protein-protein interaction studies using neuronal and non-neuronal cells we could demonstrate direct binding of alphaPIX to the E3 ubiquitin ligase c-Cbl, an enzyme that attaches ubiquitin to EGF receptors, thereby labelling them for degradation. This interaction is mediated by tryptophan 197 in the SH3 domain of alphaPIX and arginine 829 in the PKPFPR-motif of c-Cbl. EGF stimulation of H4 neuroglioma cells resulted in translocation of alphaPIX to the plasma membrane where it co-localized with c-Cbl and the EGF receptor. Indeed, alphaPIX::c-Cbl complex formation was found to be stimulated by EGF. In addition, alphaPIX and c-Cbl protein levels decrease upon EGF stimulation and this downregulation is dependent on both alphaPIX::c-Cbl complex formation and c-Cbl ubiquitin ligase activity. In line with this, our results from ubiquitylation

assays indicate that alphaPIX and c-Cbl are ubiquitylated suggesting degradation of both proteins by the proteasome system. Based on our data, we hypothesize that alphaPIX is required for temporal sequestration of c-Cbl during EGF receptor trafficking, thereby preventing ubiquitylation and subsequent degradation of EGF receptors. A similar mechanism has been proposed for betaPIX, the close homologue of alphaPIX. By applying cell surface biotinylation assays we could indeed demonstrate decreased lysosomal EGF receptor degradation in cells ectopically expressing alphaPIX. Remarkably, we uncovered a second, c-Cbl-independent alphaPIX mode of action during EGF receptor trafficking: By surface biotinylation assays we demonstrated that alphaPIX strongly enhances recycling of internalized EGF receptors back to the cell surface upon EGF stimulation, and its GEF activity is crucial for this effect. Our immunofluorescence analysis confirmed that EGF is enriched in recycling vesicles and gradually disappeared from the cytoplasm in alphaPIX overexpressing cells. Quantitative analysis revealed increased EGF receptor recycling by 5-fold and reduced degradation by 3-fold in cells overexpressing alphaPIX. In summary, our findings suggest that alphaPIX represents a double switch for controlling EGF receptor fate by accelerating recycling and inhibiting degradation. This data provides new insights in understanding the molecular functions of alphaPIX and underscores the importance of intracellular trafficking mechanisms in neuronal development.

## W9 CANCER GENETICS / DNA REPAIR

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### W9-01

#### Micro-RNA Deregulation via BCL6 Chromosome Rearrangement due to DNA Fragility

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Activation of BCL6 (at 3q27) driven by chromosomal rearrangement typifies diffuse large B-cell lymphoma (DLBCL). In germinal center DLBCL the 13q31 chromosome region may also be amplified, targeting upregulation of MIR17HG and miR-17~92 hosted within. Micro-RNA (miR) dysregulation directs tumorigenesis and oncomir loci cluster near cancer chromosome breakpoints. Oncomir rearrangements are hitherto restricted to genomic amplifications while structural changes are rare or absent unlike other oncogenes. Using long distance inverse (LDI)-PCR, we mapped and sequenced breakpoints of a complex BCL6 rearrangement t(3;13)(q27;q31)(13;12)(q31;p11) which inserts MIR17HG/miR-17~92 between ITPR2 (exons 1-13) and a cryptic upstream BCL6 transcript in SU-DHL-16 (DLBCL) cells. Reverse-transcription (RT)-PCR confirmed ITPR2-5'BCL6 mRNA fusion, while the inserted MIR17HG and miR-17~92 were transcribed independently and upregulated ~5 fold and ~15-18 fold, respectively. Genomic q(uantitative)-PCR confirmed absence of MIR17HG amplification, indicating that genomic alteration, rather than amplification, underlies upregulation. Remarkably, 5/6 breaks in the t(3;13)(13;12) occurred at AT-rich DNA superhelicity peaks, identified as stress-induced DNA duplex destabilization (SIDD) regions. In 4/5 breakpoints the translocation served to increase genome stability at SIDD peaks. MIR17HG in SU-DHL-16 but neither in unrearranged nor 13q31-amplified cells was further upregulated by histone deacetylase inhibitor treatments which spared or only transiently affected other genes of interest, including BCL6, MYC, ITPR2 and ITPR2-5'BCL6. Prompted by this result suggesting epigenetic MIR17HG regulation, we performed chromatin immunoprecipitation with antibodies against acetylated histone-H3 at breakpoints flanking the MIR17HG rearrangement. This showed increased acetylation upstream but not downstream of MIR17HG on the rearranged allele. In addition to chromosome destabilization, SIDD regions often coincide with nuclear scaffold/matrix attachment regions or replication and transcription startpoints. We, therefore, propose that MIR17HG/miR-17~92 upregulation follows formation of permissive chromatin by t(3;13)(13;12). Collectively our findings introduce fresh evidence linking oncomir activation to neoplastic chromosome rearrangement, and imply these alterations may serve to induce epigenetic changes.

**FANCP - the 15th Fanconi Anemia Gene**

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Fanconi Anemia (FA) is a rare autosomal or X-chromosomal recessive disease with great genetic and phenotypic heterogeneity. It is characterized by bone marrow failure, high cancer risk and various, non-obligatory, yet typical developmental anomalies. Hypersensitivity of FA cells to DNA crosslinking agents such as mitomycin C (MMC) results in chromosomal breakage and G2-phase arrest in the cell cycle. To date, 14 disease causing genes (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N and -O) have been reported. Their products and additional proteins interact in the FA/BRCA DNA damage response network and are required for genome stability. Despite the high number of known FA genes, there are still patients that could not be assigned to any of the known complementation groups. Our aim is to find the elusive FA genes in order to complete our understanding of the DNA repair network. With different approaches we keep screening unclassified FA patients for candidate genes. Here, we report on the identification of a novel FA gene. The gene product has already been known to be involved in DNA repair and its depletion leads to increased sensitivity to MMC – a feature typical for FA. Using gDNA and cDNA sequencing we identified four affected children of two unrelated families who carried biallelic pathogenic mutations in this gene. Thus, we detected two different frameshift-causing 1-bp deletions, one nonsense mutation and one intronic 1-bp duplication leading to a splice site change. The affected children were diagnosed with FA by chromosomal breakage and/or cell cycle analysis in the age of 7 and 9 years. One child shows typical clinical features like short stature with microcephaly, hypoplasia of one thumb and malformations of the inner and external ear. The other three patients are siblings and show a milder phenotype with only one case of renal anomaly and one case of café-au-lait spots. These cellular and clinical findings are further supported by the occurrence of the typical bone marrow failure in all four children and leave no doubt on the FA diagnosis. Finally, complementation experiments proved our results for the identification of the 15th FA gene, which is according to the common FA nomenclature called FANCP.

**MCPH1 patient cells exhibit delayed release from DNA damage-induced G2/M checkpoint arrest**

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Mutations in the MCPH1 gene cause primary microcephaly associated with a unique cellular phenotype of misregulated chromosome condensation. The encoded protein contains three BRCT domains, and accumulating data show that MCPH1 is involved in the DNA damage response. However, most of this evidence has been generated by experiments using RNA interference (RNAi) and cells from non-human model organisms. Here, we demonstrate that patient-derived cell lines display a proficient G2/M checkpoint following ionizing irradiation (IR) despite homozygous truncating mutations in MCPH1. Moreover, chromosomal breakage rates and the relocation to DNA repair foci of several proteins functioning putatively in an MCPH1-dependent manner are normal in these cells. However, the MCPH1-deficient cells exhibit a slight delay in re-entering mitosis and delayed resolution of  $\gamma$ H2AX foci following IR. Analysis of chromosome condensation behavior following IR suggests that these latter observations may be related to hypercondensation of the chromatin in cells with MCPH1 mutations. Our results indicate that the DNA damage response in human cells with truncating MCPH1 mutations differs significantly from the damage responses in cells of certain model organisms and in cells depleted of MCPH1 by RNAi. These subtle effects of human MCPH1 deficiency on the cellular DNA damage response may explain the absence of cancer predisposition in patients with biallelic MCPH1 mutations.

**Distinct DNA methylation patterns in cirrhotic liver and hepatocellular carcinoma**

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Aberrant DNA methylation is one of the hallmarks of cancerogenesis. This study therefore aimed to delineate differential DNA methylation in hepatic cancerogenesis and its putative precursor, the cirrhosis of the liver. Using Illumina's HumanMethylation27K Bead Chip the DNA methylation status of 27,578 individual CpG loci have been investigated in twelve hepatocellular carcinomas (HCC), fifteen cirrhotic controls and twelve normal liver samples.

Performing a principal component analysis (PCA) we identified 167 hypomethylated loci and 100 hypermethylated loci in cirrhosis and HCC as compared to normal controls. Thus, these loci show a "cirrhotic" methylation pattern that is maintained in HCC. In pairwise PCAs between normal liver, cirrhosis and HCC, eight loci were significantly changed in all analyses differentiating the three groups ( $p < 0.0001$ ). Of these, five loci showed highest methylation levels in HCC and lowest in control tissue (LOC55908, CELSR1, CRMP1, GNRH2, ALOX12 and ANGPTL7) while two loci showed the opposite direction of change (SPRR3 and TNFSF15). Genes found hypermethylated between normal liver to cirrhosis which maintain this methylation pattern during the development of HCC are depleted for CpG islands, high CpG promoters and polycomb repressive complex 2 (PRC2) targets in embryonic stem cells. In contrast, genes selectively hypermethylated in HCC as compared to non malignant samples showed an enrichment of CpG islands, high CpG promoters PRC2 target genes ( $p < 0.0001$ ). As a conclusion, we found that cirrhosis and hepatocellular carcinoma show distinct patterns of differential methylation with regards to promoter structure, PRC2 targets and CpG islands. Thus, the characteristics of stemness in HCC might develop only from a small number of cells present in the cirrhotic liver.

**Chromosomal aberration and gene expression patterns in diffuse large B-cell lymphoma are associated with age**

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Diffuse large B-cell lymphoma (DLBCL) constitutes about 30% of adult lymphomas and 10% of lymphomas diagnosed before the age of 18 years. The clinical outcome of DLBCL is in children much better than in adults. Several lines of evidence suggest that biological features might contribute to this age-associated difference in prognosis.

Here we aimed at comprehensively determining the molecular features of DLBCL in children and adults. A cohort of 364 mature aggressive B-cell lymphomas representing predominately DLBCL of the Deutsche Krebshilfe Network "Molecular Mechanisms in Malignant Lymphoma" were characterized by morphologic panel review, immunohistochemistry, interphase fluorescence in situ hybridization (FISH), array-CGH, gene expression profiling (GEP) and EZH2 Tyr 641 mutation analysis.

In the analyzed cohort, significant differences between children <15 years ( $n=15$ ) and adults >18 years ( $n=344$ ) were observed for chromosomal translocations affecting the IRF4 locus ( $p < 0.001$ ) and gains of 2p16 and 6p25 ( $p < 0.05$ ). The lack of a biologic rationale for the age border at 18 years led us to model the conditional probability of displaying a molecular feature given age at diagnosis using logistic regression analysis (LRA). LRA revealed a statistically significant association of ABC subtype by GEP, BCL2 protein expression, absence of IRF4 translocations, gains in 1q21, 18q21, 7p22 and 7q21 as well as changes in 3q27, including gains and translocations affecting the BCL6 locus, with increasing age. The LRA analyses reveal a significant continuous increase of the probability of several molecular features of DLBCL with patient

age rather than a strict border between age groups. Remarkably, the genetic features increasing in likelihood with age in the LRA included changes associated with both, ABC (changes 3q27/BCL6, +18q21) and GCB-type DLBCL (+1q21, +7p22, +7q21), indicating an increase in genetic complexity with age somewhat independent of the molecular lymphoma subtype. Poisson regression showed the number of chromosomal aberrations to significantly increase with age. Moreover genomic complexity, a variable proposed in many studies to be associated with unfavourable outcome, loses prognostic power if age is taken into account.

The analyses of the present cohort, which represents the largest study of DLBCL analyzed by comprehensive molecular profiling so far, challenge current pathogenetic and clinical concepts in DLBCL: On the one hand, the biologic rationale for the use and level of age borders for the stratification into clinical trials as well as the application of some age-associated biomarkers is questioned. On the other hand, our data indicate a continuous increase in genomic complexity with age suggesting an "age evolution model" of DLBCL lymphomagenesis.

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## W9-06

### **Genetic testing of uveal melanomas to predict metastatic risk of patients. A follow-up study on 347 patients.**

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Uveal melanoma (UM) is the most common eye cancer in adults. In cytogenetic and molecular cytogenetic studies it has been shown that loss of chromosome 3 (monosomy 3) in UM correlates with tumor related mortality of patients. To investigate if genetic testing of tumors by microsatellite analysis (MSA) can be used to assess patients' prognosis we determined the chromosome 3 status in > 400 uveal melanomas treated by enucleation from 1998 to 2008. We were able to collect follow-up data from 347 corresponding patients with a median follow-up time of 4,1 years. MSA was performed using 8 markers evenly distributed on chromosome 3. Monosomy 3, which is defined by loss or clear reduction of one allele at all informative chromosome 3 markers, was observed in 235 (56%) tumors. Disomy 3, which is defined by retention of both alleles in the tumor, was found in 152 (36%) samples. Sixteen tumors (3,8%) were designated as partial monosomy 3, which is assumed if only a subset of chromosome 3 markers shows loss of one allele. In a small subset of 15 tumors (3,5%) the ratio of both chromosome 3 alleles did not allow clear cut assignment to one of these groups and is therefore referred to as allelic imbalance (AI). Kaplan Meier analysis revealed similar mortality rates for patients with monosomy 3 and those with AI in their tumors. In both groups about half of the patients died of metastases. This suggests that AI depicts tumors with monosomy 3 admixed with cells with a normal chromosome 3 status. Immunohistochemistry analysis confirmed a significant portion of non tumor cells in tumor samples showing AI. Good prognosis was found for patients with either disomy 3 or partial monosomy 3 in their tumors. In both groups less than 10% of patients died of metastases. In 2008 we established MLPA (Multiplex ligation-dependent probe amplification) to investigate chromosomal gains and losses in uveal melanomas. MLPA is considered as a quick, sensitive, and cost-effective method. For purpose of validation, we analysed more than 100 tumor samples by MSA and MLPA. MLPA failed or revealed inconclusive results in about 20% of samples. In all but two conclusive samples we found complete concordance of MSA and MLPA results. Both divergent samples showed normal copy number of chromosome 3 by MLPA but loss of heterozygosity by MSA a result which is indicative of isodisomy 3. In 2009, we started offering all patients the possibility of predictive monosomy 3 testing by MLPA or MSA.

## W10 MONOGENIC DISEASE II

### W10-01

#### Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome

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Treacher Collins Syndrome (TCS, MIM154500) is a disorder of craniofacial development which is characterized by bilateral downward slanting palpebral fissures, colobomas of the lower eyelids with a paucity of eyelashes medial to the defect, hypoplasia of the facial bones, particularly the mandible, cleft palate, deformity of the external ear, atresia of the external auditory canal and bilateral conductive hearing loss. There is a striking inter- and intrafamilial variation of the clinical phenotype and reduced penetrance. The majority (about 90 %) of individuals with TCS are heterozygous for a mutation in the TCOF1 gene. The TCOF1 protein is involved in the transcription of ribosomal DNA (rDNA) by interacting with upstream binding factor.

To identify the genetic alterations underlying TCS in a 3-year-old boy who had no TCOF1 mutation, we performed genome-wide, copy number analysis using an Affymetrix GeneChip 262K NspI SNP array. We identified a 156 kb de novo deletion within chromosomal region 13q12 that resulted in a heterozygous deletion of the entire POLR1D gene and exon 1 of LNX2. This prompted mutation analysis of the POLR1D in 252 TCS patients who did not show TCOF1 mutations. We detected 10 heterozygous nonsense and seven heterozygous missense mutations. In five affected families the identified mutation co-segregated with the TCS phenotype; in four families, however, we discovered unaffected mutation carriers. POLR1D encodes a subunit of the RNA polymerase I (Poll) and RNA polymerase III (PolIII). While Poll produces 45S ribosomal RNA (rRNA), which is processed to 28S, 18S and 5.8S rRNA, PolIII synthesizes 5S rRNA, transfer RNA (tRNA) and other small RNAs.

In view of the strong interaction of POLR1D with POLR1C, another subunit of Poll and PolIII, we hypothesized that the latter is a plausible TCS candidate gene and therefore, sequenced this gene in the 252 TCS patients. In one individual with typical TCS, we detected a heterozygous nonsense mutation in exon 9; however, in the same individual we also identified a heterozygous missense mutation in exon 8 of POLR1C. Similarly, in another, unrelated individual we identified two heterozygous alterations: a stop mutation in exon 2 and a missense mutation in exon 8. Finally, a third individual and her more severely affected brother were found to be heterozygous for both a 4-bp splice donor site deletion in intron 8 and a missense mutation in exon 8. Sequence analyses revealed that each available parent carried one heterozygous mutation. These observations suggest autosomal recessive inheritance of TCS in the families with POLR1C mutations.

Reduced levels of POLR1D and loss of POLR1C are likely to affect the rate of protein synthesis in the neuroepithelium and neural crest cells at a critical time point during embryogenesis. It is plausible that analysis of other genes that contribute to ribosomal biogenesis will identify further conditions belonging to the ribosomopathies with a craniofacial phenotype.

**Mutations in RAD21 as a cause of a new cohesinopathy**

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Cornelia de Lange syndrome (CdLS) is a dominantly inherited multisystem developmental disorder that includes distinctive facial features, growth retardation, a range of limb defects and variable cognitive delay. Mutations in the NIPBL gene have been identified in approximately 75% of patients with severe classical CdLS but have been found in a far lower percentage of patients with mild or atypical features.

NIPBL, along with several other regulatory proteins, is required for the establishment of sister chromatid cohesion through the loading of Cohesin, a multimer consisting of SMC1 and SMC3 and two clasp proteins, RAD21 and STAG. Following the identification of NIPBL, several mutations in SMC1A and one mutation in SMC3 were detected in a small percent of variant CdLS cases. These patients have a milder phenotype with no major structural anomalies, although all have significant mental retardation.

Using a unique collection of nearly 350 mutation-negative individuals with CdLS and overlapping clinical phenotypes, we undertook a genome-wide copy number variation (CNV) analysis. We have identified patients with chromosomal variants and clinical phenotypes that overlap CdLS and implicate RAD21 as a candidate cohesinopathy gene. We extended this data to characterize several additional patients with overlapping deletions and have identified two de novo RAD21 mutations in patients with features that diverge from classical CdLS. We have measured the expression and activity of these missense mutations in protein interaction, DNA damage and zebrafish developmental assays. In short, one of these RAD21 mutations appears to function in a dominant-negative manner, increases binding to STAG2, and results in a more severe clinical phenotype than the other loss-of-function RAD21 mutation. These data correlate with the features observed in the patients, helping to explain why patients with deletions of RAD21 may have subtle clinical phenotypes.

We will discuss the current understanding of the mechanisms by which Cohesin mutations may alter transcriptional profiles to result in multiple variant developmental phenotypes and speculate on clinical features that appear to be unique and common between different disorders of Cohesin.

**Whole-exome sequencing in combination with simultaneous analysis of homozygous stretches of exome variants identified causative mutations in two genes in Malpuech syndrome**

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Malpuech syndrome is an autosomal recessive disorder clinically characterized by craniofacial anomalies including hypertelorism and cleft lip and/or palate, growth retardation, mental retardation, and urogenital anomalies. Other similar syndromes with overlapping features are Carnevale syndrome, Michels syndrome and oculo-skeletal-abdominal (OSA), also known as 3MC (Malpuech-Michels-Mingarelli-Carnevale) syndrome.

In our study, we identified the molecular basis of Malpuech syndrome in two consanguineous Turkish families using an exome sequencing strategy. Both index patients from each family underwent whole-exome sequencing. Coding sequences were captured using the Agilent SureSelect protocol followed by deep sequencing using an Illumina Genome Analyzer. Variant sites were called by MAQ and SAMtools programs and we used these variants throughout the genome to analyze for homozygous stretches in the index patients. Genes with novel and putative damaging variants embedded within these homozygous stretches were regarded as highly relevant candidate genes. Using this strategy, we identified a homozygous nonsense mutation, p.C573X, in MASP1 in one index patient with Malpuech syndrome. Parents were heterozygous carriers and the mutation was not found in 100 healthy control individuals. MASP1 is located on chromosome 3q27 and encodes 3 different isoforms of the mannan-binding lectin serin protease 1. The

MASP1 protein was first identified as a serine protease activating the classical complement pathway by binding to lectin. The p.C573X mutation affects the serine protease domain of isoforms 1 and 2, and the truncated protein is likely to cause loss-of-function of isoforms 1 and 2. In addition, we also found a causative homozygous missense mutation in the index patient of the second family in a different gene located within a homozygous region on chromosome 2p25. Again, this mutation was not present in 100 control individuals and is likely to disrupt protein function. Moreover, we have evidence for further genetic heterogeneity of 3MC syndrome, because no mutation was found in both genes in two additional patients.

In summary, our data demonstrated that mutation identification through exome sequencing in combination with simultaneous analysis of homozygous stretches of exome variants can be used as an efficient approach to identify genes in autosomal recessive disorders. Future functional analysis of identified genes/proteins will give insights into the molecular pathogenesis of Malpuech syndrome.

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## W10-04

### Identification of a skeletal syndrome caused by a novel recessive mutation within the ANKH gene

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Dominant mutations in the ANKH gene cause the skeletal disorders craniometaphyseal dysplasia (CMD) and familial chondrocalcinosis (CCAL2). The encoded transmembrane protein ANK is assumed to regulate extracellular pyrophosphate (PPi) levels. Thus, skull hyperostosis as well as long bone modeling defects in CMD and articular cartilage calcifications in CCAL2 are probably due to diminished or increased extracellular PPi levels, respectively. However, a human phenotype corresponding to the recessive loss-of-function mouse model progressive ankylosis (ank) has not been identified so far.

We identified the novel recessive ANKH missense mutation c.731T→C in all affected members from a large consanguineous family demonstrating ligament calcifications of small joints, progressive spondyloarthropathy, osteopenia, mixed hearing loss, and mental retardation. The resulting amino acid change Leu244Ser occurs at a highly conserved residue. Further biochemical analysis showed normal protein expression and cell surface localization in patient fibroblasts. We next performed a detailed comparison of the human phenotype with that of the ank mutant. Analysis of interphalangeal joints in homozygous ank mice at the age of 4 weeks revealed severe ligament fibrosis, cartilaginous tissue within ligaments, partial mineralization of articular cartilage, and infiltration with mononuclear cells. In the knee joints cruciate ligament fibrosis was also evident, but the articular cartilage appeared unaffected. At the age of 3 and 6 weeks homozygous ank mice demonstrated diminished relative trabecular bone values (BV/TV) in long bones, but normal values in vertebrae. These analyses corroborated the close similarity of the here identified syndrome with the ank phenotype. As the ank mouse is caused by an Ank loss-of-function due to premature protein degradation and the mutation L244S shows normal expression levels it can be concluded that this novel mutation impairs the ANK protein function.

**NEK1 mutations cause short rib-polydactyly syndrome type Majewski**

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Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in different signal transduction pathways and cell cycle coordination. We thoroughly delineated the clinical, radiographic, and histological phenotype of the autosomal-recessive short-rib polydactyly syndrome Majewski type and used parametric multipoint linkage and haplotype structure analysis in two consanguineous families. This confirmed a linked interval with a maximum LOD score (HLOD) of 2.95 representing 17.36 Mb / 18.65 cM on chromosome 4 encompassing 38 genes. Assuming an overlap of the phenotypic spectrum of our patients with other ciliopathies, we used known genes of the cilia proteome database and compared them with the genes in our candidate interval. The NIMA-related kinase 1 (NEK1) gene was highlighted as interesting candidate with homozygous mutant mice (*kat / kat-2J*) for the orthologous gene *Nek1* show polycystic kidney disease, craniofacial anomalies, and growth reduction.

Sequencing analysis of NEK1 identified a homozygous nonsense mutation (c.379C>T; p.Arg127X) in the affected individual of one consanguineous family and a homozygous splice-site mutation (c.869-2A>G) in the affected person of the second consanguineous family as the underlying cause. NEK1 encodes a serine/threonine kinase with proposed function in DNA double-strand repair, neuronal development, and coordination of cell cycle-associated ciliogenesis. With immunofluorescence analyses we confirmed that the absence of functional NEK1 significantly decreases the number of ciliated fibroblasts and alters the structural morphology of the primary cilium *in vivo* as well. Transmission electron microscopy indicated a defect in the progression from stage I after vesicular accumulation of ciliogenesis to stage II and subsequent failure of axoneme growth.

We further substantiate a proposed digenic diallelic inheritance of ciliopathies by identification of heterozygous mutations in NEK1 and DYNC2H1 in a further non-consanguineous family. Notably, these findings not only increase the broad spectrum of ciliary disorders, but also suggest a correlation between the degree of defective microtubule or centriole elongation and organization and the severity of the resulting phenotype.

**Microduplications of the HOXD locus are associated with mesomelic dysplasia Kantaputra type**

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Mesomelic Dysplasia Kantaputra Type (MDK, MIM 156232) is a rare autosomal dominant human skeletal malformation characterized by mesomelic shortening of the upper and lower limbs. Previous studies demonstrated linkage to chromosome 2q31 and the HOXD-cluster, which is known to be expressed in a time- and space-dependent manner during limb development.

In order to characterize the underlying genetic cause in a Thai family suffering from MDK we performed array CGH and detected two microduplications on chromosome 2q31.1–q31.2 separated by a 357 kb segment of normal copy number. The centromeric duplication spans 481 kb encompassing the entire HOXD-

cluster. This duplication overlaps with the inversion of the ulnaless mouse mutant which shows a similar phenotype as MDK. The 507 kb telomeric duplication encompasses long passages of non-coding regions and does not contain known limb development associated genes. Fluorescence-in situ-hybridization revealed a direct tandem orientation of both duplications.

Based on our results and previous studies of HOXD-cluster regulation we suggest a HOXD-cluster regulation defect during early limb development as a cause for MDK. In normal early limb development the control region located centromeric to the HOXD-cluster, CenR, represses the expression of the proximal encoded HOXD13 to D10. Another control element, the early limb control region (ELCR), is proposed to activate the more distal encoded HOXD genes, i.e. HOXD1 to D9. Both elements repress and activate the corresponding HOXD genes, respectively, in a promoter-independent but space-dependent manner. In case of the ulnaless mutant, which shows a MDK-like phenotype, an inversion most likely disconnects the proximal HOXD genes from CenR which results in an ectopic upregulation in the proximal limb (zeugopod).

We propose a model in which the ELCR is located within the telomeric duplication. This allows normal expression of HOXD9-1 and thus, regular development of the proximal extremities and body axis in our patients. However, the CenR 5' to the centromeric duplication fails to repress the expression of HOXD13-D10 resulting in ectopic zeugopod expression. In summary, genomic rearrangements leading to the disturbance of the HOXD-cluster regulation, i.e. the failure of 5' HOXD gene repression, cause MDK phenotype.

## W11 DISEASE MECHANISMS / THERAPY

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### W11-01

#### **Functional analysis of AMD-associated variants in 10q26 reveals alterations of expression levels in ARMS2 but not HTRA1 transcripts**

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**Introduction:** AMD is a multifactorial disease of the central retina and a leading cause of irreversible vision loss in developed countries. A total of 15 polymorphisms on chromosome 10q26 tagging a single risk haplotype and spanning two genes, ARMS2 (age-related maculopathy susceptibility 2) and HTRA1 (Htra serine peptidase 1), have been strongly associated with the disease. With the exception of a single non-synonymous variant (rs10490924), the remaining polymorphisms do not reveal any obvious functional consequences on gene or protein function. Thus far, several groups have reported contradictory results regarding risk-associated gene expression (1,2,3). In an effort to identify the functional AMD gene in 10q26, the aim of our study was to focus on the effects of the risk-associated polymorphisms on the expression levels of ARMS2 and/or HTRA1.

**Methods:** In vitro experiments were done in COS-7 cells after transfection with genomic, risk and non risk associated ARMS2 variants, as well as chimeric constructs of both variants. ARMS2 expression was compared via quantitative real-time (qRT)-PCR and immunocytochemistry. The retinal transcription start site of HTRA1 was determined via 5'-RACE. In vitro luciferase assays were performed with HTRA1 promoter constructs of non risk and risk associated haplotypes up to 4.5 kb in size. HTRA1 promoter constructs were also electroporated into mouse retinal explants. In vivo assays were done to assess an influence of the 10q26 haplotype on ARMS2 and HTRA1 expression, semiquantitative sequencing of ARMS2 and HTRA1 transcripts from human retinal/RPE samples heterozygous for the 10q26 risk haplotype was performed. In addition, qRT-PCR for HTRA1 was done with cDNA from human retinal/RPE samples, lymphocytes and placentas.

**Results:** The in vitro assays show that ARMS2 mRNA levels of the risk isoform are significantly reduced compared to mRNA levels of the non risk isoform. With our chimeric ARMS2 constructs, this effect could be assigned to an insertion/deletion polymorphism (NM\_001099667.1:c.(\*)372\_815del443ins54) in the 3' untranslated region of ARMS2. In vitro and ex vivo reporter assays with HTRA1 promoter constructs revealed no influence of the 10q26 haplotype on HTRA1 promoter activity. In vivo analyses with human retinal/RPE samples fully confirmed our in vitro results, demonstrating a 10q26 haplotype dependent decrease of ARMS2 mRNA levels, but no influence on HTRA1 expression. Interestingly, a common non risk associated polymorphism (rs2736911) leading to a premature stop of ARMS2 protein synthesis also reduced ARMS2 transcript levels in vivo.

**Conclusion:** We show that the 10q26 haplotype affects ARMS2 but not HTRA1 transcript levels. Considering the rs2736911 related reduction of ARMS2 transcript level, however, haploinsufficiency of

ARMS2 should not account for an increased AMD risk. Our data therefore challenge the role of altered HTRA1 or ARMS2 expression in AMD pathogenesis.

Literature: 1) Fritsche et al., 2008, Nat. Genet. 40:892-6; 2) Dewan et al., 2006, Science, 314: 989-92; 3) Yang et al., 2007, Plos Genet. 6:e1000836

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## W11-02

### Functional characterization of mutations in THAP1 causing dystonia 6

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Dystonias represent a group of heterogeneous movement disorders, characterized by involuntary twisting, repetitive movements and abnormal postures. Dystonia 6 (DYT6), a monogenic form of primary (isolated) dystonia, is associated with mutations in the THAP1 gene; however, its molecular pathophysiology is not well understood. THAP1 encodes a transcription factor, with a sequence-specific DNA binding zinc-finger domain (THAP domain) at the N-terminus (amino acid 1–91) and a putative nuclear localization signal (NLS) towards the C-terminus (amino acid 147–162). Recently, we demonstrated that THAP1 specifically binds to the TOR1A promoter and represses TOR1A expression. The TOR1A gene is involved in the primary dystonia 1 (DYT1).

By screening a large cohort of 700 patients with different forms of dystonia, we identified several new missense mutations located within the THAP domain as well as mutations resulting in truncated THAP1 proteins. To investigate the functional consequence of the missense mutations, we performed luciferase reporter gene assays. Thereby, we demonstrated that missense mutations within the THAP domain result in a decreased or almost abolished THAP1-mediated repression of TOR1A expression. In contrast, amino acid exchanges within the C-terminal region of THAP1 did not influence its activity in gene transcription.

In a second step, we analyzed the intracellular localization of wildtype THAP1, THAP1 with truncating mutations, and a missense mutation within the NLS (I149T). For this, we expressed THAP1-GFP fusion proteins in different cell lines and performed confocal laserscanning microscopy. We could show that truncating mutations resulted in partial or complete loss of nuclear localization. Similarly, the missense mutation I149T resulted in an impaired transport of THAP1 protein into the nucleus.

In summary, our data provide evidence that missense mutations in the DNA-binding THAP1-domain as well as mutations leading to a partial or complete loss of the NLS sequence both result in a disturbed transcription factor activity of THAP1.

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## W11-03

### SMA-therapy with VPA: Why do we have Positive- and Non-Responders?

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Spinal muscular atrophy (SMA) is the most frequent genetic cause of childhood lethality. SMA is caused by functional loss of SMN1 leading to progressive degeneration of the alpha-motor neurons in the spinal cord. Importantly, all SMA patients retain an almost identical copy gene, SMN2. However, SMN2 is unable to compensate for the loss of SMN1, since it produces only low amounts of full-length transcript, while the majority is alternatively spliced. Over the last years a considerable number of compounds has been identified which activate transcription, restore the correct splicing of SMN2 or stabilize the SMN2 protein in vitro or in vivo. We have previously shown that valproic acid (VPA), a short-chain fatty acid histone deacetylase (HDAC) inhibitor, significantly increases SMN levels in vitro, ex vivo and in VPA-treated SMA patients. However, only about 1/3 of SMA patients exhibited a positive response to VPA treatment, whereas the remaining are either non- or negative responders. Based on these results, VPA is currently used in SMA therapy and shows indeed improvements particularly in SMA patients under 5 years. To identify the underlying mechanism of differential response towards VPA treatment, we established fibroblast lines from skin biopsies of 25 SMA patients, in whom blood samples were obtained before and under VPA therapy to verify in vivo SMN2 expression. We found a strong correlation between both cell systems – fibroblasts and peripheral blood monocytes in about 65%. We employed fibroblast mRNA from positive- and negative-responders -either mock- or VPA-treated- to identify transcriptome-wide expression differences. Surprisingly, non-responders failed to show any differential expression on a transcriptome wide level, suggesting a lack of HDAC inhibition by VPA. By comparing VPA-treated cells of positive and non-responders four significantly

differentially expressed genes turned out to be highly interesting candidates based on their function in cell metabolism. Interestingly, one of these genes is involved in fatty acid metabolism suggesting a disturbed VPA turnover in non-responders. Therefore, we analyzed VPA metabolism and uptake by HPLC-MS/MS and further metabolism experiments. In addition, overexpression and knockdown studies suggest an interaction between the four candidate genes as well as directly on SMN2. Lastly, we succeeded in generating induced pluripotent stem cells (iPSC) from fibroblast lines of positive- and non-responders. Differentiating these cells into motor neurons will allow us to verify our fibroblast data in the actual SMA target cells.

In line with recent developments in pharmacogenomics, pre-therapeutic analysis of candidate genes serving as biomarkers promises to avoid side-effects and ineffective drug administration. Our data sheds first light on the molecular mechanisms and pathways influencing VPA response and may not only have a major impact on the therapy of SMA but also on other diseases like epilepsy and mood disorders, in which VPA is frequently indicated.

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## W11-04

### Gene therapy to rescue splice defects in eye diseases using U1 snRNA

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Retinitis pigmentosa (RP) initially affects the mid-peripheral retina and ultimately causes severe visual impairment. The disease is associated with mutations in over 40 different genes. Frequently, screening of affected families reveals splice site mutations as the cause of the disease. Here, we evaluate a gene therapy to correct splice defects in two patient-derived cell lines from independent families.

We performed linkage in a large consanguine RP family and candidate sequencing in a five generation family showing X-chromosomal RP. The two novel splice donor site mutations c.1245+3A>T and c.479G>A were identified in the ciliary genes of RPGR and BBS1, respectively, and found to cosegregate with the disease of RP. Both mutations cause splice defects. Affected family members show late-onset forms of RP without additional clinical features. In agreement with these mild phenotypes, we did not detect obvious ciliary defects in patient-derived cells.

To correct the splice defect, we developed a gene therapeutic approach using mutation-adapted U1 snRNA (U1). U1 is required for splice donor site (SDS) recognition of pre-mRNAs and initiates the splice process. The two RP mutations interfere with the recognition of the SDS by U1. To overcome the deleterious effects of the mutations, we generated four U1 isoforms with increasing complementarity to the SDS. Lentiviral particles were used to transduce patient-derived fibroblasts with these U1 variants. Full complementarity of U1 partially corrects the splice defects and increases recognition of the two mutated SDS. The therapeutic effect is dose dependent.

U1-based gene therapy constitutes a promising technology to treat SDS mutations in inherited diseases including autosomal recessive and X-linked RP. The technique might be applicable to many inherited gene defects.

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## W11-05

### Loss of corneodesmosin leads to severe barrier defect and atopy in autosomal recessive peeling skin disease

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Generalized peeling skin disease (PSD), also referred to as peeling skin syndrome type B, is an unusual autosomal recessive congenital ichthyosiform erythroderma characterized by lifelong patchy peeling of the entire skin and associated with pruritus and atopic disease. At the ultrastructural level PSD shows enhanced detachment of corneocytes. We have characterized a large consanguineous family with PSD using a whole-genome autozygosity analysis. We have identified a homozygous early N-terminal nonsense mutation, p.Lys59X, in CDSN, the gene encoding corneodesmosin. Western blot analysis demonstrated complete absence of corneodesmosin in the patients. Corneodesmosin is an important adhesive protein of terminally differentiated keratinocytes, located in the extracellular part of the corneodesmosomes. To further characterize the importance of corneodesmosin for the epidermal barrier, we used reconstructed skin made with primary keratinocytes and fibroblasts, which fully replicates epidermal differentiation, and generated models for PSD by knocking down CDSN in normal keratinocytes using RNAi. We observed ablation of corneodesmosin throughout the cultivation time of the model, which mimicked the patient epidermis with a decreased granular layer, strong hyperkeratosis, and a destabilized stratum corneum. The normal skin

equivalents showed intact epidermal barrier activity as shown with test substances for percutaneous absorption such as caffeine and testosterone. The apparent permeability coefficient (Papp) values with caffeine and testosterone were 15.7E+6cm/s and 8.58E+6cm/s, respectively, in patient models compared to 10.9E+6cm/s and 5.30E+6cm/s in control models ( $p < 0.01$ ). Thus we have demonstrated that PSD is caused by loss of corneodesmosine because of recessive CDSN nonsense mutations, leading to impairment of barrier integrity and keratinocyte attachment. Importantly, the pathophysiology is distinct from that of hypotrichosis simplex of the scalp (HTSS), which can be associated with particular dominant mutations in CDSN located after the first glycine loop domain. We conclude that lack of corneodesmosin accounts for the predisposition to atopic manifestations and propose PSD as a new model for atopic disorders induced by a disturbed and ineffective epidermal barrier activity.

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## W11-06

### Modelling mental retardation in Drosophila - of EHMT, Kleefstra syndrome and beyond

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Epigenetic regulation of cognition is an emerging field in Neuroscience. However, the identity of crucial regulators and underlying molecular mechanisms are poorly understood. Excellent candidates are epigenetic genes that are implicated in mental retardation disorders.

We have generated Drosophila mutants for EHMT, an evolutionarily conserved euchromatin histone methyltransferase implicated in Kleefstra syndrome. EHMT is widely expressed in the fly nervous system and other tissues, yet EHMT mutant flies are viable. Neurodevelopmental and behavioral analyses identified EHMT as a regulator of peripheral dendrite development, larval locomotor behavior, non-associative learning, and courtship memory. The requirement for EHMT in memory was mapped to a subset of neurons. Moreover, memory was restored by EHMT re-expression during adulthood, indicating that cognitive defects are reversible in EHMT mutants. To uncover the underlying molecular mechanisms, we generated genome-wide profiles of the EHMT-induced histone modification (H3K9me2) using ChIP-seq technology. Loss of H3K9 dimethylation in EHMT mutants occurs at 5% of the euchromatic genome and is enriched at the 5' and 3' ends of distinct classes of genes that control neuronal and behavioural processes that are corrupted in EHMT mutants. Our study identifies Drosophila EHMT as a key regulator of cognition that orchestrates an epigenetic program featuring two-thirds of all known learning and memory genes. Our work provides novel insights into the epigenetic control of cognition in health and disease, into the pathophysiology of Kleefstra syndrome and has potential therapeutic implications. EHMT is one of our pilot projects for systematic functional studies of >300 mental retardation genes that are currently on the way. Our goal is to provide conceptual advance in our understanding of brain development & diseases and to significantly contribute to the development of novel diagnostic and therapeutic strategies for the large and still growing group of cognitive disorders.

## EDUCATIONAL SESSIONS

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### EDU 1

#### Rechtliche Grundlagen der humangenetischen Labortätigkeit - Richtlinien der Bundesärztekammer, Teil B5 Humangenetik

*Clemens Müller-Reible (Würzburg), Karsten Held (Hamburg), Wolfgang Vogt (München)*

Mit dem Gendiagnostikgesetz (GenDG) wurde im Jahre 2009 erstmalig ein spezieller gesetzlicher Rahmen für die Tätigkeit humangenetischer Laboratorien geschaffen. Daneben existiert eine Plethora von

gesetzlichen und untergesetzlichen Regelungen, die beim Betrieb jeder Art von medizinischen Laboratorien zu berücksichtigen sind. Aktuell ist die „Richtlinie der Bundeärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen“ (RiliBÄK:2008) in den Focus der Aufmerksamkeit gekommen, weil die BÄK im Jahre 2009 unter Beteiligung der GfH eine Arbeitsgruppe zur Erarbeitung eines speziellen Teils B 5 „Molekulargenetische und zytogenetische Untersuchungen“ eingerichtet hat.

Die RiliBÄK:2008 ist ein Teil der Umsetzung der EU Richtlinie 98/79/EG („IVD-Richtlinie“), die in Deutschland durch das Medizinproduktegesetz (MPG) und die nachgeordnete Medizinprodukte-Betreiberverordnung (MPBetreibV) in nationales Recht überführt worden ist.

Im Allgemeinen Teil A der RiliBÄK, der für alle medizinischen Laboratorien gilt, wird die Einrichtung eines Qualitätsmanagementsystems gefordert, mit detaillierten Anforderungen an die Leitung des Labors und das übrige Personal, an Räume und Ausrüstungsgegenstände, an Präanalytik, Untersuchungsverfahren und Postanalytik, an die Dokumentenlenkung, die Klärung von Beschwerden, die Untersuchung in Fremdlaboratorien, die Behandlung von Fehlern und sowie die interne und externe Qualitätssicherung. Die Teile B1 bis Bn befassen sich mit den speziellen Anforderungen an die interne und externe Qualitätssicherung in den einzelnen Fachgebieten der Laboratoriumsdiagnostik, so der Teil B 5 mit den „Molekulargenetischen und zytogenetischen Untersuchungen“.

In der Sitzung werden zunächst die allgemeinen rechtlichen Rahmenbedingungen humangenetischer Labortätigkeit dargestellt und im Besonderen auf die Anforderungen eingegangen, die sich aus der RiliBÄK für die Alltags-tätigkeit eines humangenetischen Labors ergeben.

## **Implementierung der RiLiBÄK in die humangenetische Laboratoriumsdiagnostik**

*Karsten R. Held, Hamburg*

Die im Jahre 2008 grundlegend überarbeitete RiLiBÄK ist die übergeordnete Richtlinie der BÄK, die die Qualitätssicherung (QS) laboratoriumsmedizinischer Untersuchungen regelt. Die im Teil A der Richtlinie beschriebenen grundlegenden Anforderungen, z.B. zum Qualitätsmanagementsystem, gelten auch jetzt schon für alle humangenetische Laboratoriumsuntersuchungen.

Im speziellen Teil B1- Bx der RiLiBÄK sind Mindestanforderungen an die Sicherung der Qualität der Messergebnisse festgelegt. Diese Mindestanforderungen umfassen die interne und die externe Qualitätssicherung.

In der Laboratoriumsmedizin wird die Leistungsfähigkeit eines Messverfahrens durch die Kriterien analytische Empfindlichkeit, analytische Spezifität, Messgenauigkeit, systematische Messabweichung, zufällige Messabweichung, Wiederholpräzision u.a. beschrieben. Die Besonderheiten humangenetischer Laboratoriumsuntersuchungen machten die Entwicklung eines speziellen Kapitel B5 für molekulargenetische und zytogenetische Untersuchungen notwendig, da diese Kriterien nur bedingt angewendet werden können. So ist bei der Angabe von Fehlergrenzen zu berücksichtigen, dass dies nur für numerische Analyte möglich ist. Der z. B. bei einer Chromosomenanalyse zu bestimmende Analyt besteht jedoch aus zwei Komponenten. Die erste Komponente ist numerisch, nämlich die Bestimmung der Chromosomenzahl in einer Metaphasenplatte, die andere Komponente ist eine qualitative Komponente, nämlich die Beurteilung der Chromosomenstruktur nach Spezialfärbungen. Eine interne Qualitätssicherung ist damit nur für die numerischen Analyte möglich, da bei Analyten qualitativer Art keine Größenwerte bestimmt werden.

In der Sitzung wird die Entwicklung von Mess- bzw. Prüfgrößen in der humangenetischen Laboratoriumsmedizin dargestellt, die eine interne QS erlauben, weiterhin werden auf die Anforderungen an und die Durchführung der externen QS eingegangen.

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## **EDU 2**

### **Der (un)gelöste Fall**

*Dagmar Wiczorek (Essen), Gabriele Gillessen-Kaesbach (Lübeck)*

Mit Hilfe der rasanten Fortentwicklung der neuen Technologien, zu denen neben den schon länger bekannten genomweiten Array-Analysen das „Next Generation Sequencing“ gehört, gelingt es zunehmend, die molekularen Ursachen von Krankheitsbildern aufzuklären. Die Identifizierung von neuen Genen ermöglicht die molekulare Bestätigung von immer mehr klinischen Diagnosen, wie kürzlich beim Kabuki- und Miller-Syndrom, und erlaubt auch, die Phänotypgrenzen neu zu definieren. Das klinische Spektrum klassischer Krankheitsbilder wird damit breiter, da auch mildere Phänotypen sicher diagnostiziert werden können. Komplexe Signalwege werden aufgedeckt und die Zusammenhänge von Krankheitsbildern, z.B. neuro-fazio-kutane Syndrome, geklärt. Durch die neuen Methoden werden auch zunehmend neue Mikrodeletionssyndrome beschrieben, wobei der Phänotyp klinisch nicht immer sicher diagnostizierbar ist.

Diese Fortbildung soll dazu dienen, ungeklärte Fälle aus dem Auditorium zu besprechen, ein diagnostisches Procedere zu entwickeln und optimalerweise auch Diagnosen zu finden. Schön wäre es aber auch, wenn ungewöhnliche und/oder seltene gelöste Fälle aus dem Auditorium vorgestellt würden.

Die (un)gelösten Fälle (4-5 Folien) sollten bis zum 09.03.2011 per e-mail an [g.gillessen@uk-sh.de](mailto:g.gillessen@uk-sh.de) und [dagmar.wieczorek@uni-due.de](mailto:dagmar.wieczorek@uni-due.de) geschickt werden. Die Powerpoint-Vorlagen für die Präsentationen werden ab dem 1.2.2011 unter [http://www.gfhev.de/de/kongress/wissenschaftl\\_programm.htm](http://www.gfhev.de/de/kongress/wissenschaftl_programm.htm) abrufbar sein

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### EDU 3

#### **Extremitätenfehlbildungen**

*Jürgen Kohlhasse (Freiburg), Denise Horn (Berlin)*

Extremitätenfehlbildungen treten isoliert oder als Teil einer Vielzahl genetischer Syndrome auf. In dieser Veranstaltung soll ein systematisches Vorgehen zur diagnostischen Einordnung vermittelt werden. Hierzu gehören auch die Einführung in radiologische Grundlagen und pathophysiologische Betrachtungen, die wiederum auf eine Reihe beispielhaft ausgewählter Erkrankungen angewendet werden. Speziell werden Reduktionsfehlbildungen, Polydaktylien, Brachydaktylien und Syndaktylien anhand ausgesuchter klinischer Fallbeispiele behandelt.

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### EDU 4

#### **Zukunft der prädiktiven genetischen Diagnostik**

*Peter Propping<sup>1</sup>, Markus Löffler<sup>2</sup>, Christoph Engel<sup>2</sup>*

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Mit der Aufklärung des Genoms und der voranschreitenden Genotyp-Phänotyp-Analyse ist die Hoffnung groß, dass die prädiktive genetische Diagnostik als Instrument der Krankheitsprävention in Zukunft eine zunehmende Rolle spielen wird. Unter diesem Titel hat die Deutsche Akademie der Naturforscher Leopoldina gemeinsam mit der BBAW und der acatec eine Stellungnahme vorgelegt. Für eine untersuchte Person entscheidende Maße der Vorhersage sind der positiv prädiktive Wert (PPV) und der negativ prädiktive Wert (NPV) einer Genvariante für eine bestimmte Krankheit. Beide Maße hängen von der Häufigkeit der Krankheit ab, obgleich Sensitivität und Spezifität konstant bleiben. Für eine Prädiktion, die für die Prävention bzw. Therapie eingesetzt werden kann, kommen gegenwärtig nur Genotypen mit einem Hauptgeneffekt in Betracht. Anhand von HNPCC und erblichem Brust-/Eierstockkrebs wird gezeigt, wie sehr die Wahrscheinlichkeit der Identifikation einer pathogenen Mutation von der Phänotypinformation abhängt. - Die odds ratio ist ein relatives Risikomaß, mit dem der Beitrag von Genvarianten zur Entstehung multifaktorieller Krankheiten geschätzt werden kann. PPV und NPV der Genvarianten sind fast immer so niedrig, dass sie sich beim gegenwärtigen Stand des Wissens für die praktische Anwendung nicht eignen.

# POSTERS

## P-CLINICAL GENETICS

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### P-ClinG-001

#### **A new case of distal deletion of chromosom 4q35 and review of the literature**

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Terminal deletions of chromosome 4q35 comprise a variable spectrum of clinical symptoms. They are known for causing developmental delay or mental retardation, Pierre Robin sequence and a variety of structural anomalies of different organ systems including craniofacial, skeletal (mainly ray-defects of upper or lower limbs), cardiovascular and other malformations. Even normal phenotype has been reported.

A wide and clinically heterogeneous collection of numerous cases with 4q-terminal-deletion syndrome has been presented in the literature over the last decades. This heterogeneity is possibly caused by the fact, that there is a wide range of chromosomal breakpoints on the molecular level. The terminal deletions 4q concerning chromosomal bands 4q35-ter are different from the as well repeatedly reported larger deletions of chromosome 4q including more proximal chromosomal bands up to 4q31.

We report on an 11-year old girl referred to the genetic unit due to developmental delay, mild mental retardation and short stature. Clinical examination further revealed strabism, microcephaly, facial dysmorphism including hypotelorism, upslanting palpebral fissures, bulbous nose tip, long philtrum, broad mouth and prominent lips. Skeletal findings were narrow downslanting shoulders and bilateral hallux valgus formation (confirmed by X-ray analysis), the latter being very unusual for this age.

Conventional karyotyping on a 550 band level revealed a terminal deletion of the long arm of chromosome 4 (karyotype : 46,XX,del(4)(q35.1)). The deletion was confirmed by FISH-analysis using 4qter subtelomeric FISH-probes (TelVys 4q). A balanced translocation concerning 4qter could be excluded.

We will review reported cases of 4q-terminal deletion and present the data of our patient in comparison to the literature. This additional case can help to further delineate the 4q-terminal deletion phenotype.

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### P-ClinG-002

#### **Microdeletion 8q22.1 without characteristic Nablus mask-like face**

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In 2000 Teebi et al. described a patient with Nablus mask-like facial syndrome. A microdeletion 8q21.3q22.1 was shown to be the cause of this syndrome in further patients by Shieh et al. 2006, Barber et al. 2008, and Raas-Rothschild et al. 2009. A smaller deletion in a patient without a mask-like face was published by Jain et al. in 2010, who postulated a minimal critical region for the facial changes of Nablus mask-like facial syndrome.

We present a seven-year-old boy without a mask-like face but with a larger deletion of 4.1 Mb in 8q21.3q22.1 overlapping with the deletions in the patients published by Raas-Rothschild. The deletion was identified by Affimetrix Genome-Wide Human SNP 6.0 microarray and comprises 18 genes and 2422 array markers (92.358.132-96.467.774).

The patient is the first child of healthy German parents and he was born at 35+3 weeks of gestation by caesarean section with normal birth measurements. Narrow choanae, a heart defect (VSD, PDA, bicuspid aortic valve), hypospadias and cryptorchidism were diagnosed.

The boy had mild motor and speech delay (walking and talking with 18 months) and high tone sensorineural deafness. Clinical investigations at 6 and 7 ½ years of age showed normal body measurements, a long face, short palpebral fissures, a flat philtrum and prominent ears. He is only mildly mentally retarded and has a friendly demeanour.

The patient's deletion comprises the critical region for the mask-like face but his face is not characteristic. This broadens the phenotype of the syndrome and supports the necessity of array analysis in patients with mild mental retardation.

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**P-ClinG-003****Development of a skin humanized mouse model for transglutaminase 1 deficient lamellar ichthyosis**

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Lamellar ichthyosis (LI) is a severe autosomal recessive skin disease with a prevalence of about 1:100,000 and is genetically very heterogeneous. About 45% of all cases can be attributed to transglutaminase 1 (TG1) deficiency due to different mutations of the corresponding gene TGM1 on chromosome 14q11.2 of the human genome. Patients with LI due to TG1 deficiency (LI type 1) are born as so called collodion babies, encased in a tight shiny collodion membrane. Patients display a life long pronounced scaling of the skin as part of a homeostatic response to transepidermal water loss.

TG1 (EC 2.3.2.13) is a crucial epidermal enzyme that catalyzes the covalent cross-linking of numerous structural proteins of the cornified envelope (e.g. loricrin, keratin intermediate filaments, involucrin, filaggrin, SPRs) through the formation of N-(glutamyl)lysine isopeptide bonds. The activity of TG1 is initiated by binding of Ca<sup>2+</sup>. The enzyme is expressed in the upper differentiated layers of the epidermis where it facilitates the formation of the insoluble protein envelope as well as the attachment of long chain omega-hydroxyceramides to involucrin to build up the lipid envelope.

TGM1 knockout mice (TGM1<sup>-/-</sup>) have an erythrodermic and shiny skin and lack clinically visible hyperkeratosis. Instead, they display a marked impairment of the epidermal barrier and transepidermal water loss (TEWL) is dramatically increased (up to 100 fold). These mice die within 3-4 hours after birth due to extreme dehydration. Skin of transplanted TGM1<sup>-/-</sup> mice resembled that in severe LI with epidermal hyperplasia and marked hyperkeratosis contrasting to that seen in the knockout mice. At the same time TEWL in these mice was much less pronounced.

Although an animal model of LI thus exists this does not faithfully recapitulate the human disease and presents drawbacks including high mortality rates immediately after birth. Therefore, we established optimized tissue engineering and surgical conditions enabling stable human skin engraftment in immune-deficient mice as well as the modeling of LI in a humanized context. This allowed the development of a human model of LI carried on the back of athymic nude mice.

Investigations of diagnostic markers like in situ monitoring of TG1-activity and immunohistochemical staining of TG1 protein using specific TG1-antibodies showed a complete absence of TG1 in the grafts. Ultrastructural investigations revealed so called cholesterol clefts in the stratum corneum which are typical ultrastructural markers for TG1 deficient LI.

We conclude that the skin humanized mouse model faithfully recapitulates the human disease and should be an excellent tool for testing novel therapeutic approaches in the future.

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**P-ClinG-004****De novo partial trisomy 18p and partial monosomy 18q in a patient with anorectal malformation**

Bartels E.<sup>1</sup>, Draaken M.<sup>12</sup>, Kazmierczak B.<sup>3</sup>, Spranger S.<sup>3</sup>, Schramm C.<sup>1</sup>, Baudisch F.<sup>14</sup>, Nöthen M.M.<sup>12</sup>, Schmiedeke E.<sup>5</sup>, Ludwig M.<sup>4</sup>, Reutter H.<sup>16</sup>

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Anorectal malformations (ARM) encompass a broad clinical spectrum which ranges from mild anal stenosis to severe anorectal anomalies such as complex cloacal malformations. The overall incidence of ARM is around 1 in every 2,500 live births. Although causative genes for a few syndromic forms have been identified, the molecular genetic background of most ARM remains unknown.

The present report describes a patient with a de novo 13.2 Mb deletion of chromosome 18q22.1–qter and a 2.2 Mb de novo duplication of chromosomal region 18p11.32–pter located at the telomeric end of chromosome 18q. The patient presented with ARM and the typical features of 18q- syndrome (De-Grouchy syndrome). The combination of a partial duplication of the short arm and a partial deletion of the long arm of chromosome 18 has been described in 16 previous cases. However, this is the first report of an association between this complex chromosomal rearrangement and ARM.

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## P-ClinG-005

### Identification of a novel human gene for hearing loss and cochlear hypoplasia (incomplete Mondini malformation)

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The combination of hearing loss and Mondini malformation (the presence of both bilateral dilatation of the vestibular aqueduct [DVA, also called enlarged vestibular aqueduct or EVA] and cochlear hypoplasia) has been associated with recessive gene mutations in SLC26A4 (~50% of individuals affected with Pendred syndrome) and FOXI1 (~1% of affecteds, suggesting further genetic heterogeneity). Here we report on a novel gene associated with syndromic profound congenital hearing loss and cochlear hypoplasia (partial Mondini malformation). The propositus, a 6-year-old boy of Turkish origin, had computed tomography (CT) findings of bilateral single widened cochlear turn (normal are 2.5 turns) and narrowing of the internal auditory canal, as well as low-set posteriorly rotated ears, plagiocephaly/asymmetrical skull, scaphocephaly, wide/long palpebral fissures, high and very narrow palate, and sacral dimple/sinus. He also had muscular hypotonia, increased salivation at younger age, and developmental delay. Karyotyping and molecular analyses of the GJB2 and GJB6 genes were normal, but high resolution array analysis (Affymetrix 6.0) revealed a homozygous deletion sized 111 kb at chromosome 5q31.1. The unaffected consanguineous parents both showed a heterozygous deletion. The deletion predicts a functional monosomy of three genes, NEUROG1 (neurogenin-1), DCNP1 (dendritic cell nuclear protein 1), and TIFAB (TNF receptor-associated factor-interacting protein with forkhead-associated domain, family member B). None of these genes has been associated with human hearing loss previously, but there have been reports that *Xenopus ngn1* (neurogenin-1) can convert ectodermal cells to neurons and that mouse embryos lacking *ngn1* fail to generate the neuronal precursors for proximal cranial sensory ganglia (Ma et al. 1998). Moreover, *Ngn1*-null mice showed an abnormal inner ear phenotype and lacked differentiated inner ear primary neurons (Ma et al. 2000). DCNP1 and TIFAB are not likely candidate genes for hearing loss. TIFAB is highly expressed in the spleen and inhibits TRAF6-induced cellular functions such as B cell maturation and maturation of dendritic cells and macrophages, and DCNP1 is thought to function in the hypothalamus in stress response and in the pathogenesis of depressive disorders. Findings indicate that NEUROG1 gene deletions could be another cause of profound congenital hearing loss and Mondini malformation in humans. NEUROG1 maps to the DFNB60 locus for congenital autosomal recessive hearing loss on 5q22-q31, but DFNB60 has been associated with non-syndromic hearing loss (M. Hildebrand and R. Smith, personal communication) and encompasses a large area (5q22-q31; 35 Mb, >100 genes). It is therefore likely that the DFNB60 locus is heterogeneous with more than one gene involved.

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## P-ClinG-006

### De novo microdeletion 6q14.1-q14.3 in a boy with developmental delay and dysmorphism

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Proximal interstitial 6q deletions have emerged as a characteristic microdeletion syndrome, characterized by mental retardation and a distinct pattern of minor anomalies, including upslanted palpebral fissures with epicanthal folds, a short nose with broad nasal tip, anteverted nares, long philtrum, and thin upper lip. Additionally, clinodactyly, single palmar creases, joint instability, hypermobility, umbilical hernia, cardiac anomalies, and other anomalies were noted in some cases (Kumar et al. 1997).

We report on a one-year-old boy presenting with developmental delay and a characteristic dysmorphism (including upslanted palpebral fissures with epicanthal folds, anteverted nares, thin upper lip, single palmar creases, clinodactyly). Molecular karyotyping using Array-CGH analysis revealed a de novo 8.9 Mb deletion in 6q14.1-q14.3, as confirmed by FISH-analysis, and a paternal 120 kb deletion in 1p34.1.

Most cases with proximal interstitial 6q deletions described in the literature were detected by classical cytogenetic analysis. To the best of our knowledge microarray data are only available for six cases with an interstitial 6q microdeletion and a similar phenotype; one case report with an interstitial deletion in 6q13-q14.1 (Lespinasse et al. 2009) and five cases in the DECIPHER Database. Taking these microarray data into account, the smallest region of overlap could be narrowed down to a 3.3 Mb region in 6q14.1 containing 10 genes. However, two DECIPHER cases lying at the centromeric and telomeric ends of the 3.3 Mb region, respectively, do not cover the entire region. Further investigations are necessary to nominate candidate genes.

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## P-ClinG-007

### Was sind die therapeutischen Konsequenzen des genetischen Familienscreenings bei gesicherten hereditären primären Arrhythmiesyndromen

Beckmann BM.<sup>1</sup>, Greil S.<sup>2</sup>, Dalla-Pozza R.<sup>2</sup>, Vill K.<sup>2</sup>, Ulbrich M.<sup>1</sup>, Martens E.<sup>1</sup>, Clauss S.<sup>1</sup>, Netz H.<sup>2</sup>, Käab S.<sup>1</sup>

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Einleitung und Hintergrund: Die häufigsten hereditären Arrhythmiesyndrome sind das Long QT-Syndrom (LQTS), das Brugada-Syndrom (BrS) oder die katecholaminerge polymorphe ventrikulärer Tachykardie (CPVT). In 26% (BrS) bis zu 70% (LQTS) wird beim Indexpatienten derzeit eine ursächliche Mutation gefunden. Bei familiär bekannter Mutation wird ein genetisches Angehörigenscreening möglich. Untersucht wurden die therapeutischen Konsequenzen dieser Untersuchungen bei Angehörigen, die sich in unserer Spezialambulanz für angeborene Arrhythmiesyndrome vorstellten.

Ergebnisse: Zwischen 1/2000 und 10/2010 wurden 324 Angehörige genetisch untersucht (LQTS: KCNQ1, KCNH2, SCN5A, KCNE1 und – E2; BrS: SCN5A, CPVT: RYR2). Bei 158 Angehörigen von Patienten mit LQTS, BrS oder CPVT aus 103 Familien mit einer pathogenen Mutation ebenfalls die familiär bekannte Mutation gefunden. Das mittlere Alter der Angehörigen betrug zum Zeitpunkt der Diagnosestellung 28±20 (0-84) Jahre. Von diesen Angehörigen hatten vor Diagnosestellung bereits n=27 (17%) Symptome im Sinne von Palpitationen, Synkopen, Krampfanfällen oder einem Herz-Kreislaufstillstand in Unkenntnis der Ursache erlitten, bei n=2 konnte die Diagnose erst postmortem nach plötzlichem Herztod gesichert werden. Das Ergebnis der genetischen Untersuchung führte bei n= 114 Angehörigen zur Einleitung einer Therapie. Gründe für Nicht-Einleitung einer Therapie waren: Ablehnung durch Patient: n=5; LQTS 3 (n=18) oder LQTS7 (n=3), hier wurde aufgrund des unklaren therapeutischen Nutzens keine Betablockertherapie eingeleitet; verstorben: n=2. Keine Wiedervorstellung erfolgte bei n=13. Die mittlere QTc bei den betroffenen LQTS-Patienten betrug 438±25 ms (Median 439 ms) und befand sich somit bei den meisten Mutationsträgern im Normbereich, nur einer der Angehörigen von Patienten mit BrS zeigte ein spontanes, BrS-verdächtiges EKG; mindestens 2 der RYR2-Mutationsträger zeigten ein unauffälliges Belastungs-EKG.

Schlussfolgerung: Die genetische Untersuchung von potentiell betroffenen Angehörigen von Patienten mit gesichertem LQTS, BrS oder CPVT führt in einem hohem Prozentsatz auch vor Vorliegen von Symptomen oder EKG-Auffälligkeiten zur unmittelbaren Einleitung einer prophylaktischen Therapie. Die klinische Diagnostik wäre in den meisten Fällen nicht wegweisend. Für Patienten mit LQTS 1,2,5,6 sowie CPVT wird derzeit grundsätzlich eine Therapie mit einem Betablocker empfohlen, zusätzlich zu den ebenso wichtigen allgemeinen Vorsichtsmaßnahmen; bei BrS und LQTS3 ist die Betreuung schwieriger und die adäquate Therapie muss im Einzelfall individuell abgewogen werden. Allen Patienten werden regelmässige Kontrolluntersuchungen mit aktueller Risikostratifizierung und Beratung angeboten, und die Betroffenen erhalten eine stets überarbeitete Liste mit zu meidenden Medikamenten. Bei adäquater Behandlung ist die Prognose dieser sich ansonsten häufig früh manifestierender Erkrankungen gut.

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## P-ClinG-008

### Familial Reciprocal Translocation t(7;13) Associated with Maternal Uniparental Disomy 7 in a Silver-Russel-Patient

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#### Background

Silver-Russel syndrome (SRS) is a genetically heterogeneous disorder characterized by intrauterine and postnatal growth retardation, typical facial features such as a triangular face and prominent forehead and a spectrum of additional features including body and limb asymmetry and clinodactyly. Maternal uniparental disomy for chromosome 7 (mUPD7) was shown to occur in 5-15% of SRS patients. Maternal UPD7 is clinically often associated with mild SRS and the recurrence risk is considered low as almost all cases with UPD7 have been sporadic so far. In general, chromosomal rearrangements like translocations increase the likelihood of UPD for the chromosomes involved. However, SRS as the result of a mUPD7 due to an inherited chromosomal translocation involving chromosome 7 has only been reported once before. Here, we describe the second case of SRS with mUPD7 due to a familial reciprocal translocation t(7;13).

#### Clinical Report

The boy we describe is the first child of healthy non-consanguineous parents following three miscarriages. He was born at 34 gestational weeks by elective caesarean due to severe intrauterine growth retardation. At the age of 2 years, he presented with severe feeding difficulties, height and weight below the 3rd percentile, speech delay, mild facial dysmorphism including triangular face, prominent forehead and relative macrocephaly.

#### Genetic Investigations

Molecular testing for UPD7 revealed a maternal UPD7 by indicating the presence of both maternal alleles at D7S493, D7S628, D7S519, CFTR1124/1125, D7S517, D7S513, D7S507 and D7S657. With regard to the recurrent miscarriages in the past, a chromosome analysis of the parents was performed. GTG banding of the mother's metaphases showed a balanced translocation t(7;13)(q11.2;q14). Subsequently, the translocation was shown in her son with SRS as well. Fluorescence in situ hybridization (FISH) confirmed these results.

#### Discussion and Conclusion

This is the second report of a child with SRS and mUPD7 and an inherited reciprocal translocation involving chromosome 7. The cytogenetic data turned out to be of great importance for genetic counseling of the parents as well as their extended family who would have otherwise been told that the recurrence risk for UPD7 is <1%. Based on the presented results, the carrier mother has a risk for further children with SRS due to mUPD7 and a highly increased risk for miscarriages or offspring with an unbalanced translocation. An unbalanced karyotype with a deletion or duplication of 13q14-qter is expected to result in a severe clinical phenotype with profound mental retardation and various malformations. Live born children born with deletions or duplications of 7q11.2-qter have not been described before.

In summary, our report emphasizes the relevance of chromosomal analysis in SRS patients with mUPD7 to rule out chromosomal rearrangements despite their rare occurrence in SRS.

In summary, our report emphasizes the relevance of chromosomal analysis in SRS patients with mUPD7 to rule out chromosomal rearrangements despite their rare occurrence in SRS.

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#### P-ClinG-009

### **A de novo 1.1 Mb microdeletion of chromosome 19p13.11 narrows down the candidate region for split hand split foot malformation and tetralogy of Fallot to EPS15L1 and CALR3**

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We describe a 3.5 year old girl presenting with short stature, developmental delay, marked muscular hypotonia with ataxia, premature pubarche and dysmorphic features.

Cytogenetic analysis performed on the patient's peripheral blood lymphocytes revealed an apparently normal female karyotype, 46,XX, after GTG-banding (400-550 band resolution). Further analysis using the Human Genome CGH Microarray 105A platform revealed a deletion which was found to span approximately 1.1 Mb of chromosome 19p13.11 with the most telomeric deleted probe starting at chr19:16,485,524bp, and the most centromeric deleted probe ending at chr19:17,554,950bp. FISH analysis on the parents' PHA-stimulated blood lymphocytes using the BAC RP11-413M18 mapping to the deleted region showed two signals thereby confirming the deletion in their daughter to have occurred de novo.

The approximately 1.1 Mb-sized de novo microdeletion of chromosome 19p13.11 is most likely the cause for the clinical phenotype. One patient has been previously reported with an overlapping deletion in the same region. In contrast to the patient described here, this patient presented with additional clinical characteristics, i.e. tetralogy of Fallot and split hand and feet. The comparison of deleted regions in the case presented here with the one previously reported revealed only two genes, EPS15L1 and CALR3, which are not lost in the present case. As EPS15L1 has been associated with limb development previously, the presented case provides indirect evidence that this may be a new candidate gene for split hand and foot malformation (SHFM).

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## P-ClinG-010

### **Screening of the total coding region for Malignant Hyperthermia and Central Core Disease causative mutations in a cohort consisting of German, Danish and Swedish MHS patients with High Resolution Melting**

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In Malignant Hyperthermia (MH) susceptible (MHS) individuals, volatile anaesthetics and/or succinylcholine can induce a severe decompensation of muscle calcium homeostasis leading to a life threatening crisis. Central core disease (CCD) is a rare dominantly inherited congenital myopathy allelic to MHS with a highly variable clinical presentation. Patients with CCD have shown higher risk to present an MH crisis during inhalational anaesthesia.

The diagnosis of MHS is traditionally made by an in vitro contraction test (IVCT) where biopsies from musculus quadriceps are exposed to halothane and caffeine. From the result, a patient can be classified as MH-susceptible (MHS) or MH-negative (MHN). Both MH and CCD are caused by mutations in the ryanodine receptor RYR1 which is encoded by the RYR1 gene. More than 200 sequence variants have been identified and linked to MHS, CCD and other neuromuscular disorders. Due to its non-invasivity, genetic diagnostic is of course favoured over IVCT but it is hampered of the large laboratory work load and costs of screening the large RYR1 gene by sequencing, especially taking into account the fact that the de novo mutation rate is high. In addition, lack of a mutation does not entitle the diagnosis MHN.

In the present study we have applied the High Resolution Melting (HRM) technique for screening all 106 exons of RYR1. Exons with a high probability for mutation or polymorphism – as indicated by an altered melting pattern – were then further analysed by sequencing. The aim was to significantly reduce the laboratory work load and costs and still to cover the total RYR1 coding region. A cohort consisting of five German, five Danish and six Swedish MHS patients with high scores in the IVCT was created. The patients have either experienced a life-threatening MH reaction themselves or are relatives (twin or child) to a patient who has experienced a lethal MH reaction or have been IVC tested due to clinical suspicion of muscle disease.

In the study cohort 194 amplicons of 2520 (7,7 %) were identified by a significantly altered melting pattern in the evaluation of the HRM curves. By sequencing 16 sequence variants - known MH causative mutations or previously unknown amino acid changes - were found in 13 of 16 patients (81 %). A panel of 98 German, 98 Danish and 70 Swedish DNA samples from clinically healthy subjects chosen by random is currently used to assess the prevalence of the candidate mutations in the normal population. In addition, the impact of the mutations on protein function in cell-based assays to determine the causality of each mutation for MH is analysed.

As conclusion, HRM proved to be fast and cost effective for mutation screening in a very large gene as the RYR1. In a majority of the patients at least one mutation in RYR1 could be found, indicating a strong correlation of MHS to RYR1 in patients with high IVCT scores.

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## P-ClinG-011

### **Hearing loss DFNB49 in a Czech Gypsy family usefulness of homozygosity mapping on SNP chips.**

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Non-syndromic hearing loss is genetically extremely heterogenous, but in vast majority of cases follows autosomal recessive (AR) inheritance. Mutations in the GJB2 gene are the most common cause of AR non-syndromic hearing loss named as DFNB.

Roma Gypsy population is known to have a higher risk for AR diseases.

We used homozygosity mapping in a Czech Gypsy family with a deaf child and a known consanguinity of parents where mutations in the GJB2 gene were previously excluded.

The genomic DNA samples from the affected patient, his both parents and unaffected sister were hybridized on the Affymetrix GeneChip Human Mapping 250K Nspl arrays. Data were analysed in the Affymetrix software - Genotyping Console. We searched for homozygous segments in the affected patient

which were heterozygous in the parents. Homozygous segments of 2Mb size or larger were selected for further analysis.

The 18 homozygous regions of 2Mb size or larger were found in the patient and screened for the genes connected with the non-syndromic hearing loss (DFNB loci). In the largest homozygous region (9 Mb) only one gene related to hearing loss - the *Marveld2*, was found. Sequencing of all 6 coding exons and flanking intron parts of *Marveld 2* gene revealed the splice site mutation IVS 4+2 T-C. This mutation was found in the homozygous state only in the patient, the parents and patient's sister were heterozygous.

The same mutation IVS4+2 T-C was reported in 5 Pakistani families with nonsyndromic hearing loss in the recent original description of DFNB49 causal gene. This is probably the first case of DFNB49 in the Gypsy population.

Our finding may have important implications for further Gypsy patients and families with non-syndromic hearing loss. Homozygosity mapping using SNP chips followed by sequencing of candidate genes within the largest homozygous intervals may be a powerful tool for finding causal mutations in Gypsy families.

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## P-ClinG-012

### Clinical phenotype and novel mutations in *Alsin* related motorneuron disease

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*Alsin* (*ALS2*) gene mutations have been described to cause primitive, retrograde degeneration of the upper motor neurons of the pyramidal tracts, resulting in a clinical continuum from infantile ascending hereditary spastic paraplegia (IAHSP) to juvenile onset forms without or with lower motor neuron involvement (JPLS; juvenile primary lateral sclerosis or JALS; juvenile amyotrophic lateral sclerosis).

We have recruited a cohort of 11 patients from 7 Turkish families with mainly IAHSP phenotype and have performed genomic sequencing of *ALS2* after linkage to its locus.

Our study unveiled five novel and one known pathogenic mutations in the *ALS2* gene in six families (6/7). The age range at clinical assessment was 5-20 years. The disease began mostly with delayed walking around 2 years of age accompanied by tip-toe walking. Interestingly, one patient was reported to be floppy shortly after birth and another one never achieved independent walking. All patients had developed upper limb involvement during disease course, although weakness and spasticity were more prominent in the lower limbs with clear associated pyramidal signs. Severe dysarthria or even anarthria was a common feature in the first decade of life, accompanied by facial weakness and pronounced dysphagia. Cerebral MRI and EEG were normal in all but one patient, who had epilepsy.

*Alsin* functions as guanine nucleotide exchange factor and it is involved in endosomal dynamics. So far fourteen mutations have been reported in *ALS2*, being mostly small indels. Here, we report a splice site mutation (IVS5+1G>A), three missense mutations (p.Cys157Tyr, p. Ala573Glu and p.Leu1054Pro), one nonsense mutation (p.Tyr348X) and one insertion (c.4574insG), all in homozygous state. There was no obvious genotype-phenotype correlation. Our study indicates a quite high detection rate of *Alsin* mutations in clinically well characterized infantile onset motorneuron disease. Genetic investigations could provide accurate diagnosis for patients and families; additionally contribute to better understanding of the pathophysiology of HSP, PLS and ALS.

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## P-ClinG-013

### Toriello-Carey Syndrome in a female patient

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Toriello-Carey syndrome (TCS; OMIM 217980) is a multiple congenital anomaly syndrome characterized by the common manifestations of corpus callosum agenesis, cardiac defects, cleft palate/Robin sequence, muscular hypotonia, mental retardation, postnatal growth retardation and distinctive facial dysmorphism (including micrognathia, telecanthus, small nose and full cheeks). In order to increase awareness of this rare condition and to broaden the phenotypic spectrum, we would like to present a patient with TCS.

We are presenting a case of a four year old girl with multiple congenital anomalies: anal atresia, complex cardiac malformations, malformation of two vertebral bodies and hypoplastic corpus callosum. She was born

after an uneventful pregnancy with birth weight and length within a low normal range. Psychomotor retardation and muscular hypotonia were noted from the beginning. She developed epilepsy, hyperopia and bilateral deafness.

When she first presented to the outpatient clinic at the age of four, she tried to interact nonverbally, reached a ventral position, but was not able to sit unsupported or crawl. She had no speech yet. The girl had marked muscular hypotonia and bilateral hyperopia (+4 dpt). Both her weight and length were below the third centile. She was microcephalic (OFC 47 cm), and her face was characterized by a flat profile, short palpebral fissures (18 mm), and a small nose with hypoplastic nasal alae. Additionally, a high and narrow palate and scoliosis were noted.

Routine Chromosome analysis and Affymetrix 500K SNP array analysis gave normal results.

TCS was first described by Toriello HV and Carey JC in 1988. So far, more than 40 patients with TCS have been reported. The underlying gene is unknown, thought to be possibly autosomal recessive. The core phenotype consists of developmental delay, telecanthus/hypertelorism and/or short palpebral fissures, small nose, micrognathia, palatal abnormalities, corpus callosum anomalies, cardiovascular anomalies, postnatal growth retardation, hypotonia, with additional features present in a minority of cases.

We believe that the manifestations of our patient are consistent with a diagnosis of TCS, and in particular the facial gestalt is very suggestive. Dr. John Carey agreed with this clinical diagnosis. Epileptic seizures as seen in our patient are uncommon in TCS but some patients are reported to suffer from seizures. Besides clinical findings typical for this condition, our patient showed additional traits expanding further the phenotypic spectrum: vertebral and anal malformations are not typical for TCS, and the patients described so far had less severe mental retardation than our patient.

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## P-ClinG-014

### **Computational Methods for the Study of Human Disease Manifestations: Decomposing the puzzle of copy number variations in human disease**

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In genetic diseases caused by copy number variations (CNV) such as microdeletions or microduplications clinicians often struggle with the concern whether the observed CNV is pathogenic or a benign variant. In case of pathogenicity the question arises which of the genes included in the CNV might be causative for which of the phenotypic abnormalities of the patient. Databases such as DECIPHER (<https://decipher.sanger.ac.uk/>) which catalogue CNVs as well as clinical data, present invaluable help when interpreting CNVs.

In the past computational analysis of phenotypic as well as cytogenetic and molecular genetic data from databases such as DECIPHER has been hampered by the fact that most databases either used their own small sets of vocabularies or unstructured pre-existing ones such as the London Dysmorphology Database (LDDb) terms for describing phenotypes. The Human Phenotype Ontology (HPO) developed by our group provides a detailed standardised vocabulary for the description of human phenotypes. In cooperation with DECIPHER we created a mapping between the LDDb vocabulary and the HPO that will allow DECIPHER phenotypes to be compared to the phenotypes of monogenic diseases described in OMIM, for which to date over 50,000 HPO annotations are available.

We have developed computer readable logical definitions that link the human phenotype to anatomy, gene ontology, cell types and small molecules and allows the computer to "understand" the semantics of phenotypic abnormalities seen in human hereditary disease. Similar definitions have been developed by collaborating groups for mouse and zebrafish phenotypes thereby providing the foundation for a computational comparison of human and model organisms. In 2010 the International Mouse Phenotyping Consortium (IMPC) started the project to identify the function of every gene in the mouse genome by performing systematic phenotypic screens. Phenotype ontologies and computational methods of interspecies comparisons will enable us to harness this data for improving our understanding and knowledge about human disease.

Here we will demonstrate the utility of our approach by using interspecies phenotypic comparisons in order to identify previously unrecognized genotype-phenotype associations for rare as well as common CNV disorders. For common CNV disorders described in the DECIPHER database we successfully reproduced the already known genotype-phenotype correlations and uncovered significant additional information about

previously unrecognised genotype-phenotype associations. For example, Williams syndrome is annotated to 94 HPO terms representing phenotypic abnormalities. Some of these features are identical with features of single-gene disorders of genes contained within the microdeletion, for instance, supraaortic stenosis can be observed in Williams syndrome and also in patients with ELN point mutations. Automatic computational mapping identified 16 features previously postulated in the literature, and additionally identified candidate genes for another 15 features in ELN, BAZ1B, MLXIPL, LIMK1, NCF1, GTF2IRD1, FKBP6 and FZD9. We will present current results and concepts demonstrating the advantages and possibilities of a computational analysis of human and mouse phenotypes for our understanding of CNV disorders.

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## P-ClinG-015

### Refinement of the interstitial microdeletion in chromosome 6q25 associated with mental retardation

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Interstitial deletions of chromosome 6q are very rare findings. Deletions range from 0.3 Mb to several megabases spanning multiple chromosomal bands. Some associated clinical features are commonly seen including mental retardation, hypotonia and facial dysmorphism, while others like microcephaly, brain malformation, micrognathia, hearing loss, hernia and heart malformation are only seen in some cases. The high clinical variability is explained by the large divergence in the overlap and gene coverage of the deletions.

We report on a 28 month old infant with normal female karyotype, mental retardation (MR), facial dysmorphism, strabismus alternans, plagiocephaly, hyperopy and body measurements on 10th centile. After exclusion of known aberrations with various commercial MLPA-kits, Angelman Syndrome with methylation specific PCR and Rett Syndrome (MECP2 point mutations) we conducted a genome-wide analysis of copy number variation (CNV). We performed molecular karyotyping in the patient and both parents with high density Affymetrix Genome-Wide Human SNP 6.0 microarrays.

In addition to a paternally inherited 121 kb duplication on chromosome 12 we detected a de novo deletion of 2.3 Mb on chromosome 6q25. This deletion encompasses only 5 known genes and lies within the previously reported deletion region. We hypothesize that haploinsufficiency for a gene within the 2.3 Mb deleted region may impair normal development. In order to identify the phenocritical gene we are screening these five positional candidates for point mutations in 144 patients with moderate to severe MR from our MRNET-cohort.

To date we found a sequence alteration in one of the genes, highly expressed in brain. One patient of our screening cohort showed an exonic 11 bp deletion leading to a frameshift resulting in a premature stop codon, which would shorten the protein by 58 amino acids. Sequencing of both parents to prove de novo origin of the mutation is ongoing as well as mutation analysis in further patients and controls.

This study is part of the German Mental Retardation Network ([www.german-mrnet.de](http://www.german-mrnet.de)).

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## P-ClinG-016

### Paroxysmal Tonic Upgaze in a boy with 7p14.1 deletion

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Paroxysmal tonic upgaze (upward eye deviation) with ataxia (Omim 168885) was first described by Ouvrir and Billson in 1988 (J. Child Neurol.3: 177-180) as a benign syndrome with favourable long-term outcome and no apparent neurologic sequelae. Until now the etiology of this clinical distinct feature is not clear and further case reports showed that abnormal neurologic findings, learning disabilities and mental retardation can be associated. To our knowledge only three cases have been described so far with an underlying genetic disorder including a child with Beckwith-Wiedemann syndrome, a boy with partial tetrasomy 15q and a family with CACNA1A mutation.

We report on the clinical and molecular characterisation of a patient with a de novo 7p14.1 deletion. Paroxysmal tonic upgaze in this 8-year old boy started at the age of 3.5 months. The episodes of upward eye deviation usually become apparent in the morning after awaking and last approximately 1.5 hours. Diagnostic evaluation included normal cranial MRT, EEG and ophthalmologic examination. With the exception of megaureter treated by ureterocystostomy, he had no further organ malformations. Body measurements were within normal ranges and he showed no significant dysmorphic signs. Intelligence

testing revealed mild mental retardation. Ataxia and dysarthria were apparent from childhood on. The family history was unremarkable.

A 7p14.1 deletion was detected by whole genome microarray analyses (Agilent 44k Oligo-array and Affymetrix 6.0 SNP-array). The size of the 7p loss could be defined as approximately 640 kb containing the genes CDC2L5 and C7orf10. Parental chromosomes and quantitative PCR results for 7p14.1 were normal. Searching for similar reports revealed no comparable cases.

We suppose that paroxysmal benign tonic upgaze is not a distinct entity, but a clinical feature which can be attributed to very different causes. Although in the majority of cases the clinical outcome is favourable, the prognosis for patients with underlying chromosomal defects remains to be seen.

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## **P-ClinG-017**

### **An unusual manifestation of autosomal dominant Dravet syndrome**

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**Background:** Dravet syndrome or severe myoclonus epilepsy of infancy (SMEI) is a severe form of epilepsy which is often refractory to medication. It is characterized by generalized tonic-clonic seizures beginning in the first year of life and often triggered by fever. After an initial normal psychomotor development, cognitive functions become progressively delayed in the second year of life. The disease is often associated with ataxia or hypotonia. Heterozygous mutations in the voltage-gated sodium channel SCN1A gene are the main genetic cause of Dravet syndrome. More than 150 mutations have been identified which mostly arise de novo.

**Case report:** We describe a family with at least 2 half-sibs affected by Dravet syndrome who had the same father but at least two different mothers. The affected children were mentally retarded (no active speech) and had epilepsy from early infancy. The father was known to have had grand-mal attacks in his childhood associated with episodes of tonsillitis and was free of attacks since his 14th year of life. His mental function was entirely normal. The familial clustering of Dravet syndrome in our family could only be explained by an autosomal dominant inherited disorder with variable expression or somatic mosaicism in the father. Molecular genetic analysis of the SCN1A gene revealed a heterozygous deletion of the exons 18 and 19 in one affected daughter and in the father while the other patients remain to be investigated.

**Discussion:** Epilepsies which are caused by mutations of the SCN1A gene are associated with a wide phenotypic variability including moderately affected to syndromal patients. An explanation for the intrafamilial phenotypic variability could be the involvement of multiple genetic and environmental factors. Genetic analysis of the SCN1A gene should therefore not only be considered in classical Dravet syndrome but also in families suggestive of autosomal dominant epilepsy, in particular if mental impairment is present and if seizures are triggered by infections.

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## **P-ClinG-018**

### **Hepatoblastoma in infant sister and brother: Could APC gene screening be useful?**

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#### **Background**

Hepatoblastoma is the most common liver tumour in childhood and occurs more frequently in various genetic diseases including familial adenomatous polyposis (FAP), chromosomal aberrations, glycogen storage diseases and Beckwith-Wiedemann-Syndrome. FAP is a colon cancer predisposition syndrome in which hundreds to thousands of polyps of the colon and rectum develop and without surgical treatment colon cancer is inevitable. Germline mutations in the APC gene are found in most patients with classical FAP. However, it is still controversial whether APC gene screening is beneficial in cases of childhood hepatoblastoma without a family history of FAP. We describe a family with hepatoblastoma in two siblings, which later turned out to be obvious the first manifestation of a FAP.

#### **Clinical Report and Genetic Findings**

The first child of healthy parents, a girl, was diagnosed with metastatic hepatoblastoma at the age of 3 ½ years in 1987 and died shortly thereafter. Eight months later the second child was born. He developed a hepatoblastoma at the age of 17 months and was treated with partial hepatic resection followed by chemotherapy with ifosfamid, cisplatin and adriamycin. Genetic counselling of the parents was performed. Chromosomal analysis of the patient and both parents was normal. Family history did not show evidence for FAP or any genetic disease associated with hepatoblastoma. More than 17 years after the hepatoblastoma

in the patient had been successfully treated, he presented with rectal bleeding. Multiple colorectal polyposis and an advanced rectal carcinoma were found. Colonoscopy of both parents was normal. The patient underwent total proctocolectomy with ileoanal anastomosis after neoadjuvant chemotherapy. Now, two years later, he remains in complete remission. Mutation analysis of the APC gene of the patient showed the frameshift mutation c.3183\_3187delACAAA in the patient's blood sample. Molecular genetic testing of a DNA sample isolated from the hepatic tumour tissue of his sister is pending.

#### Discussion

The clinical association between hepatoblastoma and FAP is well established. In FAP families with hepatoblastoma most mutations are found in the 5' end of the APC gene. However, a negative family history for FAP in a child with hepatoblastoma does not exclude an APC mutation, because of the age dependent penetrance and high de novo mutation rate of ~20%. The frequency of APC germline mutations in hepatoblastoma without family history of FAP is still unclear and ranges from 0% to 10%. There are no standardized guidelines for diagnostic testing in these cases. As screening for APC mutations in patients with hepatoblastoma could allow early diagnosis of FAP and early colorectal surveillance in the patients and family members at risk, testing should be considered even in suspected sporadic hepatoblastoma cases. In families in which more than one child develops hepatoblastoma, systematic screening of the APC gene may be appropriate. Had our patient's older sister or the patient himself been tested for APC mutations in childhood, the patients advanced colorectal cancer very likely would have been prevented.

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#### P-ClinG-019

### **Alström syndrome: An example for efficient genetic testing in cilia-related disorders (ciliopathies)**

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Ciliary dysfunction has been shown to underlie a broad range of clinically and genetically heterogeneous phenotypes, the so-called ciliopathies. Literally all organs can be affected, frequent cilia-related manifestations are cystic kidney disease, retinal degeneration, deafness, skeletal disorders, and brain malformations, occurring either isolated or as part of syndromes. While there are some overlaps between different ciliopathies, genotype-phenotype correlations allow for time- and cost-efficient targeted testing approaches. We demonstrate this in two patients with Alström syndrome in whom we were able to identify loss-of-function mutations in the ALMS1 gene on chromosome 2p13. The phenotype in Alström patients overlaps with Bardet-Biedl syndrome and typically shows obesity, retinal dystrophy, sensorineural hearing loss, and endocrinological features, such as e. g., hypogonadism, diabetes mellitus, hypothyroidism, and hyperlipidemia. Other features that may point to the correct diagnosis are dilated cardiomyopathy and progressive pulmonary, hepatic and renal failure. Importantly, most patients have normal intelligence. Here we present our data on Alström syndrome and an algorithm for efficient genetic testing approaches in a wide range of ciliopathies.

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#### P-ClinG-020

### **Breakpoint characterization and genotype-phenotype correlation in a patient with an unbalanced 46,XX,der(7)t(7;17)(q36;p13)**

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Molecular karyotyping by DNA array analysis has improved the characterization of cryptic chromosome rearrangements as well as their genotype-phenotype correlation. Here, we report on a 16-year old girl with severe mental retardation and dysmorphic features. Pregnancy was characterized by intrauterine growth retardation and oligohydramnios. The child was born at 39 weeks of gestation. Birth measurements were below 3rd centile. From the beginning, growth and global development were greatly delayed. At the age of 16 years height (110 cm), weight (16 kg), and occipito-frontal head circumference (45.5 cm) were still below 3rd centile. A hiatus hernia, agenesis of the corpus callosum, a cervical rib, a tethered cord, a lipoma in the spinal canal, coccyx hypoplasia, and scoliosis were noted. Multiple intestinal obstructions were treated surgically. Dysmorphic features included a narrow forehead, down-slanting palpebral fissures, ptosis (right > left), epicanthus (right), a slightly beaked nose, a broad nasal bridge, small nostrils, prognathism, short phalanges with poorly developed nails, and bilateral minimal 2-4 syndactyly of the toes.

Subtelomere screening and SNP microarray analysis (Illumina HumanOmni1-Quad v1.0) revealed a 46,XX,der(7)t(7;17)(q36;p13) karyotype and defined the 17p breakpoint between rs6503076 and rs2304908

and the 7q breakpoint between rs11543936 and rs2316539. The 17p duplication has a size of about 8 Mb and the 7q deletion a size of about 4.4 Mb including MNX1, the gene for sacro-coccygeal malformations and Currarino syndrome.

Review of the literature indicated that the major anomalies of the patient such as the sacro-coccygeal malformations, the lipoma of the conus, the tethered cord, or the agenesis of the corpus callosum are the typical features of del(7)(q36.1-qter) whereas only few anomalies, like the hiatus hernia and some of the facial dysmorphisms have been described for dup(17)(pter-p13).

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#### **P-ClinG-021**

### **A complex chromosome rearrangement with mosaic deletion 1p13.1-p13.2 and triplication 1p13.3 in a female patient with developmental delay, microcephaly, short stature, Peters' anomaly, heart defect, cleft palate and dysmorphism**

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**Patient report:** We report on a female patient born at 39 3/7 weeks of gestation with low birth weight, microcephaly (OFC 31 cm, 1.5 cm < 3rd centile), a cleft palate and a ventricular septal defect, which closed spontaneously during the first weeks of life. She was found to have a Peters' anomaly, strabism, and hypertelorism. On examination at the age of 11 months, she showed developmental delay and her body measurements were still below the 3rd centile. Her facial phenotype showed overlapping features with Peters'-Plus Syndrome: a round face, hypertelorism, long philtrum and small upper lip. We decided to look for chromosome aberrations and to exclude Peters'-Plus Syndrome.

**Methods:** Following routine chromosome analysis we performed SNP-array analysis (Affymetrix® Cytogenetics Whole-Genome 2.7M SNP-Array) to identify a chromosomal imbalance and fluorescence in situ hybridization for further characterization. Molecular genetic analysis of Peters'-Plus Syndrome was performed by direct sequencing of all coding regions and the adjacent intronic regions of the B3GALTL gene and quantitative RealTime PCR to exclude larger deletions.

**Results:** Chromosome analysis showed two de novo translocations, one between chromosome 1 and 15, and the second between chromosome 1 and 12. The karyotype was designated as 45,XX, der(1)t(1;15)(p11.1;q11.1),der(12)t(1;12)(p11.1or13.3;q24.1or24.33),-15. FISH analyses using chromosome specific painting probes confirmed the two translocation chromosomes der(1)t(1;15) and der(12)t(1;12) in all 28 metaphases examined. Using molecular karyotyping, chromosomal imbalances could be detected only in the breakpoint region of chromosome 1 with a de novo deletion of about 4 Mb in region 1p13.1-p13.2 followed by a triplication 1p13.3 of 1.2 Mb. However, both imbalances could only be confirmed in about 57% of the analyzed metaphases by FISH with locus-specific BAC clones. Another cell clone (about 18%) showed signals indicating a balanced situation and a third cell clone (about 25%) showed a complete deletion of about 5.2 Mb of the whole region 1p13.1-p13.3, indicating a mosaic situation. There are 103 genes within this whole region. The VANGL1 gene lies within the deleted region and is associated with neural tube defects.

No mutations in the B3GALTL gene could be detected.

**Conclusion:** We suggest that it is very likely that the complex chromosome rearrangement is the cause of the observed clinical findings in this female patient. The genes responsible may either be situated in the observed deleted / triplicated regions or be disrupted at the described chromosomal breakpoints.

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#### **P-ClinG-022**

### **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.

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## P-ClinG-023

### Nonrandom distribution of JAG1 mutations in patients with Alagille syndrome

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Background: Alagille syndrome (ALGS) is an autosomal dominant multisystemic disorder with extremely varying severity. Main symptoms are chronic cholestasis, congenital heart anomaly, skeletal defects, eye and kidney abnormalities and a characteristic face. Penetrance and expressivity were found to be extremely variable. Approx. 80% of the cases are associated with mutations of Jagged 1 (JAG1: +601920, ALGS1: #118450), some few cases are associated with NOTCH2 (\*600275) mutations (ALGS2: #610205). Whether there are clusters of mutations within the JAG1 gene or preferences for any mutation type has been debated, yet. In the study presented we analysed the feature of JAG1 mutations in a sample of JAG- mutation carriers.

Patients and methods: As a result of a stepwise mutation screening (microsatellite analysis, cyclic sequencing, MLPA analysis) we investigated frequency and distribution of intragenic JAG1 mutations in a sample of 93 heterozygous JAG1-mutation carriers. All were unrelated and with varying clinical severity.

Results: All translated regions and the flanking splice-site motifs (AG, GT) of the JAG1 gene were studied. We found 30 cases with small deletions, 19 with insertions and 44 cases with point mutations altering the amino acid code. Only 7 mutations are recurring and were found in more than 1 patient. The most frequent mutation was a deletion of 4 base pairs within exon 17 (c.2122-2125delCAGT) detected in 6 unrelated patients. Our results also indicate three clusters of base pair exchanges, i.e. ~39% were located within exon 18 to exon 23, ~34% within exon 2 to exon 5 and ~ 16% within exon 9 to exon 13. In contrast to the wider range of base pair changes insertions and deletions were preferentially clustered in one region of the gene, insertions in exon 9 and deletions in exon 17. All these mutations affect either EGF-like domains significant for receptor-ligand interaction or the DSL domain (exon2-exon4), respectively. Long spanning genetic variations (insertions/ deletions of more than 10 base pairs) were only found at the terminal regions (exon 1, 2, 23, and 24).

Conclusion: Our data suggest a nonrandom distribution of mutations within the JAG1 gene with several clusters depending on the mutation type.

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## P-ClinG-024

### De novo MECP2 duplication in two females with mental retardation and random X-inactivation

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Xq28 duplications including MECP2 are a well known cause for severe mental retardation in males with encephalopathy, muscular hypotonia, progressive spasticity, seizures, poor speech and recurrent infections

that often lead to early death. Usually female carriers show a normal intellectual performance due to skewed X inactivation. We report on two mentally retarded female patients, seven and 19 years old, with a MECP2 duplication. Both patients have the de novo duplication occurring on the paternal allele and both show a random X-inactivation which can be assumed as the triggering factor for the phenotype. The phenotype appears to be unspecific with mild to moderate mental retardation and autistic features as well as recurrent infections in early childhood, constipation, and spastic hypertonia in early adulthood.

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#### **P-ClinG-025**

### **Schimke immuno-osseous dysplasia in a 2 year old German girl and a 5 year old Libanese boy with homozygous mutations in the SMARCAL 1 gene**

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#### Introduction:

Schimke immuno-osseous dysplasia (SIOD) (OMIM 242900) is a rare autosomal recessive syndrome which is known to result from mutations in the SMARCAL1 gene in about 50 to 60% in affected individuals. Main clinical features are spondyloepiphyseal dysplasia (SED) resulting in disproportionate short stature, steroid resistant nephropathy, and T-cell deficiency as well as cerebral ischaemia.

#### Case Reports:

Patient 1 is the first child of German parents who were grown up in different regions of Russia. Parental consanguinity was denied. However, the ancestors of both migrated from Germany to Russia in the times of Catherine the Great.

We identified in exon 17 of the SMARCAL1 gene an already described homozygous stop mutation c.2542G>T; p.Glu848X. This mutation was also verified in the heterozygous state in both parents as well as in the sister of the father.

Patient 2 is the fourth child of consanguineous Libanese parents. We identified in exon 11 of the SMARCAL1 gene an already described homozygous nonsense mutation c.1736C >A; p.S579X.

Early growth failure and mild signs of spondyloepiphyseal dysplasia as well as slightly retarded psychomotor development was evident in both patients. T-cell deficiency was evident in both patients in peripheral lymphoid organs. Patient 2 suffered from diffuse arteriosclerosis followed by multiple cerebral infarcts. He died by a severe B-cell lymphoma associated anemia.

Comment: We could verify the diagnosis in patient 1 in an very early stage, before onset of proteinuria-at the age of 2 9/12 years, the therapy with Enalapril (angiotensin converting enzyme inhibitor) was then initiated in order to diminish the proteinuria. Prenatal diagnosis is now available for both families.

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#### **P-ClinG-026**

### **Expanding the clinical spectrum associated with defects in CNTNAP2 und NRXN1**

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Heterozygous copy-number and missense variants in CNTNAP2 and NRXN1 have repeatedly been associated with a wide spectrum of neuropsychiatric disorders such as developmental language and autism spectrum disorders, epilepsy and schizophrenia. Recently, homozygous or compound heterozygous defects in either gene were reported as causative for a severe mental retardation disorder resembling Pitt-Hopkins syndrome.

We now screened 99 patients with severe mental retardation and resemblance to Pitt-Hopkins syndrome and/or suspected recessive inheritance for mutations in CNTNAP2 and NRXN1. Molecular karyotyping was

performed in 45 patients. In 8 further patients with variable mental retardation and heterozygous deletions in CNTNAP2 or NRXN1 the remaining allele was sequenced.

In the group of severely retarded patients we identified a heterozygous deletion in NRXN1 in one patient and heterozygous splice-site, frameshift and stop-mutations in CNTNAP2 in four patients, respectively. Neither in these patients nor in 8 patients with heterozygous deletions within NRXN1 or CNTNAP2 we could identify a defect on the second allele. One NRXN1 deletion occurred de novo, in another family the deletion was also identified in the mother who had learning difficulties, and in all other tested families one parent was shown to be healthy carrier of the respective deletion or mutation.

We therefore report on patients with heterozygous defects in CNTNAP2 or NRXN1 associated with severe mental retardation that was previously described to be caused by recessive defects in either gene. The large variability between severely affected patients and mildly affected or asymptomatic carrier parents suggest the presence of major modifiers, not necessarily located in the same gene.

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## P-ClinG-027

### **Nephrogenic syndrome of inappropriate antidiuresis caused by a gain-of-function mutation in the vasopressin V2 receptor gene**

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Nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is an X-linked disorder of water/electrolyte balance caused by an AVPR2 gain-of-function mutation that leads to constitutive activation of the encoded vasopressin V2 receptor. Defective urine dilution results in eu-/hypervolemic hyponatremia, a phenotype that resembles the syndrome of inappropriate antidiuretic hormone hypersecretion (SIADH).

We report a Turkish family with NSIAD. Index case was a six-month-old girl hospitalised for obstructive bronchitis. Initial serum sodium was 131 mmol/l, after intravenous fluid infusion it declined to 126 mmol/l. High amounts of NaCl (about 9 mmol/kg) had to be continuously substituted. Her low serum osmolality (273 mosmol/l) was in contrast to high urine osmolality (424 mosmol/l). After discharge, NaCl substitution was continued, first a salt wasting tubulopathy was supposed which was in contrast to euvolemic hydration status.

The girl's father had a hyponatremic seizure in infancy; a salt losing tubulopathy was supposed in him too. Hyponatremia recently re-occurred during surgery, but otherwise he gets along by drinking only very little. The father's brother also experienced recurrent hyponatremia, first observed at the age of seven months. At 13 years of age, he was hospitalised with recurrent episodes of hyponatremia (120 mmol/l) and pseudohypoaldosteronism was assumed and salt substitution started.

We suspected NSIAD in this family, sequenced the AVPR2 gene, and were able to identify the heterozygous missense mutation c.409C>T (p.Arg137Cys) in the proposita, subsequently shown to segregate with the phenotype in her father and uncle. This mutation has already been described in the literature and is one of the typical AVPR2 gain-of-function mutations leading to NSIAD. The patient's sister also carries the mutation, but is clinically fine most likely due to favourable X-inactivation of the mutant allele, recently corroborated by an unremarkable water loading test performed in her.

Given that patients benefit from NSIAD treatment with fluid restriction and osmotic diuresis with orally taken urea, an early diagnosis is helpful to protect them from episodes of severe hyponatremia. In conclusion, NSIAD should be considered in patients with recurrent or persistent eu- or hypervolemic hyponatremia and can be easily confirmed by sequencing of the small AVPR2 gene.

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## P-ClinG-028

### **Interstitial deletion Xq26.2 characterised by array CGH in a foetus with Simpson-Golabi-Behmel syndrome**

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Simpson-Golabi-Behmel syndrome (SGBS) is a rare X-linked overgrowth disorder characterised by polyhydramnios with prenatal and postnatal macrosomia, distinctive coarse facial appearance, visceromegaly and a broad spectrum of congenital malformations including diaphragmatic hernia, supernumerary nipples, congenital heart defects, renal and genital anomalies. SGBS is caused by mutations

and deletions of the GPC3 gene. We describe the antenatal features of an affected foetus, who presented at 12 weeks with increased nuchal translucency. The karyotype of the foetus was 46,XY. Further ultrasound examination revealed foetal macrosomia, macrocephaly, asymmetric bilateral mild ventriculomegaly, low set ears, a flat profile, downturned corners of a permanently opened mouth and polyhydramnios. The pregnancy was terminated at 23 weeks. The foetus was subsequently diagnosed with SGBS by array CGH showing a small interstitial deletion on chromosome Xq26.2 encompassing the genes GPC3, GPC4 and CCDC160: arr Xq26.2(132,191,191-133,257,323)x0. The deletion was confirmed by FISH analysis, the mother is also carrying the deletion on one of her X-chromosomes. The GPC3 gene was previously found to be deleted together with the GPC4 gene in one family with SGBS. The phenotype in this family was complicated by renal tract anomalies and hydrocephalus. The CCDC160 gene is so far not linked to any human disease.

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## P-ClinG-029

### **Molecular genetic analysis of patients with amyloidosis at the Amyloidosis Center at the University Hospital of Heidelberg**

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The main goal of the Amyloidosis Center of the University Hospital of Heidelberg, founded in October 2008, is the interdisciplinary diagnosis, counselling, and treatment of patients with amyloidosis. Amyloidosis is a rare and life-threatening disease with variable, often unspecific, clinical appearance invoked by misfolded proteins or peptides aggregating into insoluble fibrils in the extra- or intracellular part of various tissues and organs. Amyloidosis may be acquired or inherited in an autosomal-dominant manner. Treatment of amyloidosis is dependent on this distinction. For example, in immunoglobulin light-chain amyloidosis (AL), the precursor protein is an immunoglobulin light chain produced by clonal plasma cells and standard treatment is chemotherapy. Contrarily, in hereditary amyloidosis, the mutant protein is often expressed in liver and treatment with chemotherapy is inappropriate. Here, liver transplantation is the standard procedure to correct the underlying metabolic defect. Therefore, accurate diagnosis of amyloidosis has implications for treatment, prognosis, and genetic counselling for the patient and family members. The fact that even in a patient with negative family history, hereditary amyloidosis may be the underlying cause due to variable penetrance, emphasizes the importance of molecular genetic analysis.

Since October 2009, we have analysed 119 patients presented with amyloidosis for hereditary variants by sequencing of all coding exons and adjacent intronic regions of the TTR, FGA, and APOA1 gene. In 14 patients we identified mutations in one of the analyzed genes: TTR p.Asp38Glu (common nomenclature: p.Asp18Glu), p.Val40Ile (p.Val20Ile) 2 x, p.Val50Met (p.Val30Met) 5 x, p.Leu78His (p.Leu58His), p.Ile88Leu (p.Ile68Leu), p.Glu109Gln (p.Glu89Gln), APOA1 p.Leu99Pro (p.Leu75Pro) 2 x, and FGA p.Glu545Val (p.Glu526Val).

Here, we present a 45 years old male patient with a 6-months history of proteinuria and uncontrolled hypertension in whom amyloidosis was diagnosed by kidney biopsy. Because of slightly increased plasma cell content in the bone marrow AL amyloidosis was suspected and steroid treatment was started. For a recommendation concerning further chemotherapy he was referred to the Amyloidosis Center. Our investigations revealed an isolated kidney amyloidosis and no monoclonal gammopathy. Molecular genetic analysis of the peripheral blood showed a heterozygous mutation p.Glu545Val (p.Glu526Val; c.1634A>T) in the FGA gene confirming the diagnosis of a hereditary fibrinogen-alpha amyloidosis. Instead of further chemotherapy a liver transplantation has been recommended to the patient.

These results support the importance for routine molecular genetic analysis in patients with amyloidosis to prevent misdiagnosis and treatment failure.

**Pregnancy outcome after maternal treatment with mycophenolate - Results of a European multicenter study**

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Introduction: Mycophenolate is mainly used for immunosuppression after organ transplantation. It is increasingly prescribed for systemic lupus erythematosus (SLE) and other autoimmune diseases, thus also in women of childbearing age. After maternal exposure to mycophenolate in pregnancy a high number of both fetal losses and a specific pattern of birth defects consisting of microtia, cleft lip and other features were reported. However, prospective data on pregnancy outcome allowing quantitative risk assessment are missing so far. Methods: 50 prospectively and 14 retrospectively reported pregnancies after maternal therapy with mycophenolate (mycophenolate mofetil or mycophenolic sodium) during pregnancy were identified by European Teratology Information Services through their risk consultation process. Results: In the 50 prospectively ascertained pregnancies treatment indication for mycophenolate was organ transplantation in 21, SLE in 19 and other autoimmune diseases in 10 pregnancies. The median gestational age at first call was 7 weeks, the median maternal age 27 years. Outcome of the 50 prospective pregnancies was as follows: 13 spontaneous abortions, 11 elective terminations of pregnancy (ETOP, 2 with mycophenolate embryopathy), and 26 live born infants. 4/26 live born infants children had major birth defects: (1) isolated external auditory canal atresia, (2) tracheo-oesophageal atresia, (3) hydronephrosis requiring surgery, and (4) atrial septal defect. Thus, the percentage of major birth defects is 21% (6/28 informative pregnancies, including malformed aborted fetuses). The probability of spontaneous abortion, estimated using survival analysis technique as described in Meister et al. (2008) was about 50% (0.53, 95% CI 0.27-0.84). Among the 14 retrospectively reported pregnancies 3 fetuses and 1 live born had a phenotype consistent with mycophenolate embryopathy, one of these also with trigonocephaly. Conclusions: We present the first prospective case series on mycophenolate exposure in pregnancy, confirming the high risk for spontaneous abortions and major birth defects. Six fetuses/infants of our prospective and retrospective case series had a clinical phenotype consistent with mycophenolate embryopathy, 3 of them had been published as case reports. Trigonocephaly has not been described so far and might be an additional feature of the mycophenolate embryopathy. In some of our prospective and retrospective cases only a partial phenotype of mycophenolate embryopathy (like isolated cleft palate or tracheo-oesophageal atresia) was observed and might also have occurred by chance. Apart from exposure with mycophenolate the underlying maternal disease and concomitant medication may also have contributed to the pregnancy outcome.

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**P-ClinG-031****Partial NFIA gene deletion in a patient with mild intellectual deficiency**

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NFIA is a transcription factor which controls transition from neurogenesis to gliogenesis in developing spinal cord. Nfia knockout mice show perinatal lethality, hydrocephalus and agenesis of corpus callosum. Lu et al. (2007) suggested that NFIA haploinsufficiency causes a distinct brain and kidney malformation phenotype with agenesis of corpus callosum and developmental delay of unspecified severity as consistent features and variable other brain and spinal cord malformations, seizures and kidney anomalies in human. However, all 6 patients known in literature in addition to NFIA haploinsufficiency have other genes interrupted or deleted with deletion sizes ranging from 2-12 Mb (Lu, 2007; Koehler, 2010). NFIA mutational analysis in a total of 219 patients revealed no apparently pathogenic mutation.

We now report the first patient with a de novo microdeletion limited to the NFIA gene. This patient, a 12 years old girl, showed mild intellectual deficiency with macrocephaly and unspecific minor anomalies, but in contrast to the published cases normal MRI scans. Molecular karyotyping using a 6.0 GeneChip array showed a 128 kb deletion on chromosome 1p13.3. The deletion encompassed 88 array markers and was flanked by the markers CN\_493514 and CN\_493558. This in frame deletion removes exon 3 to 6 of the NFIA gene and thus destroys the nuclear localization signal. MLPA analyses with self-designed probes for the deleted region confirmed the deletion in the patient and excluded it in the parents, thus suggesting de novo origin. A deletion of NFIA was not observed in 667 control individuals. By sequencing the coding exons of all 4 isoforms in 190 patients with mild mental retardation and in 95 non-microcephalic patients with severe mental delay we detected no obvious mutation, but a few variants, which are currently under further investigation. In summary we suggest that defects limited to the NFIA gene cause only a mild mental phenotype.

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## **P-ClinG-032**

### **Homozygosity for a MECP2 gene mutation in a girl with variant Rett syndrome**

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The X-linked dominant MECP2-related neurodevelopmental disorders include classic and variant Rett syndrome and mild learning disabilities in females, and neonatal encephalopathy and intellectual disability in males. We report a 7 year-old girl with normal prenatal and perinatal history, night-time screaming and a loss of acquired speech at the age of 30 months. She had normal body measurements (head circumference between the 10th and 25th centile), preserved hand skills and no hand stereotypies. Based on these clinical findings a variant Rett syndrome was diagnosed. Molecular testing of MECP2 gene revealed a homozygous mutation c.1159\_1195del37bpins8bp in the patient causing a premature stop-codon after seven changed amino acids (p.P387RfsX8). MECP2 analysis in the parents showed a de novo occurrence of the mutation in the patient. Buccal smear analysis did not indicate a mosaic for the mutation. The possibility of a partial or larger deletion affecting the MECP2 gene was excluded by MLPA analysis and Affymetrix 6.0 SNP array. Haplotype analysis indicated biparental inheritance ruling out uniparental disomy. Gene conversion remains as a possible explanation for this unusual finding. Homozygosity for MECP2 mutations is a very rare finding. Up to now only three cases with classic Rett syndrome were reported in the literature. One case occurred de novo and was due to a mosaic mutation. Surprisingly the other two cases were identified by MECP2 analysis in 15 patients with typical clinical features of Rett syndrome but segregation could not be further investigated. Remarkably the phenotype of all four homozygous females is not on the severe end of spectrum as one might anticipate from male patients.

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## **P-ClinG-033**

### **Mosaic type-1 NF1 microdeletions as a cause of both generalized and segmental neurofibromatosis type-1 (NF1)**

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Mosaicism is an important feature of type-1 neurofibromatosis (NF1) on account of its impact upon both clinical manifestations and transmission risk. Using FISH and MLPA to screen 3500 NF1 patients, we identified 146 individuals harbouring gross NF1 deletions, 14 of whom (9.6%) displayed somatic mosaicism. The high rate of mosaicism in patients with NF1 deletions supports the postulated idea of a direct relationship between the high new mutation rate in this cancer predisposition syndrome and the frequency of mosaicism. Seven of the 14 mosaic NF1 deletions were type-2, whereas 4 were putatively type-1, and three were atypical. Two of the 4 probable type-1 deletions were confirmed as such by breakpoint-spanning PCR or SNP analysis. Both deletions were associated with a generalized manifestation of NF1. Independently, we identified a third patient with a mosaic type-1 NF1 deletion who exhibited segmental NF1. Together, these three cases constitute the first proven mosaic type-1 deletions so far reported. In two of these three mosaic type-1 deletions, the breakpoints were located within PRS1 and PRS2, previously identified as hotspots for

non-allelic homologous recombination (NAHR) during meiosis. Hence, NAHR within PRS1 and PRS2 is not confined to meiosis but may also occur during postzygotic mitotic cell cycles.

Importantly, although NF1-deletions are identified using aCGH or MLPA analysis, these techniques are inadequate to the task of identifying their mosaic nature, especially when the fraction of non-deleted alleles is low. Hence the use of FISH analysis, on blood or other cell types (e.g. from buccal smears, urine samples etc), is advisable as an additional detection technique in potential cases of mosaicism.

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#### **P-ClinG-034**

### **Ischio-vertebral dysostosis**

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Hypoplasia of the ischia is a rare congenital malformation which has been reported as an isolated anomaly or as a component of only a few congenital malformation syndromes. We here report on a further patient with the combination of vertebral defects.

At the age of 18 years our female patient presented with flat face, depressed nasal bridge, anteverted nostrils, highly arched palate, small chin, mildly webbed neck, severe kyphoskoliosis, pectus carinatum, narrow thorax, arachnodaktyly, and normal mental development. Because of her severe kyphoskoliosis she was confined to a wheelchair. Radiographic examination demonstrated thoracolumbar skoliosis with segmentation defects of the vertebrae and absent ossification of the corpus ossis ischi and parts of the ramus inferior ossis pubis. The patellae were normal in size and shape and the fingernails and toenails were normally grown without ridging. Laboratory examination was non-contributory, and chromosome analysis showed a normal female karyotype. Her length was 153 cm (3rd perc.), weight 37 kg (3rd perc.) and head circumference 57 cm (90th perc.)

In 1999 Nishimura et al. described 5 unrelated patients with the previously unrecognized combination of ischial hypoplasia and spinal malformation resulting in short-trunk dwarfism and in a part of the patients neurological and respiratory complications. He called this new entity ischio-spinal dysostosis. All cases were sporadic. In the same journal, Cohen et al. (1999) presented 8 patients with less severe vertebral defects, but additional features like micrognathia, highly arched palate, cleft palate and laxity of fingers. One affected family showed autosomal dominant inheritance. In both syndromes the malsegmentations of the spine rapidly lead to severe kyphoskoliosis. Presence or absence of facial dysmorphism, skin changes or heart defects may suggest genetic heterogeneity. Spranger et al. (2001) added nephroblastomatosis and rib gaps to the spectrum of ischio-spinal dysostosis. Ischio-patellar hypoplasia and ischiadic hypoplasia with renal dysfunction, immunodeficiency and polydaktyly have to be included in the differential diagnosis.

Cohen PA et al. (1999) *Pediatr Radiol* 29: 131-134

Nishimura G et al. (1999) *Pediatr Radiol* 29: 212-217

Spranger J et al. (2001) *Clin Dysmorphol* 10: 19-23

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#### **P-ClinG-035**

### **A Novel Homozygous Missense Mutation in the Potassium Channel related Gene KCTD7 in a boy with Therapy-resistant Myoclonic Epilepsy and Severe Developmental Delay**

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Progressive myoclonic epilepsy (PME) is a heterogeneous group of epilepsies characterized by myoclonic seizures and progressive neurological dysfunction like ataxia and cognitive impairment. Well recognized causes for PME include Unverricht-Lundborg disease, myoclonic epilepsy with ragged-red fibers (MERRF), Lafora body disease, sialidosis and the neuronal ceroid lipofuscinoses. However, these disorders are rare and account only for a small number of PMEs. Hence, in many cases of PME the cause remains elusive.

Here we report on a 5 year-old boy with early-onset therapy resistant PME and severe developmental delay. He was born to healthy consanguineous Turkish parents. Epilepsy started at the age of 10 months after a period of normal development. The main seizure type is multifocal myoclonic seizures with tonic episodes. The boy also shows muscular hypotonia and a cerebral movement disorder characterized by dystonia and dyskinesia. At the age of 5 years he is unable to sit or speak; however, visual interaction is preserved. Conventional cytogenetic analysis as well as high-resolution molecular karyotyping (Human1M-Duo, Illumina, Ca) were normal but SNP array analysis showed an increased number of autozygosity

regions. As the parents are consanguineous an autosomal recessive cause for the disorder was suspected. Genome-wide parametric linkage analysis under a recessive model with complete penetrance identified several candidate regions with LOD scores >1,9 (Illumina HumanCytoSNP12 Array, genotyping of grandparents, parents, the affected boy and his healthy sister). Data mining on the basis of gene function and known PME causing genes indicated the potassium channel tetramerization domain containing 7 gene (KCTD7) in the 7q11.21 linkage region as a suitable candidate (Van Bogaert et al., 2007). Sequence analysis of KCTD7 revealed a novel homozygous missense mutation in a highly conserved segment of exon 2 encoding a functional arginine to tryptophane residue change at codon 94 (p.R94W). This mutation was not present in 100 control samples. This is the second family with PME caused by KCTD7 mutations and it is tempting to speculate that KCTD7 mutations may be a recurrent cause of PME.

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#### **P-ClinG-036**

### **A de novo t(5;14)(p15.33;q12) Translocation Associated with Severe Mental Retardation**

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We report on a 5-year-old female patient diagnosed with microcephaly, partial agenesis of the corpus callosum, hypoplasia of the temporal lobe and a delayed myelination. The patient suffers from severe mental retardation and a pronounced developmental delay including speech impediment. Seizures were additionally diagnosed at the age of 15 months. The facial gestalt of the patient was considered to fit into the spectrum of Cri-du-Chat-syndrome. Cytogenetic analysis using high resolution GTG-banding revealed an apparently balanced de novo translocation described as t(5;14)(p15.33;q12). Using fluorescence in situ hybridization (FISH) mapping and subsequent "mini-FISH" refinement, a delineation of the breakpoint regions to 27.5 and 25.5 kb respectively could be achieved, but sequencing of a breakpoint-spanning long-template PCR product was not successful. Additional copy number variation studies using the Affymetrix 6.0 SNP platform were without pathological findings. Both chromosomal breaks were located in regions without annotated genes and the breakpoint on chromosome 5 corresponds to the mild mental retardation region of Cri-du-Chat syndrome, hence it is unlikely to explain the phenotype. SYBR-Green based expression analysis of selected genes from the breakpoint regions revealed a decreased expression of nine genes located in the affected region on chromosome 5 (SEC6L1, BDR9, NDUFS6, MRPL36, PDCD6, ZDHHC11, CEP72, POLS, TPPP). Interestingly, the chromosomal break on chromosome 14 was found to be located in a relatively gene poor region only 0.5 Mb downstream of FOXP1, which encodes a brain-specific transcription factor. FOXP1 is an important factor of brain development and was previously associated with autosomal dominant Rett-like syndrome. Due to the comparable severity of our patient's phenotype we hypothesize the pathomechanism is based on FOXP1 haploinsufficiency. This hypothesis is under further investigation.

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#### **P-ClinG-037**

### **Clinical characterization of a patient with a de novo microdeletion 3p14.1-p13 of about 5.6 Mb and comparison with a previously reported case with similar chromosomal breakpoints.**

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Interstitial microdeletions of the human genome are observed with a great variation regarding frequency of chromosomal sites involved. Particular loci containing so called low copy repeats are obviously more likely to be affected and recurrent rearrangements including deletions are more abundant under these circumstances. Several additional mechanisms have been identified, but not all of them are yet as well understood. By application of high resolution array CGH it has become feasible to quickly determine the size of distinct genomic imbalances and to characterize the chromosomal sites involved.

Here we want to report another patient with a de novo microdeletion 3p of more than 5.6 Mb causing a loss of about 15 genes including one copy of the MITF-gene and the PROK2-gene. These two genes can cause disorders following an autosomal dominant mode of inheritance, like Waardenburg syndrome 2A and Kallmann syndrome 4 in case of haploinsufficiency. The patient is a boy of currently 3,5 years of age. He was born at the 38+2 week of gestation by spontaneous delivery. Length at birth was 50 cm, weight 3180 g,

OFC 36.2 cm. Apgar was 9/9/9. Within the first postnatal week several abnormal findings including an atrial aneurysm, cardiac arrhythmia, distinct facial dysmorphism, bilateral cryptorchism and hearing impairment were recorded. Subsequent clinical examinations revealed a bilateral brain atrophy, a partial optical nerve atrophy and a severe psychomotor retardation. His phenotype shows striking similarities with a patient previously reported by our group. We are currently performing array CGH analysis of the reported patient to find out the precise overlap of the two deletions. Thus far only one further patient with a similar microdeletion, however with slightly different chromosomal breakpoints is listed in the Decipher database. It will be of interest to compare phenotypic features with our two patients to see if a specific new microdeletion syndrome can be defined.

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## **P-ClinG-038**

### **Oculodentodigital Dysplasia (ODDD) and cardiac hypertrophy**

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#### Background

Oculodentodigital Dysplasia (ODDD) is a very rare autosomal dominant syndrome. Main clinical features are characteristic facial dysmorphism such as blepharophimosis, epicanthus, short and small nose with hypoplastic ala, morphological abnormalities of the eye (e.g. microcornea, microphthalmia, glaucoma, cataract), dental abnormalities (microdontia, partial anodontia, enamel hypoplasia, multiple caries, early tooth loss) and bilateral syndactyly of the (third), fourth and fifth finger. The syndrome is caused by mutation of the connexin-43 gene (gap junction protein, alpha 1, GJA1) on chromosome 6q21-q23.2.

#### Patient and methods

We describe a newborn boy with Oculodentodigital Dysplasia. The patient showed the typical clinical findings of ODDD. He had a short nose with hypoplastic nostrils, blepharophimosis and microphthalmia. Bilateral syndactyly of the third, fourth and fifth finger was noted. Both feet showed syndactyly of the second, third and fourth toe. The hair was sparse. Glandular hypospadias was diagnosed. In addition to the characteristic symptoms cardiac examination revealed alterations of the heart with hypertrophy of the left ventricle, stenosis of the mitral valve and a low-voltage electrocardiogram with ventricular extrasystoles.

As the clinical symptoms were characteristic of ODDD, molecular analysis of the connexin-43 gene (GJA1) was initiated. Sequence analysis showed a heterozygous mutation c.20T>C, p.L7S, in exon 2 of the GJA1 gene. This mutation has not been described before, but as a similar mutation was already known, and the mutation was predicted as probably damaging by "Polyphen", the mutation was considered to be likely pathogenic. Both parents were analyzed, and the finding that neither was mutation carrier supported the assumption that the mutation is de novo and responsible for the clinical picture of ODDD in this patient.

#### Conclusion

While patients with a GJA1 gene mutation might have a left heart hypoplasia or other defects of the heart, cardiac hypertrophy has not been described in patients with ODDD. Experimental studies suggest an influence of mutations in the connexin-43 gene on the development of the heart and the myocardial cell-cell coupling.

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## **P-ClinG-039**

### **Pontocerebellar Hypoplasia suspected by Magnetic Resonance Imaging and confirmed by Mutation Detection**

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Pontocerebellar hypoplasias (PCH) represent a group of autosomal recessive neurodegenerative disorders which are characterised by severe mental and motor impairments, hypoplasia of the cerebellum and ventral pons, microcephaly, and variable degrees of cortical atrophy. PCH are subdivided by neuroradiologic, neurologic, and molecular genetic findings into six types (PCH1 to PCH6). To date, five genes are known to cause PCH in homozygous or compound heterozygous state: TSEN54 (PCH2 and PCH4), TSEN2 and TSEN34 (PCH2), VRK1 (PCH1), and RARS2 (PCH6). For PCH3 and PCH5, until now, no underlying genes are known.

The most common type of PCH is PCH2 accounting for about 90% of all cases. The type 2 of PCH features progressive microcephaly from birth combined with extrapyramidal dyskinesia, lack of voluntary

motor or mental development, severe chorea, and frequently epilepsy. In contrast, signs of spinal anterior horn involvement, as seen in PCH1, optic atrophy, observed in PCH3, or respiratory chain defects, typically for PCH6, are lacking. Most children with PCH2 die before age ten years, although survival beyond age 20 has been reported. Typical findings in PCH2 in cerebral Magnetic Resonance Imaging (cMRI) are, beyond ventral pontine atrophy/hypotrophy, cerebellar hypoplasia whereby the cerebellar hemispheres are more affected than cerebellar vermis with a relative sparing of the flocculi. These cMRI features were termed as dragonfly-like cerebellar pattern and recognised as pathognomic for TSEN54 mutations. This cMRI pattern is in contrast to PCH5, in which the vermis is more affected than the hemispheres. PCH4 subjects have a similar cMRI as PCH2 patients, but a more severe phenotype. Accordingly, most of PCH4 patients die as neonates.

PCH2 is caused by biallelic mutations in TSEN54, TSEN2, or TSEN34. The TSEN54 mutation c.919G>T (p.Ala307Ser) has been identified in approximately 90% of PCH2 cases in homozygous state. Furthermore, homozygous mutations in TSEN2 and TSEN34 have been detected in PCH2 patients of Pakistani and Turkish origin, respectively. In addition, c.919G>T in TSEN54 was found in a heterozygous state with a truncating TSEN54 mutation in patients with PCH4.

The p.Ala307Ser mutation in TSEN54 is thought to be a founder mutation descending of a single couple that lived in the seventeenth century in Volendam, The Netherlands. Heterozygous studies confirmed that the allele frequency for this mutation is at highest in the Volendam region (2/136, 1.47%) but occurs also in the general Dutch population (5/451, 1.1%), as well as in a considerably fraction of Germans (1/279, 0.36%).

Here we report on two female patients aged 3.5 months and 12 years, respectively. Both children were born by non consanguineous parents of German descent. After an uneventful pregnancy and birth, they developed neurological signs resembling to PCH. Cranial MRI displayed typical pattern of PCH2/PCH4 in both patients. Phenotypically, PCH2 was more likely than PCH4 since both children had a relatively good neonatal period. Sequencing of TSEN54 revealed heterozygosity for the c.919G>T mutation in both patients.

Conclusions: The underlying cause of secondary microcephaly should always be clarified. Cranial MRI should be performed also in cases, in which cranial ultrasound imaging was normal. When cMRI gives suspicion to Pontocerebellar Hypoplasia diagnosis should be confirmed at molecular level by analysing TSEN54 as well as TSEN2, TSEN34, and, eventually, also TSEN15, VRK1, and RARS2.

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#### **P-ClinG-040**

### **A case report of autosomal recessive hypophosphatasia showing an unexpected intrafamilial variability of the phenotype**

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Hypophosphatasia is a hereditary metabolic disorder of the bone due to impaired activity of the tissue-nonspecific isoenzyme of alkaline phosphatase. Five clinical forms are distinguished depending on the age of diagnosis. Early onset of the disease normally accompanies a severe clinical course that is transmitted as an autosomal recessive trait.

Here, we present a family with two affected siblings. Analysis of the ALPL gene revealed the heterozygous mutations c.526G>A; p.Ala176Thr in exon 6 and c.1282 C>T; p.Arg428X in exon 11. The compound heterozygous status was proven by family investigation. The brother shows the typical picture of infantile hypophosphatasia with dysproportioned short stature including shortening of the humerus and femur as well as bone deformity and relative macrocephaly, resulting in a body height of 146cm. Diagnosis was made soon after birth due to the clinical picture. Despite of the identical genotype, the body height of his sister is in the normal range and proportionate. So far, she does not show skeletal manifestations of hypophosphatasia except for an insufficiency fracture of the metatarsal V of the right foot without significant trauma.

Interfamilial variability of hypophosphatasia is of wide range and can be explained by different patterns of inheritance and the variety of causal mutations in the ALPL gene. Most publications describe only little intrafamilial variation, however, in this reported family an extreme variability of the phenotype is demonstrated. Therefore, results of prenatal diagnostics have to be interpreted with caution and should be confirmed by ultrasonographic findings.

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**P-ClinG-041****Concurrent Real-time Expression Study of Mutant and Wildtype Alleles in Smith-Lemli-Opitz Syndrome**

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The Smith-Lemli-Opitz-Syndrome (SLOS) is an autosomal recessive metabolic and malformation disorder. It is caused by mutations in the DHCR7 gene encoding the  $\Delta 7$ -sterol reductase which catalyses the last step of cholesterol biosynthesis. About 90% of mutations reported in this gene are predicted to cause amino acid alterations, however the impact on splicing or RNA stability is unknown. Aim of the study was (1) to establish a technique to analyse the transcription efficiency depending on mutations in the gene, and (2) to study the effect on transcription of frequent SLOS causing mutations. The newly designed method compares transcription rates of the wildtype and mutant allele of the DHCR7 gene with probe based real-time PCR technique. The technique is based on dual-labeled MGB-probes that bind sequence specific either the wildtype allele or the mutated allele. Because of its sequence specificity only the particular PCR product generates a fluorescent signal which will be detected and assigned into the calculation of comparative Ct method. The applied method gives the possibility to calculate the input amount of the two transcripts of which conclusions about the transcription efficiency can be made.

Transcriptional activity of the DHCR7 gene was assessed by real-time PCR in heterozygous fibroblast cell lines from parents of SLOS patients. Because of high variability in expression of wildtype DHCR7 in human cell lines it became necessary to establish an approach that allows comparison of mutated versus wildtype allele in the same cell. Transcriptional activities of different mutated DHCR7 gene loci were monitored by real-time PCR. The experiments have shown that the mutated alleles are transcribed in the same amount as the wild type alleles for the splice site mutation c.964-1G>C, the missense mutations p.Thr93Met, p.Trp182Cys, p.Glu224Lys and p.Gly410Ser. The splice site mutation c.964-1G>C leads to a premature stop codon, but does not trigger nonsense mediated decay due to the fact that the mutation is located in the last intron of the gene. There is also no other apparent mechanism to eliminate mutated transcripts.

Our real-time PCR technique allows the analysis of transcription levels of two different alleles separately in the same cell independently of transcription level of the investigated gene in general. Transcript stability of the alleles carrying splice site mutation c.964-1G>C, the missense mutation p.Thr93Met, p.Trp182Cys, p.Glu224Lys and p.Gly410Ser are not impaired compared to the normal allele. With the more sophisticated real-time PCR technique the impact of mutations on the RNA level can be observed easily. This technique allows also declaring about the RNA stability in the cell.

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**P-ClinG-042****A novel MTM 1 gene mutation in two Czech brothers with severe congenital myopathy - X-linked myotubular myopathy.**

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Introduction: Myotubular myopathy 1 is an X-linked recessive, usually lethal disorder of muscles caused by mutations in the myotubularin gene (MTM 1). Clinical symptoms include neonatal generalized hypotonia and muscle weakness together with respiratory failure often resulting in dependency on ventilatory support. Cryptorchidism is frequently present. Central localization of nuclei is a prominent feature in muscle biopsy. Therefore a synonymous name - Centronuclear myopathy is sometimes used. The rather serious phenotype is due to the crucial function of the MTM1 gene in a process of maturation of a muscle cell.

Case report: We describe two patients, dizygotic twins. Boys were born in the 28th week of gestation. Muscle weakness, generalized hypotonia and areflexia dominated the phenotype. Both were soon after birth dependent on the ventilatory support and intensive care. Cryptorchidism and prenatal chylothorax were present. Needle EMG showed no signs of neurogenic disorder. Creatine kinase levels in serum were normal. Progressive renal and cardiac insufficiency was the leading cause of death after one month of life.

Muscle biopsy was examined, showing hypotrophic muscle bundles and fetal myotubes together with nuclei localized centrally.

All these data supported the clinical diagnosis of X-MTM 1. Afterwards, we decided to sequence all coding exons and adjacent intronic regions of the MTM1 gene. A deletion c.82delA was found in the exon 2 in both patients in homozygous state. It is a frameshift mutation with a premature stop codon at the position 43. It is a novel, not yet described mutation. However, because of the nature of the deletion, we think this mutation is causal and pathogenic for X-MTM 1. Mother of patients was also tested, she is a heterozygote carrier of c.82delA mutation in the MTM 1 gene, with a 50% probability for her sons being afflicted by X-MTM 1.

Summary: The above-mentioned features of the floppy infant males are useful clues for the clinical diagnosis of X-MTM 1. The testing for mutations in the MTM 1 gene has not been available in the Czech Republic yet. According to our knowledge, this is the first report of molecular- genetic diagnosis of X-MTM 1 done in Czech Republic. The availability of the MTM 1 gene testing may be used also in other families where the clinical suspicion of the X-MTM 1 is stated.

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## P-ClinG-043

### Compound Heterozygosity for Two PTPN11 Mutations in a Patient with Noonan Syndrome

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Noonan syndrome (NS) is a relatively common autosomal dominant disorder characterized by typical facies, short stature, congenital heart defects and possible developmental delay. Mutations in various genes encoding members of the RAS/MAPK pathway have been found to be causative for this syndrome. The most common gene mutated in NS is PTPN11. Mutations in this gene may also cause a similar syndrome called LEOPARD syndrome (LS) or NS with multiple lentigines. Phenotypically NS and LS can be hard to distinguish, especially in very young children.

We analyzed leukocyte DNA samples from three family members affected with Noonan syndrome, a father and his two children (9 year old boy and 14 months old girl). Mutation screening was performed by high resolution melting (HRM) analysis on a LightCycler480 (Roche Diagnostics). The three samples differed from the normal control for PTPN11 exon 13. Direct sequencing revealed a missense mutation, c.1471C>G (p.P491A), segregating with the Noonan syndrome phenotype in this family. This variation has not previously been reported, but several causative amino acid exchanges are known at position 491 (p.P491S, p.P491L, p.P491H, p.P491F). Only in the daughter an additional mutation, c.1492C>G (p.R498W), was found in the same exon of the PTPN11 gene, which obviously occurred de novo. This variant is a known mutation causative for LS. Cloning of the PCR product into a TOPO TA vector confirmed that the mutations are on different alleles. All three affected individuals of this family were clinically re-evaluated. Father and son were found to have a relatively mild phenotype including typical NS facies and mild short stature, while the daughter has a heart defect (pulmonary stenosis). Due to her young age many of the more LS typical symptoms, like lentigines, may not have developed yet.

This is the second observation of compound heterozygosity for two PTPN11 mutations and the first in a living patient. One case of early fetal death associated with compound heterozygosity for two NS-causative mutations (p.Y63C and p.N308S) has been described in the literature and it was suggested that the presence of two mutated alleles has deleterious effects on early development and fetal survival. In the present case, it is remarkable that co-existence of mutations of both alleles is quite well tolerated. This may be attributed to milder functional consequences of the paternally transmitted mutation p.P491A, consistent with the relatively mild phenotype in the family members carrying only this mutation. It may also be speculated that the perturbing effects of two mutations on the RAS/MAPK pathway may not necessarily be additive. Finally, undetected somatic mosaicism for the p.R498W mutation cannot be excluded.

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## P-ClinG-044

### Development of a Macroarray DNA-Chip Platform for the Detection of a Disease Panel relevant in Nutrigenomics

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Background:

Nutrigenetics is a branch of the new science nutrigenomics. Since the completion of the human genome mapping in 2003, scientists have discovered that certain genetic predispositions can influence the tolerance a certain foods. Nutrigenetics is the discipline that determines how the individual genetic predisposition influences the metabolism or intolerance towards certain foods, thus providing different health conditions among people following the same diet.

Enzymatic food intolerance is an adverse food reaction resulting from enzymatic defects. Symptoms manifest preferentially in the gastrointestinal tract (GIT), where the functional enzyme is expressed and commonly include unspecific symptoms like vomiting, diarrhea, flatulence or abdominal pain.

The most prominent and well known disease in this panel is lactose intolerance, which is caused by a SNP in the regulatory region of the lactase gene. This mutation leads to decreased binding affinity of several transcription factors and eventually to a lower protein synthesis and less available lactase in the GIT.

Other prominent food intolerances include celiac disease, alcohol dysmetabolism, favism (Glucose-6-phosphate dehydrogenase deficiency), hemochromatosis, fructose intolerance and sucrase-isomaltase deficiency.

DNA macroarrays offer the possibility to test several genetic variants simultaneously. Hence, they are an ideal tool to analyze a person's genetic disposition, and diagnose therefore the likelihood for the development of certain food intolerance. Moreover, macroarrays can help to pinpoint the genetic reason for the food intolerance, a difficult task to accomplish when based on the symptoms alone.

#### Methods:

We hereby present a macroarray DNA-Chip with a precipitation based detection method and an analytical software. No specific handling know-how is required and results are quickly obtained and easily interpreted. The chip, carrying the immobilized probes, is integrated into a common 1.5 mL reaction tube enabling handling with common equipment found in every laboratory.

#### Results & Discussion:

Genotyping with respect to allele discrimination was performed, including markers for favism, alcohol dysmetabolism, hemochromatosis, fructose intolerance, sucrase-isomaltase deficiency, celiac disease and lactose intolerance. All results were validated by realtime PCR assays.

A Nutrigenomics macroarray assay was successfully developed, enabling simultaneous detection of multiple food intolerances at once. By comparing the results with the realtime PCR reference system, 100% accordance of genotypes was detected.

#### Conclusion:

The described method enables an easy, fast and cost-effective genotyping, which can be used for differential diagnosis. This is especially helpful for physicians as several of the above mentioned food intolerances show similar symptoms and a one-by-one screening approach can be a lengthy and uncomfortable procedure for the patient.

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## P-ClinG-045

### **Somatic mosaicism for a partial TRPS1 deletion in a healthy women causes recurrent children with TRPS I**

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The tricho-rhino-phalangeal syndrome type I (TRPS I) is a dominantly inherited syndrome with craniofacial and skeletal anomalies. It is caused by deletion or mutation of the TRPS1 gene on 8q23.3, and the expression of the phenotype is considered fully penetrant in mutation carriers.

Here, we present the first case of recurrent children with TRPS I born to apparently healthy, non-consanguineous parents. Especially, the characteristic facial features and anomalies of the hand skeleton were absent in both parents. Three children are affected with TRPS I and two are healthy. Because point mutations in the TRPS1 gene cause the condition in the majority of cases, we first sequenced the protein coding part of the TRPS1 gene in one of the affected children, but did not detect a mutation. An analysis of the index case on a 250 k Nspl SNP array (Affymetrix) then revealed a heterozygous 74-150 kb deletion comprising exons 2-5 of the TRPS1 gene. This deletes the translation start signal and most of the open reading frame, and thus, must be considered the disease causing mutation. We then examined all seven family members by multiplex ligation-dependent probe amplification (MLPA) with the SALSA MLPA Kit P228 TRPS-LGCR by MRC Holland. This confirmed the deletion in the three affected sibs and intact TRPS1 in the unaffected father and the two unaffected children. However, the dosage for the seven probes representing the TRPS1 exons 2-5 was found slightly reduced in the mother's DNA. This suggested that the mother is a somatic mosaic, and that the deletion in the affected children is of maternal origin. To further investigate this, we performed analyses with TRPS1 intragenic and flanking polymorphic microsatellite markers. All three affected children share the same maternal haplotype, whereas the two unaffected share the other.

Unfortunately, the family is not informative for the single microsatellite that is located within the deleted segment, because father and mother share one allele of identical size, but both are heterozygous at this particular locus. Interestingly, one allele appeared reduced in intensity in the mother's DNA as compared to the three healthy individuals in the family. This again suggested mosaicism for the mother. The results of the microsatellite analyses furthermore suggested correct paternity and correct maternity.

To determine the degree of mosaicism precisely, we will perform quantitative PCR analyses on DNA from blood and skin fibroblasts of the mother. In contrast to the two known mosaic cases with TRPS II who have the deletion in 100 % of fibroblast cells, and fully express the phenotype, we expect a lower degree of mosaicism for the mother's fibroblasts. Our results will have an important impact on genetic testing and counselling regarding the recurrence risk for TRPS I in families.

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#### **P-ClinG-046**

### **NOVEL MUTATIONS IN THE DCC GENE IN PATIENTS WITH CONGENITAL MIRROR MOVEMENTS**

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Mirror movements are contralateral involuntary movements that mirror voluntary ones. Usually they are present in hands, fingers and forearms of affected individuals, but they can also be present in toes and feet, observable during foot tapping and movement of toes. Mirror movements are occasionally found in young children, but persistence beyond age 10 is unusual except in some disorders of nervous system crossing such as Klippel-Feil and Kallmann syndrome. Two reports of large families with congenital mirror movements and no other neurologic abnormalities provided evidence for autosomal dominant inheritance with incomplete penetrance. Interestingly, more males than females are affected. In several affected individuals, transcranial magnetic stimulation (TMS) demonstrated the existence of abnormal ipsilateral corticospinal projection. Recently, heterozygous mutations, a splice-site mutation and a 1-bp duplication, in the DCC (deleted in colorectal carcinoma) gene in 18q21.2 have been identified in affected individuals of two families with isolated congenital mirror movements. DCC belongs to a family of transmembrane proteins with immunoglobulin domains and fibronectin type III repeats. DCC is expressed on commissural axons and growth cones, where it functions as a cell-surface receptor for netrin-1. Netrin-1 is a diffusible protein which plays a significant role in commissural axon guidance during vertebrate brain development. Mutation or inhibition of Dcc receptors resulted in the failure of commissural axons to reach the midline in *Caenorhabditis elegans*, *Drosophila*, and mice. We ascertained two sporadic individuals with mirror movements, both with TMS evidence of ipsilateral cortico-spinal projections, as well as a three-generation family with three affected females. Sequencing of the 29 DCC exons detected the heterozygous missense mutation c.817T>C (p.W273R) in the female index patient, her mother and her maternal grandmother, all presenting with mirror movements. The mutation was not found in her healthy sister. Tryptophan at position 273 is a highly conserved residue in the immunoglobulin C-2 type domain of DCC and in silico protein analysis using five different programs predicted the p.W273R change to be deleterious to protein function. In a 15-year-old male with mirror movements, we identified the heterozygous in frame deletion-insertion mutation c.2858\_2865delTCATTACTinsGG. This alteration results in loss of three amino acids (V953, I954, and T955) and gain of a glycine. The apparently asymptomatic mother of the patient also carries the mutation. In the second sporadic patient, a 13-year-old female, we could not detect a sequence change in the DCC gene; further studies are ongoing to identify the genetic alteration. Our data confirm that mutations in the DCC gene underlie congenital mirror movements and may contribute to ipsilaterally misdirected corticospinal projection.

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#### **P-ClinG-047**

### **Is FISH analysis still necessary for the interpretation of array CGH results? The clinical case of two mentally retarded siblings with a gain of 5p14.1-p15.2 derived from a familial balanced rearrangement ins(13;5)(q13;p14.1p15.2)**

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BACKGROUND: Array CGH analysis has become a major tool for the detection of submicroscopic copy number changes in mentally retarded patients. The great advantage of array CGH analysis is the high

resolution to detect small duplications and deletions up to 100 kb. However, the chromosomal localization of the detected gains and losses in the patient is not clear.

**CASE REPORT:** We report on a family with two developmentally retarded siblings. The index patient, a boy of 10 years of age with mild mental retardation, speech impairment, growth retardation, and facial dysmorphism, was referred to us for genetic evaluation including array CGH analysis. His 4 year old sister showed a similar phenotype with developmental delay, growth retardation and, in addition, focal epilepsy and MRI anomalies raising a suspicion of septo-optic dysplasia. Also the mother's sister and a paternal aunt of the mother were mentally retarded. Array CGH analysis revealed a de novo gain of 12 Mb of chromosomal region 5p14.1-p15.2 in the index patient. Subsequent FISH analysis could show that the 5p duplication actually was an unbalanced insertion of 5p14.1-p15.2 material into the long arm of chromosome 13 in the region 13q13. The karyotype of the patient was defined as 46,XY,der(13)ins(13;5)(q13;p14.1p15.2). Retrospectively, the 12 Mb insertion of chromosome 5p material was not clearly detectable by G-banding analysis. FISH analysis of the patient's parents showed that the mother is a carrier of a balanced rearrangement with a deletion 5p14.1-p15.2 in one chromosome 5 and insertion of this 5p material in chromosome 13. The same balanced rearrangement was found in the mother's father. FISH analysis of the patient's sister showed the same unbalanced karyotype with an insertion duplication of the chromosome region 5p14.1-p15.2.

**CONCLUSION:** Array CGH analysis is a powerful tool to detect small deletions and duplications. But one should keep in mind that it does not provide any information on the chromosomal position of the observed imbalances and can not detect balanced rearrangement in the parents. Our data showed that it is of great importance to verify array data by FISH analysis on metaphase-chromosomes in order to be able to allow a comprehensive genetic counselling in the family.

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#### **P-ClinG-048**

### **Instant genetic diagnosis in newborn infants - Examples of early diagnosis of rare genetic syndromes**

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Diagnosis of rare genetic syndromes is very peculiar especially in young infants. Due to either not fully blown phenotype, non specific symptoms or phenotype variability, clear, unequivocal diagnosis is frequently not established within the first 2-3 years of life. Looking for specific phenotypic signs, however, in some instances diagnosis of specific genetic syndromes can be evolved even within the first days of life.

We here present three patients with Freeman-Sheldon syndrome (FSS), Rubinstein-Taybi syndrome (RTS) and vascular Hamartosis e.g. Klippel-Trenaunay syndrome (KTS), in which specific genetic syndrome diagnosis was supposed at the first day of life and diagnosis was subsequently proven by gene test in the first two cases. None of the newborns showed the full pattern of typical malformations, but all three presented with rather specific symptoms allowing to establish the diagnosis. Leading symptoms were distinct facial appearance (FSS), characteristic thumb and big toe malformation (RTS) and extensive vascular malformation (vascular hamartosis e.g. KTS). These patients demonstrate, that a close cooperation between neonatologists and geneticists is important for early diagnosis of rare genetic syndromes.

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#### **P-ClinG-049**

### **Detection of small deletions in 2q24.3 involving the SCN1A gene in two patients with severe epilepsy and developmental delay**

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Microdeletions in the region 2q24.3 involving the SCN1A gene are probably more common among patients with epilepsy than previously thought. Interstitial deletions of chromosome 2q encompassing this region have been reported in patients with epilepsy and additional features including microcephaly, craniofacial dysmorphism, brachysyndactyly, and developmental delay. Point mutations in the SCN1A gene, located within the SCN cluster in 2q24.3, have been identified in patients with generalized epilepsy with febrile seizures plus (GEFS+), a benign childhood-onset epileptic syndrome, but also in patients with severe epileptic syndromes including myoclonic epilepsy in infancy/Dravet's Syndrome (SMEI/DS). Here we present the clinical and molecular findings of two patients with overlapping microdeletions in the region 2q24.3 that

involve the SCN1A gene to compare the phenotype with other patients reported in the literature. Patient 1 is a 3 year old girl with small stature, microcephaly, mild ataxia and developmental delay, febrile seizures, and grand-mal epilepsy in infancy. She showed mild facial dysmorphism including a small chin, a small upper lip and a short nose. No anomalies of brain or heart were found. Patient 2 is an unrelated 34 year old man with progressive epilepsy since the age of nine months with up to 30 seizures per months. After a period of normal development in infancy psychomotor delay was seen. At the age of 34 years, the patient shows moderate mental retardation; in addition Crohn's disease was diagnosed at the age of 24 years. In both patients, sequencing of the whole coding region of SCN1A including exon/intron boundaries was performed but did not detect any pathogenic point mutation. Nevertheless, it was remarkably, that all known Single Nucleotide Polymorphisms (SNPs) in SCN1A appeared apparently to be "homozygous". Subsequently MLPA analysis of SCN1A revealed a heterozygous deletion of the whole SCN1A gene in both patients. Thus, the apparently "homozygous" SNPs are actually in a hemizygous state. Supplementary array CGH analysis in patient 1 showed a 944 kb deletion, in patient 2 a 524 kb deletion in the region 2q24.3. The minimal region of overlap involves four genes, SCN1A, SCN9A, GALNT3 and TTC21B. The deletion in patient 1 in addition contains the SCN7A gene. SCN9A may act as a genetic modifier of severe myoclonic epilepsy of infancy in conjunction with SCN1A. GALNT3 causes autosomal recessive hyperphosphatemic familial tumoral calcinosis. TTC21B modulates mouse Sonic hedgehog signal transduction and affects intraflagellar transport in cilia. SCN7A is likely to function in the central sensing of body-fluid sodium in the brain. In patient 1, in addition a 46 kb deletion in the region 22q12.3 was detected that leads to haploinsufficiency of the APOL3 gene which plays a central role in cholesterol transport. Comparison of the presented phenotypes with other reported patients can help to further link clinical features to specific genes in the region 2q24.3.

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#### **P-ClinG-050**

#### **Identification of a 4.3 Mb microdeletion in 1p36.22p36.21 unmasking a hemizygous PLOD1 mutation in a boy with a complex phenotype resembling EDS VIA**

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We report on a patient who is the first child of a mother from Russia and a father from Germany. He was born after an uneventful pregnancy. He was first referred for genetic evaluation at the age of 18 month because of significant muscular weakness, hypotonia and motor development delay. He showed joint hypermobility, kyphoscoliosis, strabism, mild facial dysmorphism, and a large hematoma after recent surgical correction of inguinal hernia. Because of the prominent hypotonia and delay in gross motor development, spinal muscular atrophy was supposed, but no SMN1-gene deletion was found. A conventional cytogenetic analysis revealed normal male karyotype.

Four years later, follow-up examination revealed global developmental delay, a marfanoid stature, wide based gait, hypermobility of multiple joints, as well as skin hyperelasticity and fragility. Furthermore, marked muscular atrophy of distal lower extremities and flat feet were recognized. Finally, abnormal scars with hemosiderin, a marfanoid habitus, and progressive kyphoscoliosis raised the suspicion of a connective tissue disorder.

A skin biopsy revealed rarefaction and highly variable diameters of collagen fibres and supported the diagnosis of Ehlers-Danlos syndrome. Based on these ultrastructural findings in conjunction with the clinical phenotype the kyphoscoliotic type of EDS (EDS VIA) was suspected. HPLC analysis of urinary pyridinoline cross-links showed a decrease of the hydroxylysyl pyridinoline lysyl pyridinoline ratio (HP/LP <0,17) thus confirming the diagnosis of EDS VIA. Molecular genetic testing of the PLOD1 gene revealed a hemizygous mutation p.Ala667Thr in exon 18, while the second allele harboured a deletion of at least the 3' terminal half of the PLOD1 gene as evidenced by MLPA (SALSA-Kit P143-B1, MRC-Holland). As this kit contains additional probes for the MFN2 (mitofusin 2) gene localized directly adjacent on chromosome 1p36.2 a dosis reduction of both genes indicated a contiguous gene syndrome. Oligo-Array-CGH using a CytoChip Oligo array ISCA 4x180K allowed to map the deletion to a 4.3 Mb interval on 1p36.22p36.21. This genomic region contains 61 annotated genes including nine disease associated genes.

In conclusion, the initially unsuspected molecular findings now explain the complex phenotype in this patient. It consists at least of the following, partially clinically overlapping features: EDS VIA due to a paternally inherited PLOD1 mutation in conjunction with a de novo deletion on the maternal allele, hereditary motor neuropathy due to haploinsufficiency of MFN2, as well as non-specific developmental retardation and dysmorphism due to haploinsufficiency of non-specified genes in the deleted region of 1p36.

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**P-ClinG-051****Deleterious effects of the TMEM43 mutation p.S358L found in a German family with arrhythmogenic right ventricular cardiomyopathy**

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a clinically and genetically very heterogeneous heart muscle disease leading to progressive loss of predominantly right ventricular myocardium, terminal heart failure, and frequently sudden cardiac death (SCD) which might even be the first clinical manifestation. The pattern of inheritance is autosomal dominant. Disease causing mutations in genes encoding for desmosomal proteins have been reported in up to 40 % of ARVC patients.

We present a German ARVC family who lost three male members aged 26 to 36 years due to SCD within a few months before the disease could be diagnosed. Another early death of unknown cause in one male ancestor was reported. Affected females have shown a rather mild phenotype. Our aim was to find the disease causing mutation in this family and to establish genetic diagnosis in clinically unclear cases or to perform predictive testing in apparent healthy relatives. After a non-informative segregation analysis, the plakophilin 2 (PKP2) gene was sequenced in one living patient of our family but no mutation was identified. This investigation was followed by sequencing of the genes desmoglein 2 (DSG2), desmocollin 2 (DSC2), desmoplakin (DSP), and plakoglobin (JUP) with each inconspicuous results.

Recently, a specific mutation (c.1073C>T; p.S358L) of another gene, TMEM43, had been identified by linkage analysis of 15 unrelated Canadian ARVC families from Newfoundland. This mutation was estimated to be fully penetrant and to affect male patients more severely than female ones. It was proposed to be a founder mutation. Nevertheless, the TMEM43 gene was analyzed also in our case. Thereby, the same C to T transition could be detected cosegregating within the affected family members including available DNA from autopsy samples of two individuals with SCD. In addition, we investigated 382 anonymous blood donors as a control group but the mutation p.S358L was not found in one of them. To our knowledge, it has not yet been reported from European or other than Newfoundland ARVC patients. Ancestors or relatives of our family in this region are not known. Thus, this mutation seems to be not solely confined to Newfoundland but also to be present on the European continent, possibly in rare cases only. However, the TMEM43 gene should be included in genetic screening of ARVC patients.

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**P-ClinG-052****MYH9 disorders revisited - morphology of leukocyte inclusion bodies predicts the position of the mutation**

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**Purpose:**

Myosin heavy chain 9 (MYH9)-related platelet disorders belong to the group of autosomal dominantly inherited macrothrombocytopenias caused by heterozygous mutations in MYH9. The MYH9 gene consists of 40 coding exons and encodes non-muscular myosin heavy chain IIA (NMMHC-IIA), a cytoskeletal contractile protein. Four overlapping syndromes, known as May-Hegglin anomaly (MHA), Epstein syndrome (EPS), Fechtner syndrome (FS), and Sebastian platelet syndrome (SPS), describe different clinical manifestations of MYH9 gene mutations. These syndromes differ in their risk to develop additional clinical manifestations such as high tone hearing loss, presenile cataracts, and interstitial nephritis leading to renal failure. We have shown that the risk for these complications depends on the position of the mutation within the MYH9 gene. Mutations in the C-terminal coiled-coil region have been associated with a hematological-only phenotype while mutations of the head ATPase domain have frequently been related to nephritis and/or hearing loss. Mutations of other regions show an intermediate expression of non-hematological characteristics. Genetic testing is instrumental in defining the mutation, but sequencing of all 40 coding exons is elaborate. We aimed to narrow down affected exons by defining phenotype-genotype correlations.

**Methods:**

Air dried blood smears from peripheral blood of 81 patients with macrothrombocytopenia were fixed, permeabilized, stained using a monoclonal antibody against NMMHC-IIA and visualized by immunofluorescence. Mutation analysis of the MYH9 gene was performed by standard methods using PCR-RFLP/SSP and Sanger sequencing.

**Results:**

In all 81 patients, MYH9 disorders could be identified by immunofluorescence (sensitivity 100%). In 39 cases mutation analysis was performed. We found a clear correlation between the position of the mutation and the phenotype of NMMHC-IIA clusters in neutrophils: small speckled clusters: exon 1 (n=5); snow flurry clusters: exon 16 (n=4); small round or slightly oval-shaped clusters: exon 26-30 (n=20); one or two large oval-spindle-shaped inclusion bodies: exon 38-40 (n=10).

Conclusions:

The position of the mutation in the MYH9 gene determines the phenotype of NMMHC-IIA clusters in granulocytes. It is likely that the different conformations due to the position of the mutation also explain the varying functional consequences and thereby the risk for manifestation of comorbidities. Prescreening by immunofluorescence allows targeted and less expensive genetic analysis of MYH9 disorders.

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## P-ClinG-053

### Case report of a CFC patient with a novel BRAF mutation

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Cardiofaciocutaneous syndrome (CFC syndrome; MIM 115150) is a rare multiple congenital anomalies / mental retardation syndrome characterized by congenital heart defects, short stature, a characteristic face, and abnormalities of the skin. The hair is typically sparse and curly, the eyebrows may be absent. Mental retardation usually is moderate to severe. CFC syndrome belongs to a family of syndromes that also includes Noonan and Costello syndromes. These three conditions show considerable phenotypic overlap and share a similar pathogenetic mechanism. The protein products of the causative genes all belong to the RAS–MAPK pathway, an mitogen-activated protein kinase pathway that regulates cell differentiation, proliferation and apoptosis. CFC syndrome is caused by mutations in four different genes: BRAF, KRAS, MEK1, and MEK2.

We report on a four years old girl with facial peculiarities suggestive of CFC syndrome (high forehead, bitemporal narrowing, hypoplasia of the supraorbital ridges, very distinct epicanthal folds, depressed nasal bridge and slightly anteverted nares, low-set and posteriorly rotated ears, short neck), sparse and curly hair, sparse eyebrows, moderate motor development delay and pronounced speech development delay. Divergent from the typical phenotype, our patient has no cardiac abnormality, her height and head circumference are within the normal range.

Sequence analysis of the BRAF gene identified a de novo heterozygous nucleotide change c.1780G>T in exon 15 (p.D594Y) that was not described before. The affected amino acid is highly conserved. Several amino acid changes in close vicinity were described as pathogenic mutations in other CFC patients and different nucleotide changes at the same position were reported as somatic mutations in tumor cells.

The clinical findings in our patient confirm the broad phenotypic variability of CFC syndrome. Cardinal features like cardiac abnormalities and skin findings may be absent. Furthermore, CFC shows considerable phenotypic overlap with Noonan and Costello syndromes. In questionable cases molecular analysis can be helpful. Finally, this is the first description of the BRAF mutation c.1780G>T (p.D594Y) causing CFC.

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## P-ClinG-054

### Ciliopathies: Experiences with SNP array based homozygosity screening as a rational diagnostic approach in consanguineous families

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Ciliopathies are a growing group of diseases which are characterized by defects of primary cilia function. Clinically they show overlapping phenotypes. Cystic kidneys, structural brain abnormalities and liver fibrosis are common findings in the mostly severe ciliopathies as Meckel syndrome, Bardet-Biedl syndrome or Joubert-related disorders. Many genes have been shown to be involved in the etiology of these diseases. Therefore identifying the underlying gene defects for the mainly autosomal recessively inherited ciliopathies is challenging. A diagnostic approach based upon mutation frequencies in the respective genes, phenotypic disease presentation or a linkage based preselection of genes seems reasonable in principle. Classical linkage analysis consists of separate STR marker analysis for each locus. However, due to the phenotypic overlap and the extreme heterogeneity of ciliopathies STR marker analysis is often labor intensive and time consuming. Therefore, in consanguineous families presenting with a ciliopathy syndrome a SNP array

(Affymetrix Genome-Wide Human SNP 6.0-Array) based homozygosity screening facilitates and accelerates the identification of the underlying gene defect by subsequent direct sequencing of the genes located in the identified regions of homozygous haplotypes. SNP arrays are used for miscellaneous applications as homozygosity mapping, determination of copy number variations (CNVs) or association studies. In patients with additional unspecific clinical findings, besides the determination of homozygous haplotypes for the known ciliopathy loci, an ancillary analysis of CNVs is therefore possible and often useful to rule out the chromosomal imbalances. Furthermore, though depending on marker coverage, exonic deletions in the considered ciliopathy genes can be detected as well. We analyzed about 20 consanguineous families – partly retrospective as positive controls – by SNP array. In all of the eight positive control patients homozygosity for the genes, in which pathogenic mutations had been identified previously, could be confirmed. Although the analysis is still ongoing, in four families the identified candidate genes have been sequenced so far and homozygous mutations in these genes were detected. None of the patients showed larger deletions in the candidate genes. Moreover an analysis of the array results regarding chromosomal imbalances has been performed in families with additional symptoms not common for the initially suspected diagnosis. However no clear pathogenic chromosomal aberration could be detected in these patients. In two families direct sequencing was not started because the array analysis did not reveal homozygosity for the known genes corresponding to the presumptive diagnosis. Therefore our data show that in consanguineous ciliopathy patients SNP array based homozygosity testing is a rational approach to identify the underlying gene defect.

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#### **P-ClinG-055**

### **Autosomal recessive osteopetrosis with neural involvement: Severe phenotype in a family due to a homozygous deletion including the OSTM1 gene and SEC63 promoter**

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The first child of consanguineous parents developed seizures on the 3rd day of life and had low grade fever and hepatomegaly. Investigations revealed low total calcium, increased PTH, anemia and thrombocytopenia, and chest x-ray revealed osteopetrosis. An MRI of the brain showed generalized brain atrophy, ischemic changes in basal ganglia and hypoplasia of the corpus callosum. Despite treatment with systemic antibiotics and blood transfusion the baby deceased at 6 weeks of life. Another maternal relative had a baby who presented with a similarly severe phenotype. This child died due to multiorgan failure at the age of four months.

In order to identify the underlying cause of this particularly severe form of autosomal recessive osteopetrosis with neural involvement we started sequencing analysis of the OSTM1 gene. However, the coding exons of OSTM1 could not be amplified in the DNA of the affected child, but only in the DNA of his parents. Quantitative PCR analyses revealed a homozygous deletion including the entire coding sequence of the OSTM1 gene as well as the promoter region of the SEC63 gene in the affected child and confirmed heterozygosity for the deletion in both parents. SEC63 is part of a trimeric complex involved in posttranslational transport of newly synthesized proteins. Mutations in the SEC63 gene lead to autosomal dominant polycystic liver disease.

In summary, we report on the clinical and molecular findings of two affected children from the same extended family with autosomal recessive osteopetrosis with neural involvement due to a novel homozygous deletion of the OSTM1 gene. This deletion includes the promoter region of the SEC63 gene, which might contribute to the distinct severity of the observed phenotype.

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#### **P-ClinG-056**

### **Pulmonary arterial hypertension – study of a large German cohort**

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Background: Pulmonary arterial hypertension (PAH) is a rare vascular disorder in which occlusion of small pulmonary arteries leads to increased pulmonary vascular resistance and right heart failure. PAH can

be sporadic or familial or associated with other conditions as connective tissue diseases, congenital heart diseases, portal hypertension, drug or toxin exposure. Heterozygous germline mutations in the bone morphogenetic protein type 2 receptor (BMP2) have been identified as underlying cause in approximately 10 to 40% of patients with apparently sporadic PAH and in 58% to 74% of patients with familial PAH. Patients carrying a BMP2 mutation may have a distinct phenotype and develop the disease approximately 10 years earlier than non-carriers, with more severe hemodynamic changes and a reduced response to acute vasodilator testing.

The aim of this study was to evaluate hemodynamic parameters assessed by right heart catheterization and genetic status in a large cohort of patients.

Methods: Detailed family history and pedigree analysis have been obtained from each investigated patient. We compared the age at diagnosis and hemodynamic parameters between carriers and non-carriers of BMP2-mutations. In non-carriers with familial aggregation of PAH further genes/gene regions as the BMP2 promoter region, the ACVRL1 gene, Endoglin and SMAD8 have been analysed. Mutation analysis was performed by sequence analysis of all coding exons and surrounding intronic regions and Multiplex Ligation Dependent PCR Amplification (MLPA).

Results: Of the 231 PAH index-patients 22 revealed a confirmed familial aggregation of the disease, 209 patients had sporadic IPAH. In 49 patients mutations of the BMP2 gene have been identified (in 86.3% of patients with familial PAH and in 14.3% of sporadic IPAH). Twelve BMP2 mutations and 3 unclassified sequence variants have not been described before. Mutation-carriers have been significantly younger at diagnosis than non-carriers and had a more severe hemodynamic compromise.

Conclusion: This study identified new BMP2-mutations, which have not been described before and confirmed previous findings, that mutation-carriers are younger at diagnosis with a more severe hemodynamic compromise in a large prospectively assessed cohort of PAH-patients.

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## P-ClinG-057

### **RCL2-fixed paraffin embedded tissue enables quantitative MGMT – DNA methylation testing of glioblastomas**

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Glioblastoma multiforme (GBM) is a rare malignant brain tumour. GBM originate from astrocytic cells and is responsible for 12-15% of all brain tumours. The peak of incidence is between 55 and 74 years and survival rates are poor. Mortality rates in patients with diagnosed GBM are over 70% within the first year and more than 97% within 5 years [1, 2]. Mean survival rates are about 12-18 months.

The current standard therapy protocol involves maximal safe neurosurgical tumour resection and postoperative combined chemoradiotherapy with the alkylating agent temozolomide. Although a benefit of concomitant chemotherapy was only observed in patients with intratumoural promoter methylation of the O6-methylguanine-methyltransferase gene (MGMT). Therefore MGMT methylation testing could serve as useful biomarker for therapy response prediction. Although most MS-PCR-assays are currently qualitative methylation tests, reliable quantification of MGMT-methylation would be preferable and necessary for elucidation of any correlation of methylation-ratios with therapy response.

We investigated 1) using fresh frozen versus paraffin embedded tissue using either RCL2- and formalin-fixed samples, for 2) development of a methylation sensitive restriction enzyme (MSRE) based method for detection and quantification of MGMT methylation in tumour-DNA of GBM patients, and 3) defined additional methylation markers capable of distinguishing GBM from normal brain tissue. This latter panel is thus suitable as sample specific internal reference for quantifying MGMT methylation with respect to the tumour content of GBM samples known being invasive. For developing the MSRE assays and internal reference markers we employed autopsy brain tissue (5 different brain regions) derived from 5 individuals without any neoplastic disease and GBM tissue (n=21).

DNA-methylation values of RCL2-fixed paraffin embedded samples were identical with values when using DNA from fresh frozen samples. Using the GBM samples we confirmed that the MSRE based MGMT-qPCR test is a highly reliable and sensitive (limit of detection 0.12% methylation; cutoff: 0.33%) as well a simple and fast way for MGMT methylation quantification. Upon a methylation screen targeting 360 genes, we identified 4 genes enabling differentiation of GBM versus normal brain tissue. These markers were confirmed by qPCR (upon MSRE digestion); in addition quantitative measures correlated well ( $r=0.85$ ) with quantitative bisulfite deamination methylation values derived from Sequenom's based mass spectrometry assay. Herewith we confirm that quantitative methylation testing on RCL2-fixed samples is reliable and simple using MSRE-qPCR. Hence, our assay is a further step and a contribution to personalized medicine, which allows accurate measurements for improving therapeutic decisions.

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**P-ClinG-058****A family with X-linked mental retardation based on copy-number gain at Xq28***Riedel S.<sup>1</sup>, Reichenbach H.<sup>2</sup>, Eichhorn B.<sup>1</sup>, Fahsold R.<sup>1</sup>*

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Mental retardation is not only a symptom of complex syndromes, rather it is an independent disease known as non-specific mental retardation. Non-specific X-linked mental retardation (MRX) is a very common disorder affecting males more often than females.

Here we report on a family with an X-linked mental retardation based on copy-number gain on chromosome Xq28 including the gene GDI1. The gene encodes for the Rab GDP-dissociation inhibitor 1. GDI1 is an evolutionarily conserved protein playing an essential role in the recycling of Rab GTPases. This enzyme is required for vesicular transport and release of the neurotransmitter. Rab3 proteins are also substrates for the GDI1. Therefore GDI1 is a potential candidate for MRX. The copy number of GDI1 correlates with the severity of clinical features. The development of this copy number variation is caused by different mechanisms. A recombination in this specific region on Xq28 is one underlying mechanism. The index patient, a 2 year old boy, shows slight facial dysmorphic features and a motor and mental retardation. We identified the submicroscopic triplication by MLPA (Multiplex Ligation-dependent Probe Amplification) analysis. Further family members were examined and showed also a gain of copy number of GDI1 with different clinical features.

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**P-ClinG-059****Analyze of T-13910C polymorphism in the LCT Gene in population with high consanguinity***Sadr Nabavi A.<sup>1</sup>, Alizadeh M.<sup>2</sup>*

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**Background and Aim:**

Adult type hypolactasia also known as lactose intolerance is the most common enzyme deficiency in the world. hypolactasia is a common autosomal recessive condition resulting from reduction in the lactase-phlorizin hydrolase (LPH) activity in intestinal cells after weaning phase. Symptoms of lactose intolerance are different in severity, individual sensitivity. Symptoms usually include flatulence, nausea, osmotic diarrhea and abdominal pain. Several SNP (single nucleotide polymorphism) have been identified for lactase persistence and non-persistence traits, C/T polymorphism is the most common SNP which is located on 13910 bp upstream of the lactase gene (LCT) at chromosome 2q21-22. The C/C-13910 genotype associated with lactase non-persistence.

Genetic test of C/T (-13910) polymorphism can be used as a diagnostic test as a first stage screening test during childhood and for adult-type hypolactasia, however the prevalence of adult type hypolactasia is different among populations, in some sub-Saharan African groups, C/T-13910 variant can not serve as a predictor of lactase persistence. In this study we try to validate a genetic test for adult type hypolactasia in Iranian population.

**Methods:**

Number of 30 patients that were suspect to hypolactasia clinically and number of 60 healthy individuals as a control groups were invited to participate. DNA was extracted from leukocytes of peripheral blood; LCT-13910C>T polymorphism was analyzed by PCR-restriction fragment length polymorphism.

**Results:**

In LPH Group allelic frequency for SNP T-13910C (C, T) respectively is 83%, 17% and in control group allelic frequency for C and T is respectively 92%, 7%. ( $p=.035$ ). Also genotype frequency in LPH group (CC, CT, TT) is 65%, 35%, 0%. And genotype frequency in control group (CC, CT, TT) is 87 %, 12 %, 1 %.( $p=0.01$ )

**Conclusion:**

Based on our primary data the frequency of the C allele are high in both groups, probably due to high consanguinity in Iranian population. We are continuing our research by increasing the sample size and use of Hydrogen breath test (HBT), to achieve more data that are applicable.

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**P-ClinG-060****Microarray studies in deletion-based Prader-Willi-Syndrome (PWS)**

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**Background**

Prader-Willi Syndrome results from loss of paternally expressed genes in the 15q11-q13 region, which is caused in 70% by deletions of different lengths. Depending on the proximal breakpoint of this deletion, two subtypes can be distinguished, the smaller deletion (type II) comprising about two thirds of the patients. Because of a quite variable extent of the clinical features even in the same subgroup, it was suggested that microarray investigation might be informative regarding additional submicroscopic anomalies that modify the cognitive and behavioural phenotype of the patients.

**Aim of the study**

Our ongoing study aims to evaluate the potential of high density array technology for fine-mapping of genotypes and identification of possible novel genetic associations in deletion based PWS. Here we report on technical challenges and preliminary results.

**Methods**

After genotyping of PWS patients by methylation specific PCR (MS-PCR) and subtyping by microsatellite analysis and multiplex ligation dependent probe amplification (MLPA) we introduced the Affymetrix Cytogenetics Whole-Genome 2.7 array for detailed characterization of chromosome aberrations at a high resolution level (analysis software: Chromosome Analysis Suite 1.1.0). We also compare various DNA extraction methods with respect to differences in the genotyping results.

**Results**

Fine-mapping of PWS deletions showed subtle differences for the positions of breakpoints 1 and 2 (proximal, defining deletion subtypes I and II, respectively) and breakpoint 3 (distal) between patients of one subtype, but also between DNA samples extracted from the same patient with different methods. To date, of 14 deletion PWS patients tested, one showed major differences in deletion patterns for DNA extracted with Qiagen Puregene (qp) compared to Roche MagNA Pure Compact (mp) and Promega Maxwell 16 Blood DNA Purification Kit (ma). Additionally, in 3 out of 14 patients we detected duplications proximal from breakpoint 1, a region not covered by fluorescence in situ hybridization (FISH) or MLPA subtyping.

**Discussion**

High quality DNA is required to obtain reliable results in microarray technology. Surprisingly, our results indicate that a "classical" salting-out DNA purification protocol appears to be superior to the two automated DNA extraction methods tested. Referring to the additional duplications upstream breakpoint 1, they are likely not significant for the phenotype because isodicentric marker chromosomes proximal to the PWS region were shown to be clinically irrelevant. More genotype-phenotype correlation studies are needed to explain the phenotypic variability in PWS patients and the changing expression of features across the life span. Currently we look for correlations of deletion patterns with psychological outcome of growth hormone-treated PWS patients. These results are still pending.

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**P-ClinG-061****Multiple myxoid neurothekeoma in a 3-generation family**

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Neurothekeomas (NTK) are rare, benign cutaneous tumors of putative nerve sheath origin, first described in 1969 by Harkin and Reed as nerve sheath myxomas. Neurothekeomas usually arise in the skin of the head and neck region and upper extremities with a remarkable predilection for young females. Less frequently, the tumors have been described in extracutaneous sites, such as oral cavity or hypopharynx. Histopathological examination can distinguish three types of tumors: the myxoid or classical type (MyNTK), the cellular type (CNTK) and the mixed type (MiNTK). Fetsch et al. (2007) described more than 170 patients with neurothekeoma, who all were sporadic. The rarity of this skin tumor and the unusual occurrence in a 3-generation family prompted the following report.

Our index patient was a 36 years old male. Since puberty, he noticed disseminated asymmetric papillomatous, partly pale, partly erythematous nodules in the face, hands, knees and feet. He described them as asymptomatic with slow progression of the tumor size, but they are cosmetically troublesome. Histopathologic examination showed a myxoid nerve sheath tumor. Immunohistological studies displayed

strong immunoreactivity for S100, NSE and Vimentin. Treatment with multiple local excisions and laser therapy showed only temporary success. The daughter (8 years) of our index patient only had small nodular lesions on her hands and knees. The mother (54 years) of our index patient had multiple nodular lesions on her hands, nose and oral mucosa as well as in the genital region.

To the best of our knowledge, we could not find familial cases of neurothekeomas in the literature (also no OMIM number). We speculate that the tumors are, similar to those in neurofibromatosis, the result of a second somatic hit of a tumor suppressor gene. As the tumors are asymptomatic in most cases, we presume that many familial cases are not recognized.

Fetsch JF et al. *Am J Surg Pathol* 31 (2007): 1103-1114

Gallager RL, Helwig EB. *Am J Clin Pathol* 74 (1980): 759-764

Harkin JS, Reed RJ in Firminger HI(ed). *Tumors of the peripheral nervous system* (1969): 60-64

Jaffer S et al. *Am J Surg Pathol* 33 (2009): 905-913

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## P-ClinG-062

### **Sweating ability and genotype in individuals with X-linked hypohidrotic ectodermal dysplasia**

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Background: X-linked hypohidrotic ectodermal dysplasia (XLHED), the most common type of ectodermal dysplasia, is caused by EDA gene mutations. Reduced sweating contributes substantially to XLHED-associated morbidity and mortality. To characterize the genotype-phenotype relationship, sweat gland function was assessed non-invasively in XLHED patients and healthy controls.

Subjects and methods: In 36 genotyped XLHED patients and 29 control subjects aged 0-57 years, pilocarpine-induced sweat volume, palmar sweat pore density and palmar skin conductance before and after stimulation were determined.

Results: Among 31 XLHED males, 14 had neither detectable sweat pores nor inducible sweating, 10 showed a few sweat pores but absent sweating, and 7 produced reduced sweat volumes (1-11 µl) as compared with controls (38-93 µl). Two of the low-sweating XLHED subjects had normal sweat pore counts. In all 5 heterozygous females, some sweat was detected, but generally less than in female controls. Basal and stimulated skin conductance readings were reduced in 23 of 24 non-sweating, but only in 3 of 12 low-sweating XLHED subjects. There was no correlation between sweat production and number of missing teeth.

Conclusions: In contrast to prior reports on non-genotyped HED populations, this study confirmed a consistent, quantifiable defect of sweat gland function in male XLHED subjects as a disease biomarker. Among 26 different EDA genotypes, specific mutations were shown to be consistently associated with anhidrosis, implying that systematic mapping of EDA mutations together with the analysis of objective clinical data may help to distinguish functionally crucial mutations from those allowing residual activity of the gene product.

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## P-ClinG-063

### **Second report of a 17q11.2 microduplication with phenotypic features of Rubinstein-Taybi syndrome**

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Rubinstein-Taybi syndrome (RSTS) is a genetically heterogeneous disorder characterised by postnatal growth retardation, microcephaly, specific facial features, broad thumbs and halluces and mental retardation of variable degree.

Using CREBBP and EP300 mutational analysis 50-70% of patients show a mutation, suggesting genetic heterogeneity. Gervasini et al. (2010) reported seven out of 26 (27%) microduplications and microdeletions in patients clinically diagnosed with RSTS.

We report on a 14-month-old boy diagnosed elsewhere with RSTS based on the combination of microcephaly, broad halluces, generalised muscular hypotonia, and marked global developmental delay. He also had minor facial dysmorphisms including flat midface, broad nasal base, and low set dysplastic ears. The distal phalanges were missing on both little fingers. Family history was negative for RSTS, but his mother and one of his three maternal half-sisters had a learning disability, and his other half-sisters had mental retardation. His half-sisters lived elsewhere and were not available for study. Chromosomal analysis revealed a normal male karyotype of 46,XY. MLPA and sequence analysis of the CREBBP and EP300 genes was normal. High resolution SNP-microarray analysis (Affymetrix 6.0) identified a microduplication of

3.14 Mb at chromosome 17q11.2 spanning from 24,552,217—27,692,532 (hg18, NCBI build 36.3, March 2006), which included the complete common NF1 microdeletion syndrome region. A subsequent microarray analysis of the parents was normal, indicating that the microduplication had occurred de novo. In their report of microduplications and microdeletions in 7 out of 26 patients clinically diagnosed with RSTS, Gervasini et al. (2010) described a man with mental retardation, skeletal anomalies including broad thumbs and halluces and a 400 kb microduplication of 17q11.2 resulting in functional trisomy of only six genes: LRRC37B2, CRLF3, ATAD5, C17orf42, ADAP2 and RNF135 (from 17pter->qter). The microduplication in our patient comprised the same six genes and 29 additional genes including NF1 (gene for neurofibromatosis type 1). In another large family with seven individuals and microduplication of 17q11.2 including NF1 and the aforementioned six genes, the affected showed variable phenotypes from mild learning disability to apparent mental impairment, but no broad thumbs and halluces (Grisart et al. 2008). Haploinsufficiency of the RNF135 gene (OMIM 611358) has been described to be associated with overgrowth, facial dysmorphisms, and learning disability in rare cases (Douglas et al. 2007). Therefore the functional trisomy of RNF135 could be discussed as possible cause of the broad thumbs and halluces and the phenotypic overlap with the RST syndrome. In addition to NF1 and the six genes duplicated in the patient of Gervasini et al. (2008), the microduplication of this patient comprises NUFIP2, TAOK1 (expressed especially in brain), SLC6A4, EVI2A (from 17pter->qter) and further genes that could possibly have contributed to mental retardation and/or other clinical features. SNP-array analyses of additional patients diagnosed with RSTS and normal findings after CREBBP and EP300 mutation analysis have been initiated (results pending).

Douglas et al, Nat Genet, 2007, 39, 963-5

Gervasini et al, Eur J Hum Genet, 2010, 18, 768-775

Grisart et al, Eur J Hum Genet, 2008, 16, 305-311

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## P-ClinG-064

### Variable expression of FMR1 gene in patients with POF syndrome is not dependent on number of (CGG)<sub>n</sub> triplets in exon 1

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In the literature increased FMR1 expression in blood cells has been claimed to be associated with a variable number of (CGG)<sub>n</sub> triplets in the 5' untranslated region (UTR) of FMR1 exon 1. It has also been shown, that if the number is outside its normal range (n=26-34) increased CGG triplet numbers including that of the so called premutation range (n=55-200) have a risk of <26% to impair woman's ovarian reserve eventually leading to primary ovarian insufficiency (POI) and premature ovarian failure (POF).

DNA and RNA samples were therefore isolated from 74 idiopathic POF patients to evaluate in parallel and quantitatively expression of FMR1 in their blood cells and the number of CGG triplets on both FMR1 gene alleles by sequence analyses. FMR1 expression levels were compared to that of women with normal menstruation history after having normalized both to the level of HPRT expression in each experiment.

A large variance of the FMR1 transcript level was found in the leukocyte RNA samples but only in the POF patient population. Surprisingly, this level was not associated with the variance of CGG triplet numbers found on both alleles of FMR1 exon 1 (19<n>90). Some CGG triplet numbers in normal range were associated with a larger increase of FMR1 expression than found in the samples with a heterozygous CGG triplet number in the premutation range.

During fetal female germ cell development the FMR1 encoded protein (FMRP) is known to be expressed in the oocyte. With aid of immunohistochemical experiments we show that in contrast of this during postnatal normal folliculogenesis, FMRP is predominantly expressed in granulosa cells.

Our data suggest that proper function of FMRP in granulosa cells seems to depend on an optimal controlled transcript level. Expression of the FMR1 gene during human folliculogenesis is therefore probably a quantitative trait. If this holds true, the high variation of the FMR1 transcript level found in blood cells of only POF patients might be diagnostic for a functional drawback of FMRP in granulosa cells because these cells control the oocyte maturation process.

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**P-ClinG-065****Affymetrix resequencing array technology - application of RetChip v1.0 for high-throughput DNA testing in Stargardt disease**

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**Introduction:** Degenerative retinal dystrophies are a major cause of blindness and visual impairment in developed countries. The high genetic and phenotypic heterogeneity of these pathologies is a major challenge in offering comprehensive DNA diagnostics. To overcome these difficulties and to provide a platform for fast, reliable and economic DNA testing of patients, we designed a microarray platform, termed RetChip v.1.0 which enables modular and parallel resequencing analysis of the complete coding regions and exonic / intronic boundaries of large sets of retinal disease genes. Here, we report on the results of routine diagnostics in the Stargardt disease module addressing the three genes ABCA4, ELOVL4 and CNGB3, all associated with Stargardt disease, one of the most frequent forms of hereditary macular dystrophy with juvenile onset.

**Methods:** Based on the Affymetrix resequencing array technology, we have designed and implemented a fast and reliable platform for complete or modular sequence analysis of up to 72 retinal disease genes.

**Results:** The potential of the RetChip v1.0 to identify known and novel mutations has been assessed in a project in which samples from 80 Stargardt patients were resequenced with RetChip v1.0. Besides the detection of 39 unique already published mutations or unclassified variants (UVs), 16 novel mutations and 4 UVs were identified.

**Conclusions:** As demonstrated in the analysis of 80 Stargardt patients, the RetChip v1.0 proved to be a powerful tool with an accuracy for base substitutions comparable to the golden standard of Sanger sequencing. The integration of this technology into routine DNA testing will significantly support state-of-the-art patient care bridging clinical and scientific research. It will not only facilitate differential diagnosis but also enable predictive testing, identification of modifier variants and determination of genotype-phenotype correlations for retinal disease phenotype with high genetic heterogeneity.

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**P-ClinG-066****Is the genetic variability in genes of the IL-1 cluster associated with the subgingival occurrence of periodontopathogens?**

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**Background:** Periodontitis is initiated by the subgingival occurrence of periodontopathogens. It is triggered by specific host dependent immune response which is influenced by the genetic predisposition. Polymorphisms in the interleukin 1 (IL-1) gene cluster have been suggested to influence the pathogenesis of periodontitis. **Patients and methods:** A total of 159 periodontitis patients (chronic: n=73, aggressive: n=86) and 89 periodontitisfree controls were included in the study. Polymorphisms IL-1a61537; (rs1800587), IL-1b61538; (rs16944, rs1143634), IL-1 receptor (rs2234650), and IL-1 receptor antagonist (rs315952) were determined by polymerase chain reaction with sequence-specific primers (PCR-SSP). Subgingival bacterial colonization was assessed using a polymerase chain reaction (PCR)/DNA probe test (micro-Ident®). Haplotype block structure was determined using Haploview 4.2. Statistical analyses were performed applying SPSS 17.0 considering dominant, recessive and codominant genetic models. **Results:** In this case-control-study no association between genomic variants of the IL-1 gene cluster and the occurrence of severe periodontitis could be shown. Carriers of the rare genotypes of rs1800587 (pcorr.=0.009), rs1143634 (pcorr.=0.009) and composite genotype (rs1800587+rs1143634) (pcorr.=0.031) had a 2-fold higher risk for subgingival occurrence of *A. actinomycetemcomitans*. In forward stepwise binary logistic regression analyses considering age, gender, smoking, approximal plaque index as potential confounders these significant associations were proven. **Conclusions:** Despite the genetic background of IL-1 gene cluster could be shown to be associated with subgingival colonization of *A. actinomycetemcomitans* there is no evidence that it is an independent risk indicator for periodontitis.

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**P-ClinG-067****Deletion of 2p24.3-23.3 mimics Coffin Lowry syndrome associated with overgrowth***Shoukier M.<sup>1</sup>, Schröder J.<sup>2</sup>, Zoll B.<sup>1</sup>, Brockman K.<sup>3</sup>, Burfeind P.<sup>1</sup>*

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Interstitial deletions of chromosome 2p are a very rare event. We are aware of five cases published previously (Francke et al. 1976, Zou et al. 2007, Lo-Castro et al. 2009, Türköver et al. 2009 and Rajcan-Separovic et al. 2009). Here we report on a 13 year-old male patient presented with severe psychomotor retardation and dysmorphic features reminiscent of those found in Coffin Lowry syndrome (CLS), but also with overgrowth and dilatative cardiomyopathy. In our patient a heterozygous deletion of approximately 8.97 Mb with breakpoints in bands 2p23.3-24.2 was detected by array-comparative genome hybridization (array-CGH). The deletion was confirmed and the breakpoints were narrowed down by using quantitative real time PCR. The subsequent microarray analysis of the proband's parents revealed normal chromosomes 2, thus indicating a de novo deletion in the child. The deletion of 2p23.3-24.3 comprises 66 genes and hypothesised transcripts. Among these we suspect DTNB (dystrobrevin-beta, OMIM 602415) and FKBP12.6 (OMIM 600620) as candidate genes responsible for mental retardation and dilatative cardiomyopathy in our patient, respectively. DTNB is known to be part of the dystrophin-glycoprotein complex. Its expression is markedly reduced in patients with Duchenne muscular dystrophy and is supposed to be relevant for cognitive dysfunction in this disorder (Blake et al. 2008). The FKBP12.6- gene has been found to be necessary for stabilization of calcium release channels in myocardial cells and knock-out mice show exercise-induced cardiac ventricular arrhythmias. Decreased expression of FKBP12.6 in dogs was found to be associated with arrhythmogenic right ventricular cardiomyopathy (Wehrens et al. 2008).

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**P-ClinG-068****Identification of a new FLNA splice mutation in a girl initially misdiagnosed as tuberous sclerosis***Siebers-Renelt U.<sup>1</sup>, Hehr U.<sup>2</sup>, Bohring A.<sup>1</sup>, Wieacker P.<sup>1</sup>*

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We report on an 18 year old girl suffering from focal epilepsy since the age of 5 years. At the age of 6 years a cerebral CT was performed and the diagnosis of tuberous sclerosis was suspected. Neither the parents nor the 15 year old sister ever experienced seizures. The girl was born preterm (37th week of gestation) and at the age of 10 months a persistent ductus Botalli was ligated. At birth a single neonatal tooth was present. Psychomotor development was mildly impaired. Clinically, on the first presentation at the age of 17 years no depigmented areas of the skin were visible in the Wood light. An MRI of the cerebrum revealed a periventricular nodular heterotopia leading to the diagnosis of a filaminopathy. Hypertelegorism and brachydactyly in addition suggested a mild FLNA associated skeletal manifestation. Mutation analysis of the FLNA gene identified a heterozygous 4bp deletion including the last two bp of exon 20 and the first two bp of intron 20. Although this mutation (c.2943\_2944\_+2delGAGT) has not been described previously, it is presumably disease causing because it comprises the splice donor site. Since it is well known that patients with FLNA mutations are prone to stroke and other cerebral vasculopathies, a Doppler investigation of the cerebral arteries was performed with normal results. Our case illustrates that MR imaging is superior to CT in the detection of neuronal migration disorders. Moreover, it emphasizes the need of a subtle workup of anamnestic and clinical details because minor findings like the neonatal tooth in our proposita may be a hint at the correct diagnosis.

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**P-ClinG-069****Homozygous deletion of chromosome 15q13.3 including TRPM1 causes mental retardation, seizures and bilateral amblyopia in siblings***Spielmann M.<sup>12</sup>, Reichelt G.<sup>3</sup>, Hertzberg C.<sup>3</sup>, Mundlos S.<sup>12</sup>, Horn D.<sup>1</sup>, Klopocki E.<sup>12</sup>*

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The heterozygous 15q13.3 microdeletion syndrome (MIM #612001) was first described by Sharp et al. in 2008. The phenotypic spectrum of this microdeletion is highly variable ranging from mental retardation and

epilepsy to complete absence of clinical symptoms. So far three patients with 15q13.3 BP4–BP5 homozygous microdeletions of ~1.5 Mb could be identified. The patients presented with visual impairment, refractory epilepsy, hypotonia, intellectual disability and/or autistic features.

Here we report for the first time a non-consanguineous family with two affected siblings carrying a 15q13.3 BP4–BP5 homozygous microdeletion of ~1.5 Mb. They presented with congenital bilateral amblyopia, which is defined as reduced and uncorrectable vision in a structurally normal eye. Further clinical symptoms included: refractory epilepsy, encephalopathy, severe intellectual disability, absent language development, hand stereotypies and gradually worsening hypotonia leading to feeding difficulties. Dysmorphic facial features included synophrys and bilateral proptosis. Both came to medical attention shortly after birth because of poor visual tracking behaviours, feeding difficulties and muscular hypotonia. The funduscopy showed no fundus abnormalities, however visual evoked potential testing was markedly abnormal with poor electrographic activities and the full-field electroretinography (ERG) revealed a “negative ERG”. It was proposed that the patients suffer from bilateral amblyopia. Seizures developed in infancy and since the age of around 7 years the electroencephalogram in both children showed a therapy refractory bioelectric status epilepticus. Their mother who is suffering from learning difficulties and seizures was identified as a heterozygous carrier of this deletion.

These two siblings like the previously reported cases carry a homozygous microdeletion 15q13.3 of ~1.5 Mb including seven annotated genes: ARHGAP11B, MTMR15, MTMR10, TRPM1, KLF13, OTUD7A, and CHRNA7. The absence of CHRNA7 has been suggested as a cause of refractory seizures. The deletion of MTMR10 and MTMR15 could be the cause for severe hypotonia.

Recently it was reported that mutations in TRPM1 cause autosomal-recessive complete congenital stationary night blindness (MIM #613216). TRPM1 could be localized in human retina to the bipolar cell dendrites in the outer plexiform layer. Interestingly one additional patient was described carrying a homozygous deletion 15q13.3 of 680kb that did not include TRPM1. The patient did not show any signs of visual impairment. Therefore the homozygous loss of TRPM1 is a possible explanation for the congenital bilateral amblyopia of the two affected siblings presented here. All three published cases so far that share the homozygous deletion of TRPM1 showed also severe visual impairment. None of the heterozygous patients were reported to be affected. In the light of these new findings TRPM1 seems to be an interesting candidate gene for congenital bilateral amblyopia.

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## P-ClinG-070

### **Missense mutations in TMC6 and TMC8: A risk factor for epidermodysplasia verruciformis-like skin lesions in HIV-infected patients?**

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Individuals affected by epidermodysplasia verruciformis (EV) have an inherited abnormal susceptibility to infections with human papillomaviruses of the beta genus (beta-HPVs), in particular HPV5 and HPV8. In EV patients infection with these viruses leads to skin lesions from early childhood onwards, which are characteristic for the disease. A clear association between beta-HPV infection and the occurrence of nonmelanoma skin cancer (NMSC) has been observed in sun exposed skin area of EV patients. Malignant transformation occurs in about one third of the patients, usually in their third or fourth decade of life. The exact mechanism of malignant transformation and how the beta-HPVs are involved is still a subject of investigation.

Analysis of families with cases of EV showed that the disorder has a recessive mode of inheritance and displays genetic heterogeneity. Most EV patients (75%) carry a homozygous nonsense mutation in either TMC6 or TMC8, two genes located on 17q25.3. Also frameshift and splice site mutations in these genes have been found in EV-affected individuals. Linkage analysis of two affected families pointed out two other loci on chromosome 2 and the X-chromosome segregating with the disease. There is evidence that also certain missense mutations in TMC6 and TMC8 might predispose for beta-HPV infection, EV and a higher risk for NMSC.

Individuals affected by lymphoma, graft-versus-host disease, and immunosuppression carry an additional risk for the development of EV-like skin lesions. How the genetic background of these individuals figures in these “acquired” forms of EV is unknown. In EV-like skin lesions of HIV patients, the prevalence of HPV infection is significantly increased and unusual HPV types, such as beta-HPVs are often detected. The risk of HIV patients for NMSC is at least fivefold increased compared to the general population.

Here we report the case of a HIV-positive patient with EV-like skin lesions and mutations in TMC6 and TMC8. Two are missense mutations, thus possibly affecting functionality of proteins through amino acid

exchange. Interestingly one of these mutations was found in the only HIV patient whose TMC6/8 genes were analyzed for mutations before. Additionally the same mutation has been shown to increase the susceptibility for beta-HPV infection and a form of NMSC in an otherwise healthy cohort.

Although EV-like skin lesions are a rare complication in HIV patients, with only 25 cases described to date, mutation analysis of TMC6 and TMC8 genes of HIV patients with EV-like lesions might allow better understanding of the development of EV and prevent cases of NMSC in patients with acquired immunodeficiency.

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## P-ClinG-071

### **Characterization of 175 children with specific language impairment in the context of genetic background**

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**Background:** Specific language impairment (SLI) is defined as an unexplained failure to acquire language skills despite normal intelligence and adequate educational opportunity. The diagnosis of SLI requires a significant discrepancy in the child's verbal and nonverbal abilities and the absence of any additional disorder. Affected children have problems articulating speech sounds, expressing themselves verbally and comprehending the speech of others. The majority of SLI studies have investigated children at the preschool and school age. Here we propose a simplified approach to diagnose SLI in children as young as three years. Furthermore we demonstrate the evidence for genetic background in a cohort of young children with SLI.

**Methods:** We have investigated 175 individuals with SLI who fulfill one of the following criteria: delay in first spoken words, reduced vocabulary, low speech production, articulation deficits, and/or dysgrammatism. Children with intellectual disability or other co-morbidity were excluded. All children were assessed for speech production (AWSTR) and language comprehension (SETK) as well as for non-verbal IQ. A detailed personal and family history of each individual was recorded.

**Results:** The first assessment of children with SLI took place at the mean of 45 months. More than one third of individuals were boys (73%). About 79% of children had a delay in first spoken words, 42% did speak any word at the age of 18 months. This delay in speech abilities has occurred gender independently. Vocabulary was reduced in all children: 61% had a vocabulary of <10 words at the age of 24 months and 68% a vocabulary of <60 words at the age of 36 months. Familiarity was mentioned in the vast majority of index patients. Overall 41% of the children had a relative with SLI, 34% with SLI and dyslexia and 5% with dyslexia. A first relative has suffered from language disorder in 46%, a second relative in 22% and a first and second relative in 7%. As the children became older and started to speak, a majority of them have developed impairment in grammar, articulation and/or phonology.

**Conclusion:** We demonstrate that diagnosis of SLI is possible and reasonable in young children. These children are well recognizable by a characteristic pattern of their language impairment. Family history and the uniform impairment of language in the face of different environmental conditions indicated the importance of genetic factors in SLI which can now be identified potentially by genome sequencing approaches.

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## P-ClinG-072

### **A comprehensive molecular genetic workup for autosomal recessive forms of Charcot-Marie-Tooth disease**

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Autosomal recessive forms of Charcot-Marie-Tooth disease (AR-CMT) account for less than 5 % of the CMT families in Western countries. However, in communities with a high proportion of consanguineous marriages, AR-CMT constitutes 30-50 % of cases. AR-CMT is genetically heterogeneous with more than a dozen known genes and loci. The identification of an ever increasing number of genes associated with AR-CMT requires an economic strategy to characterize the gene defects in families affected with the disease. We performed a systematic genetic screening of 96 AR-CMT families by a combination of linkage analysis, homozygosity mapping and sequencing. Genotyping of polymorphic markers for 14 AR-CMT loci reduced the number of genes to be sequenced to one or two candidates in 60 % of the families. Subsequent mutation analysis allowed the identification of disease causing mutations in GDAP1, MTMR2, SBF2 (MTMR13),

SH3TC2 (KIAA1985), NDRG1, PRX, IGHMBP2 and FGD4. SH3TC2 (KIAA1985) mutations were by far the most frequent identified cause of AR-CMT in our cohort. Overall, we determined the genetic defect in 37 families, giving a mutation detection rate of 38 %. Our strategy appears to be an efficient and effective means for gene-based molecular diagnosis of AR-CMT.

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#### **P-ClinG-073**

### **Novel GDI1 mutation in a large family with non-syndromic X-linked mental retardation**

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X-linked mental retardation (XLMR) is a heterogeneous disorder, and mutations in more than 90 genes have been associated with XLMR to date. We report on a large multi-generational German family in which the affected male family members suffered from non-syndromic mental retardation, i.e. they had neither abnormal body measurements nor any other clinical problems. Molecular genetic analysis revealed a frameshift mutation in GDI1 (c.1185\_1186delAG; Ser396ProfsX15) that co-segregated with the disease. GDI1 encodes for a Rab GDP-dissociation inhibitor, and only 3 families with GDI1 mutations have been reported so far. The present family confirms the lack of additional features in patients with GDI1 mutations, which renders a clinical diagnosis of GDI1-associated XLMR impossible. Thus, this family not only broadens the spectrum of GDI1 mutations but also emphasizes the need for parallel testing of all known XLMR genes in non-syndromic families.

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#### **P-ClinG-074**

### **UBR1 Missense Mutations and Oligosymptomatic Johanson-Blizzard Syndrome**

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Johanson-Blizzard syndrome (MIM #243800) is a very rare disorder that is inherited in an autosomal recessive manner. The main feature – exocrine pancreatic insufficiency – causes malabsorption and postnatal growth retardation. Further abnormalities include hypo-/aplasia of alae nasi, dental anomalies, sensorineural hearing loss, hypothyroidism, aplasia cutis congenita of the scalp, urogenital malformations and anorectal anomalies and variable mental retardation. JBS is caused by homozygous or compound-heterozygous mutations of the UBR1 gene. UBR1 encodes for a ligase of the N-end rule pathway, called ubiquitin-protein ligase E3 component N-recogin 1.

In literature, 20 different mutations have been described so far. The vast majority of them are nonsense, frameshift, or splice site mutations predicting complete loss-of-function of the gene product. By direct sequencing we have identified 32 novel mutations in 21 unrelated patients, including 15 novel missense mutations. Herein we specifically review on genotype-phenotype correlation in patients carrying novel and previously published missense mutations. All these missense changes regarded as causative mutations affect highly conserved amino acid residues and are predicted to be damaging to the UBR1 protein. We constructed mutants of the yeast *Saccharomyces cerevisiae* Ubr1 that were counterparts of three missense JBS-UBR1 alleles. Thereby, we could demonstrate that one of them (H160R) was inactive in yeast protein degradation assays, the other one (Q1224E) was nearly but not quite inactive, and the third one (V146L) exhibited a decreased but significant activity. These findings correlated with the phenotypic severity in the patients carrying the respective mutations.

15 Patients with missense mutations on at least one allele were compared regarding their clinical symptoms with the group of patients carrying biallelic nonsense or frameshift mutations. In both groups 100% of the patients had exocrine pancreatic insufficiency and dental defects (oligodontia of permanent teeth), thus identifying these features as the most consistent symptoms in JBS that may also serve as minimal clinical criteria for the diagnosis. In contrast, facial anomalies including hypoplasia of the nasal wings were quite subtle in some patients with missense mutations and deafness, short stature and mental retardation were observed in 50%, 53%, and 36%, respectively, versus 91%, 89%, and 100%, respectively, in patients with presumed complete UBR1 deficiency.

In conclusion, these findings indicate that at least some of the UBR1 missense mutations observed in patients with JBS encode mutant proteins that retain residual enzymatic activity. The presumed residual function appears to be the main modifier of the clinical phenotype. In oligosymptomatic cases that we have found to be strongly associated with non-truncating UBR1 alleles, the clinical diagnosis may be challenging. The combination of exocrine pancreatic insufficiency and oligodontia in a patient should be regarded as minimal criteria for the diagnosis of JBS.

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**P-ClinG-075****De novo duplication 17q21.33 in a patient with Dubowitz syndrome.**

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Dubowitz syndrome (DuS) is a rare syndrome characterized by pre- and postnatal growth retardation, microcephaly, distinct facial appearance, mild to moderate developmental delay with predisposition to eczema, and immune defect.

To date over 150 patients with DuS have been reported in the literature. However, the cause of this disease is still unknown. Since in about 10 % of all cases two or more siblings are affected, DuS is presumed to be an autosomal-recessive disorder.

Here we report on a four year old girl with the working diagnosis of DuS and a de novo 65,9 kb microduplication 17q21.33 in the immediate adjacency of the TOB1 gene (arr 17q21.33 (46.302.176-46.368.103)x3; NCBI36/hg18). TOB1 is considered to be a potential cell growth regulator and shows a growth suppressive activity when expressed in tissue culture. Mice carrying a target deletion of the Tob gene had an increased number of osteoblasts leading to a greater bone mass. The overexpression of human TOB1 may potentially lead to the reduction of cell numbers consequently causing growth retardation that renders the TOB1 gene an attractive candidate for DuS.

Expression analysis of TOB1 is in progress in the patient and her parents. The investigation of other unrelated DuS patients is necessary to clarify the causative role of the TOB1 gene in this phenotype.

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**P-ClinG-076****Estimation of PKHD1 deletion frequencies in a patient cohort with one previously detected sequence alteration by MLPA analysis**

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Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in the PKHD1 gene. The main characteristics of the disease are massively enlarged kidneys and congenital hepatic fibrosis. The PKHD1 gene is located on chromosome 6p12 and encodes a membrane protein called polyductin. Detection rates in the causative gene depend on the molecular genetic method of choice and the composition of the cohort. The overall mutation detection rate by DHPLC (denaturing high-performance liquid chromatography) and direct sequencing is about 42-87% depending on the clinical selection criteria. As reported in the literature in about 74% (n=75/101) of the patients with typical clinical features of ARPKD two clearly pathogenic mutations can be detected in the 66 exons of the longest open reading frame and the adjacent intronic regions. Nevertheless in about 26% (n=26/101) of the patients only one pathogenic mutation can be detected. In these patients the second change might be located in regulatory elements and/or intronic regions distant from the coding region that are not covered by routine direct sequencing. Another reason for the detection of only one mutation can be large deletions that cannot be detected by direct sequencing. In the past we established quantitative PCR (qPCR) approaches to recover these changes. However, as the detection rates were low and the procedure was time consuming we did not include this procedure into our routine diagnostic approach. Meanwhile Multiplex Ligation-dependent Probe Amplification (MLPA) is the method of choice to detect these changes. Therefore we analyzed our current cohort with previously only one confirmed change in the PKHD1 gene by MLPA analysis (Kits P-341 and P342 purchased from MRC-Holland) to determine the deletion frequency in the PKHD1 gene in these patients by this reliable and comfortable technique. In control patients we could confirm the deletions previously detected by qPCR with MLPA analysis. Although the analysis is still ongoing we have detected only one exonic deletion in our cohort of about 30 patients so far. We therefore confirmed the results generated with other techniques and those from other groups that exonic deletions play a only minor role in ARPKD pathogenesis.

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**P-ClinG-077****HDAC4 haploinsufficiency causes a recognizable pattern of facial dysmorphism and mild developmental delay**

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Chromosome deletions 2q37 cause a disease spectrum known as Albright hereditary osteodystrophy-like syndrome (AHO-like) or brachydactyly-mental retardation syndrome (BDMR, MIM 600430) which includes neurological symptoms, congenital heart defects, obesity, dermatitis and brachydactyly type E. Recently, HDAC4-haploinsufficiency has been identified as the critical genetic mechanism responsible for BDE, developmental delay and behavioral abnormalities in patients with 2q37 deletions by Williams et al. (2010). We report on a family with affected members of three generations carrying a chromosomal microdeletion 2q37 detected by array CGH analysis and verified by FISH analysis. This microdeletion comprises 800 kb and affects the HDAC4 (histone deacetylase 4) gene. HDAC4 represses transcription when tethered to a promoter by altering chromatin structure. It is known to be involved in skeletogenesis as well as in chondrogenesis and seems to have an impact on RAI expression the critical gene for Smith-Magenis-Syndrome. The index patient, a female infant, was initially referred to us because of motor development and growth delay as well as behavioral problems. Her craniofacial abnormalities include plagiocephaly, round face with midface hypoplasia, deep set eyes, high arched eye brows, mild ptosis, low set and posteriorly rotated ears, thin upper lip and pointed chin. Interestingly, the index patient, her affected mother and grandmother do not reveal a clear BDE phenotype as observed in other patients with HDAC4 haploinsufficiency but instead present distinct facial dysmorphism signs with some of the above mentioned features becoming more pronounced with age. To delineate a recognizable facial dysmorphism pattern associated with HDAC4 haploinsufficiency we compared these patients with other individuals carrying 2q37 chromosome deletions. Common facial features are round face with midface hypoplasia, deep set eyes, ptosis or narrow palpebral fissures, and high arched eye brows. As the BDE phenotype does not seem to be a fully expressed and consistent feature in patients with HDAC4 haploinsufficiency the distinct pattern of facial anomalies can facilitate syndrome diagnosis.

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**P-ClinG-078****Monozygotic twins discordant for neurofibromatosis type 1 due to a postzygotic NF1 gene mutation**

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Neurofibromatosis type 1 (NF1; MIM#162200) is a hereditary cancer predisposition syndrome characterized predominantly by neurocutaneous pathology. The main hallmarks of the disease are dermal neurofibromas (benign nerve sheath tumours), pigmentation changes such as café-au-lait spots, axillary and inguinal freckling, and Lisch nodules of the iris. Other complications occurring at increased frequency in NF1 include optic gliomas, malignant peripheral nerve sheath tumours, skeletal anomalies and learning difficulties. NF1 is caused by mutations of the NF1 tumour suppressor gene located at 17q11.2. Although NF1 is inherited in simple autosomal dominant fashion with complete penetrance, highly variable clinical expressivity is frequently observed, manifesting as marked inter- and intra-familial variation in relation to the major clinical features of the disease and the co-occurrence of complications. Somatic mosaicism resulting from a postzygotic mutation within the NF1 gene is a known cause of phenotypic variability in neurofibromatosis type 1 (NF1). We report here on a pair of monozygotic, dichorionic twins who are phenotypically discordant for NF1. DNA sequence analysis indicated a postzygotic NF1 mutation [c.4108C>T (p.Q1370X) located in exon 23-2] present in the affected twin II/1, but apparently absent in his unaffected brother, was responsible for the phenotype discordance. Heterozygosity for flanking SNP and microsatellite markers rendered it most unlikely that the observed mosaicism for the mutation was due to mutation reversion brought about by either gene conversion or mitotic recombination. Instead, we conclude that the twinning event, which would have taken place within three days after fertilization, must have preceded the c.4108C>T mutation which is therefore predicted to have occurred during the cell divisions of the blastocyst stage, leading to somatic mosaicism with normal cells lacking the mutation. This is the first reported case of monozygotic twins discordant for NF1 in whom mosaicism for a postzygotic NF1 gene mutation has been observed in the affected but not the unaffected twin.

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**P-ClinG-079****Autosomal dominant sensorineural hearing loss in a large German family due to a novel splice-site mutation in MYO6**

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Mutations in different unconventional myosins have been identified in hereditary hearing loss. Mutations in Myosin VI (MYO6) are a rare cause of autosomal recessive (DFNB37) or autosomal dominant non-syndromic hearing loss (DFNA22). Until now, six families each with a unique mutation in MYO6 have been described with autosomal dominant hearing loss. Mutations in MYO6 lead to non-syndromic hearing loss but cardiomyopathy and retinopathy with incomplete penetrance were also discussed as associated clinical features in two families. One patient with autosomal-dominant MYO6-associated hearing loss underwent cochlear implantation with favourable results.

We describe a large German family in which 23 affected individuals in three generations had autosomal dominant hearing loss. We conducted a genome scan and found putative linkage to the three chromosomal regions 3p11.1-q13.31, 20q13.33 and 6p21.1-q22.1, the latter containing MYO6. A novel splice site mutation (c.554-1G>A) leading to different alternatively spliced mRNA transcripts was found. All participating patients showed a non-syndromic, slowly progressive symmetric sensorineural hearing loss which was more pronounced in the higher frequencies. None of the participating individuals or other affected family members were reported to have clinical symptoms indicative for cardiomyopathy. Two family members underwent cochlear implantation with successful postoperative rehabilitation.

Our study expands the knowledge on MYO6-associated autosomal dominant hearing loss. Further studies should focus on the postoperative rehabilitation in patients with cochlear implants and MYO6-associated hearing loss as our results point to a favourable outcome after implantation.

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**P-ClinG-080****Identification of an unusual BBS4 two exon deletion**

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Bardet-Biedl syndrome (BBS) is a heterogeneous autosomal recessive ciliopathy characterized by obesity, polydactyly, retinopathy, mental retardation, hypogenitalism and renal and cardiac malformations. So far, 15 different BBS genes are known. Depending on the clinical findings and the ethnic origin approximately 38% of all patients with Bardet-Biedl syndrome show one of the two hotspot mutations M390R in the BBS1 gene (approx. 22%) or C91fsX in the BBS10 gene (approx. 15%). After exclusion of these hotspot mutations in a consanguineous patient we performed a SNP array analysis (Affymetrix GeneChip®Genome-Wide Human SNP 6.0-Array) to determine the genotype for the known loci because of the broad heterogeneity. Homozygous haplotypes could be detected for the BBS4 region among others. Mutations in the BBS4 gene are responsible for about 2-3% of the cases. We did not detect any mutation in the coding region of the BBS4 gene of the index patient but as in the direct sequencing polymerase chain reaction (PCR) amplification failed to amplify exons 7 and 8 we amplified this region by junction fragment PCR. Sequence analysis of this junction fragment confirmed a novel 2841 bp deletion with breakpoints upstream of exon 7 and downstream of exon 8. This type of mutation is likely to be under-reported because of the difficulty of deletion detection in the heterozygous state by the non-quantitative mutation screening methods that are used in many studies. It is postulated that Alu-elements play a role in the occurrence of deletions since one well conserved region shows similarity to the prokaryotic chi-sequence, which is thought to be involved in the stimulation of recombination in *E. coli*. The breakpoints of the deletion in our patient do not lie within one of these Alu-elements but one of them is located within an LTR-element and the other was found to occur just outside a MER1-element. Both of these elements have previously been associated with deletions in other genes and might be responsible for the deletion in our patient.

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**P-ClinG-081****Molecular analysis in a large cohort of patients with anterior segment dysgenesis***Weisschuh N., Gebhardt M., Wissinger B.*

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Anterior segment dysgenesis (ASD) comprises a heterogeneous group of developmental abnormalities affecting several structures of the anterior segment of the eye. Aniridia, Axenfeld Rieger Syndrome (ARS) and Peters' anomaly represent subgroups of ASD. The incidence is between 1/50000 (for aniridia) and 1/200000 (for ARS). The transmission of ASD is autosomal dominant. Ocular symptoms include iris hypoplasia, irido-corneal adhesions, corneal opacity and increased intraocular pressure. Axenfeld Rieger Syndrome can also be associated with systemic features including maxillary hypoplasia, hypodontia, microdontia, umbilical abnormalities, hearing defects, and congenital cardiac or kidney abnormalities.

Mutations in the genes PAX6, FOXC1, PITX2 and CYP1B1 underlie ASD. We aimed to assess the prevalence of disease-causing mutations in these genes in a cohort of 45 index patients with ASD, mainly of German origin.

The cohort consists of 45 diagnostic referrals for ASD (39 with ARS, 3 with aniridia and 3 with Peter's anomaly), derived from different ophthalmology clinics/genetics centers. Primers were designed to amplify the coding exons and intronic splice site junctions of PAX6 (NM\_000280.3), PITX2 (NM\_153427.1), FOXC1 (NM\_001453.2), and CYP1B1 (NM\_000104.3). The PAX6 gene was sequenced only in patients with aniridia, whereas FOXC1, PITX2 and CYP1B1 were sequenced in all patients with ARS and Peters' anomaly. In addition, quantitative genomic PCR of the PITX2 gene was performed in one patient with ARS.

Altogether, disease-causing mutations were identified in 17 out of 45 patients. A splice site mutation in the PAX6 gene was identified in one of the patients with aniridia. One patient with Peters' anomaly carried a homozygous mutation in the CYP1B1 gene. Another patient with Peters' anomaly carried two compound heterozygous mutations in the CYP1B1 gene. Four missense and five nonsense mutations in the FOXC1 gene were identified in nine patients with ARS. Two patients with ARS showed nonsense mutations in the PITX2 gene and two showed missense mutations, respectively. One patient was shown to harbor a heterozygous deletion of the entire PITX2 gene.

In conclusion, genetic defects in PAX6, FOXC1, PITX2 and CYP1B1 underlie 40% of our ASD cohort. However, this mutation prevalence might be an underestimation, since our mutation screening was based solely on sequencing, except for one patient, where quantitative genomic PCR was performed for the PITX2 gene. Recent mutation reports however indicate that mid-size gene deletions are frequently found in PITX2 and FOXC1. Therefore, analyses for mid-size gene deletions should be routinely performed in mutation screenings in ASD patients.

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**P-ClinG-082****Transmission of a novel SPRED1 mutation from father to son with Legius (NF1-like) syndrome***Wieland I.<sup>1</sup>, Wand D.<sup>2</sup>, Muschke P.<sup>1</sup>, Zenker M.<sup>1</sup>*

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SPRED1 mutations (MIM 609291) have been identified in several patients evaluated for neurofibromatosis 1 (NF1, MIM 162200) but without identifiable NF1 mutation. Recently, Legius (NF1-like) syndrome (MIM611431) has been introduced because patients show multiple café-au-lait spots without neurofibromas or other tumour manifestations typically found in NF1. Additional clinical manifestations are axillary freckling, lipomas, macrocephaly, learning disabilities and developmental delay. However, clinical diagnosis of Legius syndrome is difficult to make, especially in children, and needs confirmation by detection of a SPRED1 mutation. Like NF1, Legius syndrome is inherited in an autosomal dominant manner and pathologic allelic variants have been identified throughout the SPRED1 gene. They comprise primarily loss-of-function mutations including nonsense, frameshift and splice site mutations. We report a one-year old boy with several café-au-lait spots and no family history of classical NF1. Sequence analysis of DNA from peripheral blood cells including the entire NF1 coding region and multiplex-ligation probe amplification (MLPA) using two probe mixes for NF1 (MRC Holland) revealed no pathologic sequence variant and no deletion of NF1. Sequence analysis of the SPRED1 gene, however, revealed heterozygous frameshift mutation c.1151\_1152delAG (p.E384GfsX5) in the last coding exon. Evaluation of the parents identified the father to be clinically affected by Legius syndrome and to carry the mutation, which he transmitted to his son. SPRED1 encodes a member of sprouty (SPRY)-related proteins that are negative regulators of the Ras/ERK signalling pathway. Mutation p.E384GfsX5 occurred in the C-terminal SPRY domain that may lead to a

truncated Spred1 protein acting in a dominant-negative fashion. Alternatively, haploinsufficiency may be the predominant mechanism as has been proposed for other truncating mutations in SPRED1.

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#### **P-ClinG-083**

### **Clinical variability in two cases with interstitial de novo deletions of chromosome 10q21 is explained by non-overlapping microdeletions in molecular karyotyping**

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An interstitial deletion in 10q21 is very rarely described. We report on two patients with de novo deletions in 10q21 presenting with intellectual disability (ID) as well as variable other anomalies. The first patient, a 13 year old girl, presented with mild ID. Walking age was 20 months and speech development was also delayed. During her first days of life cardiac arrhythmia was noted but wasn't observed later on. Apart of obesity, hyperopia, strabism and asthma bronchiale no organic anomalies were reported. No specific facial dysmorphism was observed. Conventional karyotyping revealed a deletion in chromosome 10q21.2-q21.3. Confirmation and determination of deletion size were performed by molecular karyotyping using a 6.0 Affymetrix SNP array platform (hg18: chromosome 10:64,482,137-70,368,245: 5.9 Mb).

Patient 2, a six year old boy, showed moderate ID. His walking age was 24 months and speech started at the age of four years. After birth a tetralogy of Fallot needed cardiac surgery. Apart from recurrent bronchial infections and a mild ureter stenosis no other organic anomalies were found. Also no specific facial dysmorphism was noted. Conventional karyotyping revealed a deletion in chromosome 10q21.2-q22.1. Confirmation and size determination of the deletion were performed by molecular karyotyping using an Affymetrix Cyto2 array (hg18: chromosome 10:56,155,690-63.989.419: 7.8 Mb).

Marked differences in clinical presentation in both patients can be explained by different, non-overlapping deletions. Our findings indicate the existence of two adjacent critical regions, a 5.8 Mb harbouring a locus for mild ID and a more distal 7.8 Mb region with loci for heart defects and moderate ID. This clinical variability is mirrored in the literature and we hypothesize that different deletions of different loci in 10q21 underlie this variability.

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#### **P-ClinG-084**

### **Loss of distal MLPA Probe in PMP22 Exon 5 in a Patient with HNPP**

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#### Introduction

Hereditary neuropathy with liability to pressure palsies or HNPP is associated with a deletion on chromosome 17p11.2-12 of typically about 1.5 Mb. This is the same chromosomal site where CMT1A has been found to have duplication, and it contains an important myelin gene, peripheral myelin protein-22 gene (PMP22). HNPP results from the loss of a copy of PMP22.

The herein described case shows an isolated deletion of part of exon 5 with unknown dimension upstream of PMP22 gene. Such a deletion is not described so far.

#### Clinical description

A 28 year-old male patient presented with painful paresthesia and numbness of both hands, the right side being more severely affected. There was no history of transient paresis. Nerve conduction studies in the patient demonstrated typical feature of entrapment of both median nerves in the carpal tunnel. In addition, signs of clinically asymptomatic focal compression of the right ulnar nerve in the cubital tunnel and slowing of motor and sensory nerve conduction velocities outside entrapment sites were found on upper and lower extremities.

#### Methods

Multiplex Ligation Probe Amplification (MLPA, P033-B2 from MRC-Holland) was used to detect a probable deletion of PMP22 region. The isolated deletion of one of the exon 5 specific probes (04659-L04464) was first checked by sequencing the region corresponding to the probe specific hybridization and ligation site. Real-time PCR was established using self-designed primer and probes for PMP22 (NM\_000304.2) detecting two different sequences in exon 5 of PMP22 which correspond to the two different adjacent probes in MLPA. Probes were designed as described by Applied Biosystems and FAM or TET 5' labeled and MGB 3' labeled. PCR was running on ABI Prism SDS7000. For calculation comparative CT method was used.

#### Result

The patient carries an isolated loss of the 3' specific probe in exon 5 of PMP22 gene corresponding to a heterozygous deletion. The adjacent 5' probe in exon 5 (01465-L00930) is present in normal dose. No mutation was detected in specific probe and ligation sequences. MLPA analyses and Real-time PCR determination revealed a deletion spanning at least c.435G (p.L145) to a maximum of about 1000 kb, deduced from the next available probe in MLPA which is specific for COX10 gene being present.

#### Conclusion

This is the first time that a partial deletion of the C terminal exon 5 in PMP22 explains the HNPP phenotype. It is not possible to prove this deletion using the normal routine diagnostic procedures for HNPP. Since abnormal duplications of PMP22 region have been found previously it is not surprising that abnormal deletions do exist as well.

The presented case is new with respect to the fact that this deletion is limited to the 15 amino acids C terminal in PMP22 gene. Given the nature of the deletion it was not expected that the phenotype would differ from the phenotype in HNPP patients with the common 1.5 Mb deletion or the rare point mutations of the PMP22 gene.

Ascertainment of deletion size will be done by further real-time PCRs specific for the region in between COX10 and PMP22, array analysis with chip arrays (HumanCytoSNP-12 DNA Array, Illumina, Ca) and also by Vectorette-PCR.

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## P-ClinG-085

### Molecular karyotyping identifies CNVs in individuals with short stature

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Shortness of stature is one of the most common concerns in genetic counselling. Overall, 3 % of the population present with a body height below -2 SD score (SDS). In many cases this causes serious social and medical problems for the affected individuals and their families. After excluding chromosomal abnormalities and recognizable malformation syndromes the underlying cause remains unknown in approximately 80 % of all cases (idiopathic short stature). This aggravates an adequate medical care for most of these patients to date.

To identify novel genetic causes of growth retardation we performed molecular karyotyping in 105 individuals with idiopathic short stature using Affymetrix Genome-Wide Human SNP Array 6.0 and scored copy number variants (CNVs) with a minimum size of 10 kb and 5 markers. We found a total of 3,787 aberrations with an average of 36 copy number changes per individual. To rule out copy number polymorphisms, a conservative screening of all identified CNVs was carried out against an independent control cohort of 820 healthy individuals. Accordingly, aberrations which showed an overlap of more than 95 % in more than 15 control samples were excluded from further investigation.

To narrow down our search for potential pathogenic CNVs, we analysed all remaining 1,499 aberrations based on their gene content. This included known or assumed gene function, expression and murine knock-out phenotypes of all contained genes. A total of 46 CNVs (27 duplications, 19 deletions) in 34 patients were retained for follow-up. All of these CNVs were confirmed by MLPA analysis in affected individuals. Inheritance of 30 CNVs (2 de novo, 14 maternally and 14 paternally inherited) could be verified in 23 families. After re-evaluating the parental growth phenotypes, 10 CNVs (2 de novo, 6 maternally and 2 paternally inherited) in 9 families, including 5 duplications and 5 deletions, were scored as potentially pathogenic. These CNVs cover a range of 34 to 14,229 kb in size with only one aberration smaller than 100 kb.

This is the first report of a systematic approach using molecular karyotyping to identify novel genetic causes of shortness of stature. We identified individual potentially pathogenic CNVs in 9 % of affected individuals, where further functional testing is necessary. In conclusion, our data supports a "rare variant - frequent disease" hypothesis and confirmed a comparable frequency of CNVs as in similar studies of other entities. Screening of additional patients might further substantiate these results.

**Autosomal recessive renal tubular dysgenesis (RTD): A rare cause of Potter-Sequence which is linked to defects of the renin-angiotensin system.**

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Potter Sequence (PS) refers to the fetal consequences of an-/oligohydramnios. Intrauterine renal dysfunction is the major cause of PS most often due to renal agenesis and/or dysplasia, polycystic kidneys, severe form of nephronophthisis type and syndromes like Meckel-, Joubert- or BOR syndrome. In addition, PS may be a feature of chromosomal disorders. A thorough pathoanatomical diagnosis is the most important basis for further molecular evaluation and genetic counselling.

We report on our own observations of RTD as a rare cause of PS, which is characterized by specific pathoanatomical features with normal or only slightly enlarged kidneys, normal cortico-medullary differentiation, absence or paucity of differentiated proximal tubules as the hallmark of the disorder which may be associated with skull ossification defects and widely patent fontanels. While most RTD patients die perinatally, a few surviving patients have been reported in the literature.

RTD can be caused by mutations in genes encoding components of the renin-angiotensin system: 1. angiotensin II receptor type 1 (AGTR1), 2. angiotensinogen (AGT), 3. angiotensin-converting enzyme (ACE), 4. renin (REN), which also can cause autosomal dominant juvenile hyperuricemic nephropathy. Since only about 100 cases of RTD have been confirmed by molecular genetic testing so far, no definite information about the distribution of mutations in these genes can be given. Biochemical analysis of plasma renin activity, active renin, and active ACE-concentration may correlate with the involvement of specific defects.

Hypoperfusion of the fetal kidneys as a consequence of renin-angiotensin system inactivity has also been suggested as the mechanism of RTD by findings in the twin-twin transfusion syndrome in monozygotic twin gestations (in which the donor fetus may develop RTD), major cardiac malformations, and severe liver diseases. RTD with large fontanels has also been observed in fetuses exposed in utero to ACE inhibitors or angiotensin II receptor antagonists.

On the basis of our own observation in five families with six mutations distributed among the three RTD genes AGT, AGTR1 and ACE we will describe an algorithm of genetic assessment of PS as a basis for genetic counseling.

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**P-ClinG-087a****Four novel patients with MEF2C mutations expanding the associated phenotype**

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MEF2C encodes a member of the myocyte enhancer factor 2 (MEF2) subfamily of the MADS (MCM1-agamous-deficiens-serum response factor) gene family of transcription factors. The protein is supposed to play an important role in neurogenesis, and shows high expression levels in brain and a Rett syndrome-like phenotype in conditional knockout mice. Recently, MEF2C was identified as the phenocritical candidate gene for the 5q14.3q15 microdeletion phenotype characterized by profound muscular hypotonia, severe mental retardation and variable neurological and minor anomalies. Point mutations in this gene have been shown to cause the 5q14.3q15 microdeletion phenotype, establishing defects in this transcription factor as a novel, relatively frequent autosomal dominant cause of severe mental retardation accounting for as much as 1.1% of investigated patients. To date only 5 point mutations have been reported.

We now report on four new patients with de novo point mutations in MEF2C detected through screening of 100 patients initially considered as Pitt-Hopkins syndrome (1 mutation), 50 patients initially considered as Rett- or Angelman syndrome (2 mutations) and 15 patients suspected to have MEF2C mutations (1 mutation). While the published and 3 of the 4 novel patients had severe mental retardation with absent speech, one of the new patients with a missense mutation was able to speak several single words at the age of 4 years. Transcriptional reporter assays are currently ongoing to investigate the degree of impairment of the missense mutations.

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**P-ClinG-087b****Identification of a submicroscopic 3q29 deletion in a patient with normal intelligence, initially suspected of having Friedreich's ataxia**

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A 12 year old girl was referred for molecular genetic testing and genetic counselling.

Motor development milestones such as walking were delayed. Balance problems and ataxia were noted in early childhood. Speech became progressively slurred at the age of 11 years. Lack of social skills, lack of empathy, a formal style of speaking and dislike of any changes in routines reminded of an autism spectrum disorder. Subtle facial anomalies include a long face with high forehead, a long nose with high nasal bridge, broad nasal tip and a short philtrum. Additional features were long tapering fingers and shortened metacarpalia IV and V. Brain magnetic resonance imaging (MRI) showed an enhancement of the frontal gyri. Due to bullying at regular school her initially good school performance dropped and she showed an increasing aggressive behavior. Although her mental functions are within normal range she is now visiting a special school, where she is performing well. Initially, Friedreich's ataxia (FRDA) was suspected. In most cases Friedreich's ataxia, an autosomal recessive disease, is caused by a GAA-triplet repeat expansion within both alleles of the frataxin (FXN) gene. Analysis of the GAA-triplet region of FXN in our patient did not confirm the clinical diagnosis of FRDA. Measurements of actylgalactosamine-6-sulfatase and  $\beta$ -Galactosidase in leukocytes showed normal values excluding Gangliosidosis type I, MPS IV-A and MPS IV-B. Conventional chromosome analysis resulted in a normal karyotype but array comparative genomic hybridization (array CGH) using a 44K oligo chip (Bluegenome) revealed a 1.28 Mb deletion within chromosome 3q29 (position: 197,224,783-198,500,991 bp [Ensembl V. 54 / NCBI36]), which was confirmed by fluorescence in situ hybridization (FISH) with BAC probe RP11-296H14 (Bluegenome). The deleted region contains 11 genes with known function including TFRC, OST-alpha, PCYT1A, RNF168, FBXO45, PIGX, PAK2, NCBP2, PIGZ, MFI2 and DLG1. More than 20 patients have been described in the literature with almost identical microdeletions of 3q29. PAK2 and DLG1, the autosomal homologues genes of two known X-linked mental retardation genes (PAK3 and DLG3) and additionally FBXO45 are discussed as candidate genes responsible for autism and impaired mental development in these patients. Dysmorphic facial features are described in most, ataxia only in few patients. The clinical symptoms of the 3q29 microdeletion syndrome however are highly variable even among family members carrying the same deletion. Recently, a deletion carrier has been described with an average full scale IQ-result. Molecular karyotyping using array CGH should be considered even for patients with suspicious clinical phenotype but normal intelligence.

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**P-ClinG-087c****Coronary Artery Dilatation in Noonan Syndrome**

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Noonan Syndrome (NS) is a relatively common congenital genetic disorder with an estimated prevalence of 1 in 1000 to 1 in 2500 live births. Characteristic findings include distinctive facial features, short stature, chest deformity and congenital heart disease. It is an autosomal dominant disorder with complete penetrance but variable expressivity. Although NS is clinically and genetically heterogenous, gene mutations identified in individuals with the NS phenotype are involved in the Ras/MAPK (mitogen-activated protein kinase) signal transduction pathway and currently explain approximately 61% of NS cases. We report on a 11 year old boy with a heterozygous mutation of Exon 8 of the protein tyrosine phosphatase nonreceptor type 11 gene (PTPN11) showing the typical clinical findings: pulmonary valve stenosis, hypertrophic cardiomyopathy without subaortic stenosis, short stature, lower insulin-like growth factor levels, hypothyroidism and no cognitive delays. Also he shows marked bilateral dilatation of the proximal coronary arteries (5,2 mm) without any symptoms of ischemic heart disease. PTPN11 gene maps to chromosome 12q22-pter and encodes the human Shp2, the nonreceptor-type protein tyrosine phosphatase, which is a signal enhancing component of growth factor, cytokine and extracellular matrix receptor signaling. Shp2 is involved in the signaling cascade of the vascular endothelial growth factor and exerts mitogenic effects on vascular smooth muscle cells under the nitric oxide-induced pathway. These mechanism may explain the vascular abnormalities, including coronary dilatation in NS patients. Therefore we recommend genetic

counselling in patients with clinical findings suggestive for NS and the clinical follow up has to be planned according to the specific genotype/phenotype issues.

Coronary Artery Dilatation in LEOPARD and Noonan-Syndrome. A Child Case and Literature Review. Iwasaki Y. et al. Congenit Heart Dis. 2009; 4:38-41 .

Bilateral Coronary Artery Dilatation and Supravalvular Pulmonary Stenosis in a Child with Noonan Syndrome. Ucar T. et al. Pediatric Cardiol 26: 848-850, 2005

## **P-MONOGENIC DISEASE - FROM GENE IDENTIFICATION TO PATHOMECHANISM**

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### **P-MonoG-088**

#### **Molecular karyotyping by array CGH (aCGH) of 40 patients with malformations of cortical development**

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Array CGH was performed on a cohort of 40 patients with distinctive malformations of cortical development (MCD), including: 13 patients with microcephaly (primary microcephaly, MCPH, or microcephaly with simplified gyration, MSG), eight patients with lissencephaly (classical lissencephaly or cobblestone lissencephaly), five patients with polymicrogyria (PMG), three patients with periventricular nodular heterotopia (PNVH), six patients with rare, unclassified cortical malformations and five patients with idiopathic mental retardation (MR). All patients, except those with idiopathic mental retardation, were diagnosed by neuroimaging cMRI. In all patients with recognizable MCDs, targeted mutation analysis via DNA sequencing had excluded mutations in known genes as a cause of the disorder. CGH was performed on one of three different arrays, either the Affymetrix 6.0 SNP, or Agilent 244K or 180K arrays. For all patients, duplications and deletions detected by aCGH were checked for their possible relevance to the patients' phenotypes by comparing them with annotations in the Database of Genomic Variants, the Decipher Database, listed genes in OMIM and other online resources. For 17 patients, aCGH was also performed on the patients' parents to distinguish between inherited and de novo aberrations. A number of CNVs were found to have arisen de novo in the patients. In eight patients de novo, pathological findings could be excluded with certainty. Of the 17 patients for whom the parents were also analysed, only a single small de novo deletion on 11p15.5 that does not coincide with any known CNVs, was found. For three patients in whom an alteration was not de novo, but an autosomal recessive inheritance was likely in the family, genes interrupted by the deletion or duplication were analyzed by DNA sequencing in order to look for a second mutation. No additional mutations were found in the LDB2 and NCAPG genes on 4p15.32 (976 kb duplication), in the SCIN gene on chromosome 7p21.3 (100 kb deletion) or in the SEMA3A, SEMA3D or SEMA3E genes on chromosome 7q21.11 (1,9Mb deletion). In four patients with different MCDs, three deletions and one duplication were found in 22q11.2, within the common Di George/VCFS Syndrome region. No recurrent deletions or duplications at non-CNV loci could be identified within the phenotypic groups, thus emphasizing the extensive heterogeneity of these disorders.

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### **P-MonoG-089**

#### **The impact of mutated Fanconi-associated protein on the mitochondrial proteome**

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The renal Fanconi syndrome is characterized by the failure of the proximal tubules in the kidney to reabsorb small molecules causing urinary loss of amino acids, glucose, electrolytes, phosphate, and low-molecular-weight proteins.

Recently, Kleta and coworkers at the University College London identified a novel form of autosomal dominant renal Fanconi syndrome (LOD-Score > 3) in an extended family by classical linkage analysis. The gene encodes for a peroxisomal protein (Fanconi-associated protein, FAP) and upon mutation at the N-terminal end a negatively charged amino acid is replaced with a positively charged amino acid, which

generates a mitochondrial targeting sequence leading to the erroneous transport of FAP into mitochondria as confirmed by immunohistochemistry.

The aim of the study is the elucidation of the molecular effects of the FAPmut-mislocalization to the mitochondria. The impact of the mislocated FAPmut is analyzed using LLC-PK1 cells, which have been derived from the proximal tubulus. For this purpose, the cells were stably transfected with either FAPwt or FAPmut cDNA using the inducible Tet-On gene expression system. Cells start to express FAP within 24 hours after the addition of tetracycline and are grown for 7 days on glucose-free medium.

By means of differential proteome analysis of purified mitochondria and whole cell lysates the effect of the mutated FAP on the LLC-PK1 proteome is analysed employing two-dimensional gel electrophoresis and multi-dimensional liquid chromatography tandem mass spectrometry in combination with iTRAQ® labelling.

Whole cell lysates were analyzed by means of 2D-DIGE. First and second dimension protein separation were performed on an immobilized pH gradient (pH 3-7; pH 7-11) and a precast 24x20 cm 12.5% SDS-PAGE gel, respectively. Gels were analysed using the Progenesis SameSpots software. Preliminary results of the comparison between the whole cell lysates of the FAPmut and FAPwt transfected cell lines show 31 regulated spots with  $p \leq 0.05$  and a statistical power  $P \geq 0.8$ .

Ongoing characterization of the differentially regulated spots by nano-HPLC/QTOF-MS has led so far to the identification of proteins involved in remodelling of the cytoskeleton, e.g. actin, tubulin, and plastin, as well as proteins that are involved in energy metabolism, such as alpha-enolase and an electron-transfer-flavoprotein.

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## P-MonoG-090

### CHD8 is an interacting partner of CHD7

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Mutations in the CHD7 gene cause CHARGE syndrome, an autosomal dominant malformation syndrome. For a better understanding of the pathogenesis of CHARGE syndrome we searched for CHD7 binding partners by a yeast two hybrid library screen. CHD8 was identified as a putative interacting partner. We confirmed this result by direct yeast two hybrid studies, co-immunoprecipitation and by a bimolecular fluorescence complementation assay (BiFC-Assay). Furthermore we characterized the CHD7-CHD8 binding site and studied the effect of four CHD7 missense mutations (the known mutations p.His2096Arg, p.Val2102Ile and p.Gly2108Arg and the new mutation p.Trp2091Arg) on the binding capacity between CHD7 and CHD8. The direct yeast two hybrid analysis revealed a disruption of the CHD7-CHD8 interaction by the CHD7 mutations p.Trp2091Arg, p.His2096Arg and p.Gly2108Arg. In the Co-IP studies no influence of the mutations on the binding capacity could be observed. It is known that CHD7 and CHD8 are both components of multiprotein complexes. Our results suggests that CHD7 and CHD8 bind directly (shown by our Yeast two hybrid experiments) and indirectly via additional proteins. We identified a protein which interacts with CHD7 and CHD8 and might function as a linker protein. The characterisation of CHD7 containing complexes will help to understand the pathogenesis of the multiple birth defects seen in CHARGE syndrome patients.

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## P-MonoG-091

### Neurologic and ocular phenotype in Pitt-Hopkins syndrome and a zebrafish model

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TCF4 haploinsufficiency was recently identified to cause the Pitt-Hopkins syndrome (PTHS), a disorder characterized by severe mental and motor retardation, breathing abnormalities and distinctive facial features. The *Danio rerio* ortholog, *tcf4*, is expressed in several regions in the CNS and the retina during early development. The neurologic and ocular phenotype was characterized in a PTHS-patient with a uniallelic

TCF4 deletion and in a zebrafish model of the disease. While a cerebral MRI-scan in the PTHS-patient at age one year showed a markedly delayed myelination and ventriculomegaly, no structural cerebral anomalies including no evidence for white matter tract alterations were detected on high-resolution MRI with DTI at age 9 years. Structural ocular examinations showed highly myopic eyes and an increase in ocular length, while spectral domain optical coherence tomography (SD-OCT) imaging showed retinal layers and foveal configuration to be normal. Knockdown of *tcf4*-function by injection of morpholino antisense oligos into zebrafish embryos was performed, and the morphant phenotype was characterized for expression of neural differentiation genes *neurog1*, *ascl1b*, *pax6a*, *zic1*, *atoh1a*, and *atoh2b* resulting in a developmental delay or defects in terminal differentiation of brain and eyes, small eyes with a relative increase in ocular length and an enlargement of the hindbrain ventricle. In summary, *tcf4*-knockdown in zebrafish embryos does not seem to affect early neural patterning and regionalization of the forebrain, but may be involved in later aspects of neurogenesis and differentiation. We provide evidence for a role of TCF4/E2-2 in ocular growth control in PTHS-patients and the zebrafish model.

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#### **P-MonoG-092**

### **NOVEL CANDIDATE GENES FOR MENTAL RETARDATION THROUGH BREAKPOINT MAPPING OF CHROMOSOMAL REARRANGEMENTS**

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Apparently balanced chromosomal rearrangements are useful tools for the identification of causative genes for monogenic disorders. We report patient 1 with mental and psychomotor retardation and facial dysmorphism, carrying a balanced de novo 11;17-translocation [46,XY,t(11;17)(q13.3;q25.1)dn]. Array CGH analysis did not reveal any genetic imbalance. Both breakpoints of the translocation were fine-mapped by fluorescence in situ hybridization (FISH) using BAC and fosmid clones. The breakpoint in 11q13.1 was found to be located in intron 14 of the *SHANK2* gene. *SHANK2* encodes a member of scaffold proteins that are located in the postsynaptic density of excitatory synapses. Recently, heterozygous mutations in *SHANK2* have been described in patients with mental retardation or autism suggesting that disruption of *SHANK2* is responsible for the neurodevelopmental phenotype of patient 1. Patient 2 has mental and psychomotor retardation, postnatal microcephaly, and agenesis of the corpus callosum. Chromosome analysis revealed a de novo 5;9-translocation [46,XY,t(5;9)(q23.2;q22.2)dn]. In addition, a microduplication of ~270 kb in Xp11.22 was detected by array CGH analysis. The patient inherited the duplication from his healthy maternal grandfather indicating that this alteration most likely is not associated with his phenotype. By FISH we mapped the breakpoint in 5q23.2 to a ~20-kb region, not directly affecting a gene. Nonetheless, *SNCAIP* and *CEP120*, located 50 kb proximal and 800 kb distal to the breakpoint, respectively, are attractive candidate genes for the clinical phenotype of patient 2. *SNCAIP* encodes synphilin-1, which shows a predominant neuronal expression and is enriched in presynaptic nerve terminals during development. Synphilin-1 interacts with alpha-synuclein and alpha-synuclein (*PARK1*) was the first gene identified to cause an inherited form of Parkinson's disease. *CEP120* is a centrosomal protein that is predominantly expressed in the brain. Besides its role in maintaining the neural progenitor pool in mouse brain, *Cep120* is also required for microtubule stability and axon formation. In summary, we suggest that a regulatory mutation of either *SNCAIP* or *CEP120* might be of functional importance for the neurological manifestations in patient 2 with the 5;9 translocation.

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#### **P-MonoG-093**

### **Duplicated CYP21A2 gene with a p.Q318X mutation – functional active or not?**

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#### Introduction:

One of the most common inborn endocrine disorder is congenital adrenal hyperplasia (CAH). CAH is inherited in an autosomal recessive manner caused in more than 90 % of cases by mutations in the steroid 21-hydroxylase (*CYP21A2*) gene. The p.Q318X mutation is known to cause the classic salt-wasting (SW) form of CAH and often linked to a duplicated *CYP21A2* gene. This haplotype has been reported to a clinically unaffected phenotype in contrast to a p.Q318X mutation without a duplication of the functional gene.

#### Patients and Methods:

We report a family with two affected children suffering from hirsutism, acne and accelerated bone age.

Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding regions and corresponding exon-intron boundaries of the CYP21A2 gene. The Multiplex Ligation-Dependent Probe Amplification (MLPA) SALSA P050-B2 probe mix by MRC-Holland (detecting exon 1,3,4,6 and 8) was used for the detection of duplications and deletions in the CYP21A2 gene.

Results:

PCR and direct sequencing of the CYP21A2 gene from index patients and parents revealed compound heterozygosity for two mutations p.V281L and p.Q318X. A duplication of the CYP21A2 gene could be detected on one allele in the two affected children and the mother by MLPA.

Conclusion:

Both children showed the same genotype with the "mild" mutation p.V281L on the paternal allele and the "severe" mutation p.Q318X with a duplicated CYP21A2 gene on the maternal allele. Because of the non-classical form (NC) of phenotype in both patients, the function of the allele with the p.Q318X mutation with duplicated CYP21A2 gene could not be completely intact. An accurate differentiation of these genotypes and a comparison with the clinical manifestations is very important in these cases, particularly for genetic counselling.

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### **P-MonoG-094**

#### **Two dominantly inherited ataxias linked to chromosome 16q22.1: SCA4 and SCA31 are not allelic**

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Autosomal dominant spinocerebellar ataxias (SCAs) are heterogeneous neurological disorders characterised by cerebellar dysfunction mostly due to Purkinje cell degeneration. Genetically, 30 different loci have been identified so far whereas the corresponding gene has not yet been determined for 12 of them. The chromosomal location for the spinocerebellar ataxia type 31 (SCA31) has been mapped to chromosome 16q22.1. This region is located within the candidate interval for the spinocerebellar ataxia type 4 (SCA4), for which the underlying mutation still has to be discovered. Recently, a complex repeat insertion within the SCA31 critical region was reported to be causative for SCA31. In order to check whether the German SCA4 patients, belonging to one of the two currently known SCA4 families worldwide, exhibit a potential pathogenic mutation at the SCA31 locus, we performed molecular genetic analyses for affected as well as unaffected family members. Based on a nested-PCR approach and direct sequencing, a disease causing mutation at the SCA31 locus could be excluded for the German SCA4 kindred. However, our data impressively demonstrate the genetic instability in this chromosomal region.

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### **P-MonoG-095**

#### **Biochemical characterisation of GORAB, the protein defective in Geroderma osteodysplastica**

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Geroderma osteodysplastica [OMIM#231070] is a rare inherited progeroid disorder characterised by lax, wrinkled skin, joint laxity, jaw hypoplasia and osteoporosis leading to frequent fractures. We demonstrated that mutations in GORAB (formerly SCYL1BP1) are causative for this disease. GORAB is localised at the Golgi apparatus and interacts with the small GTPase RAB6, identifying GORAB as a golgin. Little is known about the cellular function of GORAB and thus about the geroderma osteodysplastica pathomechanism. In a yeast-two-hybrid screening for additional GORAB binding partners we identified an interaction with ARF5. The specific binding to the active (GTP-bound) forms of RAB6 and ARF5 was proven by pulldown-experiments, suggesting that GORAB is an effector of both GTPases. Analysis of truncated GORAB variants revealed that the middle part contains the GTPase binding site. We were additionally able to show that this part of the protein is necessary for the recruitment to the Golgi apparatus. It comprises two coiled-coil domains, which are good candidates for RAB6-Interaction. However, the fact that simultaneous knockdown of RAB6 and ARF5 only partially displaced GORAB from the Golgi compartment implies that additional factors determine its subcellular localisation. Since RAB6 and ARF5 are both key regulators of Golgi trafficking, loss of GORAB can be predicted to alter Golgi-related trafficking and/or posttranslational modification processes.

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**P-MonoG-096****Maturity-onset Diabetes of the Young (MODY): Analyses of the spectrum of mutated genes, description of 19 new mutations and clinical classifications as a basis for a rational optimized therapy**

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Maturity-onset diabetes of the young (MODY) is a subgroup of diabetes mellitus (DM) characterized by an autosomal dominant inheritance and early onset. MODY accounts for about 5% of all cases of diabetes, and is meanwhile diagnosed more often than before since its molecular genetic analysis enters increasingly diagnostic routines. Genetic, metabolic, and clinical heterogeneity is determined by mutations in so far known eleven different genes. In five genes occur mutations that can explain nearly 90% of all cases with MODY. Mutations in the gene coding for the glucokinase gene (GCK/MODY2), a pancreatic intracellular glucose sensor, are the cause of a relatively mild and stable hyperglycemia and reduced sensitivity towards glucose. Complications are unusual. Mutations in four genes encoding the transcription factors hepatocyte nuclear factor (HNF)-1alpha (MODY3), HNF-4alpha (MODY1), HNF-1beta (MODY5) and pancreas duodenum homeobox protein-1 gene (PDX1/MODY4), all expressed in pancreatic beta cells, lead often to a reduced insulin production and consecutively to more severe hyperglycemia. These patients are at risk for diabetes associated complications. The identification of the distinct molecular genetic alterations that are the underlying cause of the specific forms of diabetes is an important prerequisite to optimize the individual treatment of the affected persons.

We present results of the study of 156 patients, that were analyzed concerning variations in five MODY genes (MODY1-5) using exon-specific PCR amplification and sequencing of the coding DNA sequences including exon/intron boundaries. In addition, for the detection or exclusion of deletions and duplications Multiplex Ligation-dependent Probe Amplification (MLPA) was performed. Molecular genetic testing was done by stepwise analyzing MODY2, MODY3 and MLPA as a first procedure, followed by analyses of MODY1, MODY4 and MODY5 when this revealed no indication for a mutation. The cohort of patients was very heterogeneous concerning the previously assigned clinical diagnoses. It encompassed patients pre-diagnosed as type I or type II diabetes mellitus as well as clinical unclear diabetes forms. With the molecular studies for 41 persons (26,3%) mutations in MODY genes could be demonstrated. We found 4 (9,75%), 17 (41,45%), 15 (36,6%), 4 (9,75%) and 1 (2,4%) mutations in the genes for MODY1-5 respectively. Nearly 50% of the detected mutations were previously undescribed variations. Most patients with the genetically proven monogenic DM were incorrectly diagnosed clinically before. The discrepancies of clinical and molecular diagnoses in turn result in differences in treatment thus influencing negatively the individual prognosis of the patients. Proper differential diagnosis by molecular diagnostics guides to the most appropriate treatment and supports the prediction of the clinical course for the affected individuals. On the basis of our results we developed a clinical score and scheme for the pre-selection of patients with DM to increase the efficiency of molecular diagnostics of MODY.

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**P-MonoG-097****Role of mitochondrial dynamics and function in autosomal recessive cutis laxa due to PYCR1 mutations**

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Cutis laxa (CL) syndromes are highly heterogeneous. CL with progeroid features (ARCL2b OMIM #612940) as part of this spectrum is characterised by loose and lax skin, intrauterine growth retardation, corpus callosum abnormalities, mental retardation and a triangular face resulting in a progeroid appearance. Recently we showed that mutations in pyrroline-5-carboxylate reductase 1 (PYCR1) are causative for this disease. PYCR1 is described to be involved in de novo proline biosynthesis and a role in oxidative stress resistance was suggested. We found that PYCR1 localizes to mitochondria and regulates mitochondrial morphology, membrane potential and apoptosis in response to stress.

Here we present seven novel PYCR1 mutations including five missense and two splice site mutations in seven unrelated patients that fulfilled the major criteria for ARCL2b. We used siRNA mediated knock-down of PYCR1 in two cell lines to analyse mitochondrial structure and function. As in PYCR1-deficient patient fibroblasts, we observed a severe fragmentation of the mitochondrial network after PYCR1 depletion. Additionally a substantial decrease of the mitochondrial membrane potential was detectable. Overexpression of wildtype PYCR1 and various mutant forms also had a strong impact on mitochondrial tubulation.

A detailed analysis of the subcellular localization of PYCR1 suggested a presence of the protein in the mitochondrial intermembrane space. Our data suggest a role of PYCR1 in the regulation of the mitochondrial redox state, which influences mitochondrial fusion and possibly also metabolic activity.

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## **P-MonoG-098**

### **A novel nonsense mutation in TUSC3 in a consanguineous Iranian family with autosomal recessive mental retardation**

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The genetic basis of autosomal recessive mental retardation (ARMR) is extremely heterogeneous, and there is reason to believe that the number of underlying gene defects may well reach a thousand or even more. To date, however, only less than 10 genes have been implicated in unspecific/non-syndromic ARMOR (NS-ARMR). As part of an ongoing systematic study aiming to identify further ARMOR genes, we investigated a consanguineous family with three patients suffering from NS-ARMR. By linkage analysis and subsequent mutation analysis we identified three linkage intervals with LOD scores above three. The maximum LOD score of 3.72 was observed for an interval between rs1047950 and rs6991832 on Chr8, which contained the previously identified ARMOR gene TUSC3. Mutation screening in this gene revealed a novel nonsense mutation (c.163C>T [p.Q55X]) within the second exon.

This is the third MR causing defect in TUSC3 to be described and the second independent mutation in this gene observed in a cohort of more than 200 ARMOR families from the Iranian population. This argues for a more prominent role of TUSC3 in the aetiology of this genetically heterogeneous disorder as compared to most of the other so far identified ARMOR genes. Moreover, by increasing the number of patients with different mutations in TUSC3 we are now able to substantiate a common clinical phenotype characterized by severe MR, head circumference in the lower normal or borderline microcephalic range, height in the lower normal range, yet absence of any further clinical problems

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## **P-MonoG-099**

### **Investigating the possible relevance of a stop mutation in ERC2**

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Recessive defects in NRXN1 and CNTNAP2 have recently been found in patients with severe mental retardation and variable additional features resembling the Pitt-Hopkins syndrome spectrum. Recent observations in *Drosophila* indicated that the fly orthologues NrX-I and NrX-IV share a common role in synaptic organization as both proteins i) localize to synapses, ii) determine the level of active zone protein bruchpilot and iii) as overexpression of either protein results in identical morphological changes of fly synapses. As also the vertebrate homologues of *Drosophila* bruchpilot, members of the CAST/ELKs family, are involved in presynaptic function, we considered the human homologues ERC1 and ERC2 as good candidate genes for mutational screening in further patients.

We screened 180 and 268 patients with severe mental retardation for mutations in the human bruchpilot homologues ERC1 and ERC2, respectively. We found a stop mutation in exon 12 of the ERC2 gene in one patient with severe mental retardation that had initially been referred because of suspected Pitt-Hopkins syndrome. Analysis of parental samples revealed that the mutation was inherited from the apparently healthy mother. To rule out a biallelic defect in the patient we first performed molecular karyotyping with an Affymetrix 6.0 SNP array. No aberration spanning the ERC2 gene was found. The possibility of single exon deletions was excluded by MLPA analysis for all coding exons of ERC2 in the patient and her parents. Even though imprinting has not been described for ERC2 we additionally looked for methylation of CpG islands in

the promoter region of ERC2 by bisulfate conversion of the patients DNA and subsequent sequencing. However, no indication of methylation of the CpG islands could be seen.

Further studies on mRNA and protein level are ongoing to further examine a potentially pathogenic role of this mutation.

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## **P-MonoG-100**

### **A novel HRAS substitution (c.266C>G; p.S89C) resulting in decreased downstream signaling reveals a new dimension of RAS pathway dysregulation in human development**

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Costello syndrome (CS) was delineated based on its distinctive phenotype including coarse facial features, severe failure to thrive, mental retardation, cardiac abnormalities and a high risk for malignancies. CS is caused by germline mutations in HRAS encoding a small GTPase which cycles between an inactive, GDP-bound and an active, GTP-bound state. Mutations in >90% affect glycine 12 or 13 and are associated with a relatively homogeneous CS phenotype. The same amino acid substitutions occur as somatic changes in malignant tumors and result in constitutive HRAS activation and increased RAF-MEK-ERK and PI3K-AKT signal flow. A few less common germline missense mutations affecting other HRAS codons were reported in patients with a distinctive, usually attenuated or mild, CS (p.T58I, p.K117R and p.A146T/V), or in individuals with a predominant muscular phenotype (p.Q22K and p.E63K) [1]. These changes were also suspected or proven to enhance HRAS-dependent signaling.

Here we report on a novel heterozygous HRAS alteration, c.266C>G (p.S89C), in a patient presenting with severe fetal hydrops and pleural effusion, followed by a more benign postnatal course including a renal cyst and strabismus. Her apparently asymptomatic father is heterozygous for the same change. In functional studies of COS-7 cells ectopically expressing various HRAS mutants, the binding domains of the HRAS effector proteins RAF1, PI3K, and RALGEF were used to specifically pull-down active HRAS. Upon growth factor-stimulation, co-precipitation of HRAS S89C with any tested effector was decreased compared to wild-type HRAS, indicating a reduced growth factor-dependent activation of HRAS S89C. Accordingly, we detected slightly diminished MEK, ERK and AKT phosphorylation in cells overexpressing HRAS S89C. Thus, p.S89C appears to reduce downstream signaling, a novel consequence of disease-associated HRAS mutations.

The *C. elegans* RAS homologue *let-60* is essential for vulval induction and activating gain-of-function mutations, such as p.G13E, result in multivulval organisms. Notably, expression of *let-60* with the amino acid change p.S89F results in a vulvaless phenotype, thereby supporting our functional data [2].

The decreased downstream signaling effect of HRAS S89C clearly differs from those for typical CS-associated HRAS mutations. Given the patient's benign postnatal course and presence of this change in her asymptomatic father, its harmful consequences may be time limited, with the late fetal stage being most sensitive. Together, these data illustrate the wide functional and phenotypic variability of germline HRAS mutations.

[1] Kerr B. et al., *J Med Genet*, 43, 401–5 (2006); Zampino G. et al., *Hum Mutat*, 28, 265–72 (2007); Gripp KW. et al., *Am J Med Genet*, 146A, 683–90 (2008); Denayer E. et al., *Hum Mutat*, 29, 232–9. (2008); Van der Burgt I. et al., *J Med Genet*, 44, 459–62 (2007).

[2] Beitel GJ. et al., *Nature*, 348, 503–9 (1990); Han M. & Sternberg PW., *Genes Dev*, 5, 2188–98 (1991).

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## **P-MonoG-101**

### **Identification of a novel germline mutation of the HNF1B gene as a cause of MODY type 5**

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Introduction:

Maturity-onset diabetes of the young type 5 (MODY5) also known as renal cysts and diabetes syndrome (RCAD) is one of the less common forms of MODY. MODY5 is an autosomal-dominant inherited disease caused by mutations within the the Hepatic-Nuclear-Factor 1 beta (HNF1B) gene and characterized by various phenotypes including atrophy of the pancreas, renal abnormalities and abnormalities of the reproductive system.

**Patient:**

We report a 22-year-old female patient from Germany, suffered from diabetes and amenorrhea. Her medical record showed that an AICD-implantation was performed nine years ago because of HOCM. She also had a medical history of bronchial asthma. Her mother and other family members are suffered from diabetes as well.

**Methods:**

Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding region of the HNF1B gene, including corresponding exon-intron boundaries. PCR products were sequenced directly. Multiplex Ligation-Dependent Probe Amplification (MLPA) was performed using the SALSA P241-B1 probe mix by MRC-Holland for detection of deletions and duplications.

**Results:**

In the first step deletions and duplications within the HNF1B gene were excluded by MLPA. Sequence analysis of the HNF1B gene showed a novel heterozygous germline mutation p.Leu65Phe.

For clarifying its clinical relevance further investigations were performed. In a healthy control collective of 50 individuals we could exclude diabetes mellitus by medical history and laboratory results. No one showed this mutation.

**Conclusion:**

Genetic counselling and genetic testing of the HNF1B gene should be considered in these families to confirm the diagnosis of MODY and to provide the basis for diagnostic and therapeutic decisions.

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**P-MonoG-102**

**CDKL5 and NGL-1: New insight into the molecular pathomechanism of Rett-related disease entities**

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Mutations in the X-linked gene encoding the kinase CDKL5 (cyclin-dependent kinase-like 5) are a significant cause of severe infantile epileptic encephalopathy predominantly in girls (Kalscheuer et al., *Am J Hum Genet.* 2003; Tao et al., *Am J Hum Genet.* 2004). The clinical consequences of CDKL5 mutations characteristically comprise infantile spasms, early onset seizures and severe mental retardation. Clinically, there is some overlap with Rett syndrome (RTT), and female patients with CDKL5 mutations are often considered as suffering from atypical RTT or the Hanefeld variant of RTT. Other genes associated with atypical RTT are FOXP1 and MEF2C. In addition, Netrin G1 (NTNG1) was found to be truncated by a translocation breakpoint in a girl with atypical RTT (Borg et al., *Eur J Hum Genet.* 2005). The function of CDKL5 protein is largely unexplored. To date, only two interaction partners have been discussed in the literature: the methyl CpG binding protein 2 (MeCP2) and the DNA methyltransferase1 (DNMT1). More recent studies have shown that CDKL5 controls the morphology of nuclear speckles, which are thought to be storage sites for splicing factors, and that the protein is required for neuronal morphogenesis.

Considering the similar phenotypes of patients with mutations in NTNG1 and CDKL5, we hypothesized that the two genes play a role in common pathogenetic processes. Presynaptic Netrin-G1 associates with postsynaptic NGL-1, a leucine-rich repeat containing neuronal adhesion molecule, inducing synaptic differentiation. We have found that Cdkl5 localizes within the postsynaptic compartment and that it co-localizes with Ngl-1 on dendritic spines. Subsequent immunoprecipitation experiments revealed that Cdkl5 and Ngl-1 interact both in vitro and in vivo. Interestingly, knockdown of Cdkl5 altered the Ngl-1 dependent differentiation of dendritic protrusions, indicating a functional interaction of these proteins. Additional preliminary results suggest that NGL-1 is phosphorylated. Ongoing experiments aim to determine if NGL-1 phosphorylation is mediated by CDKL5. Taken together, our findings revealed that CDKL5 and Netrin-G1 belong to the same molecular pathway. CDKL5 may act as an effector of membrane to nucleus signaling, which might be triggered by cell adhesion during neuronal target recognition. Dysfunction of this signaling pathway in patients with CDKL5 mutations may contribute to the clinical phenotype.

**Detection of new SCARB2 mutations in Action Myoclonus-Renal Failure (AMRF) syndrome and evaluation of the role of SCARB2 mutations in isolated AMRF features.**

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**Background:** The action myoclonus-renal failure syndrome (AMRF) is a hereditary form of progressive myoclonus epilepsy (PME) associated with renal failure (RF). AMRF is considered to be an autosomal-recessive disease related to loss-of-function mutations in SCARB2. We studied a German AMRF family showing additional signs of demyelinating polyneuropathy (PNP) and dilated cardiomyopathy (DCM). To test the hypothesis that isolated appearance of individual AMRF features could be related to heterozygote SCARB2 mutations, we performed a mutation screening in SCARB2 in further unrelated patients showing isolated AMRF features.

**Methods:** All exons of SCARB2 were sequenced in eight family members using standard Sanger sequencing. The mutation screening of patients affected by either epilepsy (n= 103, PME or generalized epilepsy), PNP (n= 103), RF (n= 192), or DCM (n= 85) was performed as high resolution melting curve analysis of the SCARB2 exons.

**Results:** A novel homozygous 1bp deletion (c.111delC) in SCARB2 was found by sequencing in three affected homozygous siblings of the AMRF family. A heterozygous sister showed generalized seizures and reduction of nerve conduction velocity in her feet. No mutations were found in the epilepsy, RF or DCM samples. In the PNP sample, two heterozygous carriers of a SCARB2 frameshift mutation (c.111delC) were identified.

**Conclusions:** Our findings indicate that PNP and DCM may be part of the AMRF syndrome. Moreover, they suggest that in rare cases heterozygous SCARB2 mutations may be associated with isolated AMRF-like features. However, SCARB2 mutations do not appear to be a major cause of such features in the general population.

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## P-MonoG-104

### Variable structural brain malformations, including microcephaly with simplified gyration (MSG), caused by 5q14.3q15 deletions including MEF2C

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5q14.3q15 microdeletions spanning and flanking MEF2C as well as intragenic MEF2C mutations have recently been described as a cause of severe mental retardation, epilepsy and muscular hypotonia, with variable brain and other anomalies. With an increasing number of patients described, the clinical presentation of the patients appears to be relatively uniform, however the structural brain phenotypes described are variable. Here we report two unrelated patients with overlapping de novo interstitial microdeletions in 5q14.3, including MEF2C. The deletions were revealed by array comparative genomic hybridization (aCGH) and confirmed by FISH and/or quantitative PCR (qPCR).

Patient 1 is a 2 year-old boy with mental retardation, severe muscular hypotonia and epilepsy. Microcephaly was apparent at birth (OFC 31 cm, -2 SD) and at 15 months (OFC 43 cm, -2 SD). Brain MRI strongly resembled microcephaly with simplified gyration (MSG) with frontal pachygyria, corpus callosum agenesis, hydrocephalus internus, and cerebellar vermis hypoplasia. The patient shows mild dysmorphic features including a broad nasal bridge, telecanthus and wide nostrils; as well as inverted mammillae, a small sacral dermal appendage and a café au lait spot on the back. Array CGH and deletion mapping using qPCR revealed a 4,0 Mb deletion on 5q14.3 spanning the MEF2C and ARDC3 genes.

Patient 2 is a 4 year-old boy with severe psychomotor retardation, autistic behaviour, absence of speech, myoclonic epilepsy, muscular hypotonia, strabismus divergens and mild dysmorphic features. MRI abnormalities were subtle with enlarged pericerebral spaces, a shortened corpus callosum and frontal polymicrogyria. Array CGH and deletion mapping revealed a 1,9 Mb deletion on 5q14.3, including MEF2C and the proximal part of GPR98.

MEF2C has important functions in hematopoiesis, cardiogenesis, neurogenesis and neuronal migration. Furthermore, MEF2C is required for craniofacial development and can lead to cranial and facial dysmorphic features. Although many of the genes included in the 5q14.3 deletions have described functions compatible with some of the pathologies described in the patients, haploinsufficiency of MEF2C alone is apparently capable of producing the full phenotypic spectrum described in patients. Thus the deletions on 5q14.3 apparently do not cause a contiguous gene deletion syndrome and it is not yet clear what other factors modify the variable structural brain phenotype.

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## P-MonoG-105

### Impact of ATP6V0A2 on endosomal transport

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ATP6V0A2 encodes the vesicular H<sup>+</sup>-ATPase subunit a2 which is deficient in autosomal recessive cutis laxa type 2 (ARCL2) Debré type/wrinkly skin syndrome (OMIM #219200). The a-subunit connecting the V-ATPase V1 and V0 domains, is responsible for proton translocation and might also directly mediate vesicle fusion. The a2 subunit has been detected in endosomes and the trans-Golgi network. Its deficiency causes several cellular alterations: a delay of Golgi-ER trafficking after BFA treatment, a disrupted and swollen Golgi network, abnormal cargo vesicles and increased apoptosis rates. A role of a2 in Golgi function is mirrored by the presence of a congenital glycosylation defect (CDG type II) in affected individuals.

Our current work focuses on the suggested involvement of the a2-subunit in endocytosis. We observed a striking dyslocalisation of the endosomal compartment after RNAi-mediated knockdown of a2 in HeLa cells. Comparative immunoblotting of cell lysates detected decreased early endosome antigen 1 (EEA1) protein levels in ATP6V0A2 deficient cells. Since the EEA1 protein has been proposed to regulate formation and fusion of early endosomes we further examined the impact on receptor mediated endocytosis using Transferrin (Tf) and human epidermal growth factor (EGF) as marker proteins. We found an impairment of both endocytotic pathways after internalization upon loss of ATP6V0A2.

This demonstrates that loss of a2, besides glycosylation and secretory cargo transport, also affects receptor-mediated endocytosis. The role of this defect in the mechanism of this complex disorder remains to be determined.

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## P-MonoG-106

### Transcriptional regulation and retinal knock-down of Fam161a, the mouse ortholog of the human RP28 gene

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Retinitis pigmentosa is an inherited degenerative retinal disease leading to progressive loss of vision. The RP28 locus was previously mapped to chromosome 2p11-15 in a consanguineous Indian family (Gu et al. 1999). A complementary approach, integrating Crx chromatin immunoprecipitation (Corbo et al. 2010) and massively parallel sequencing of genomic DNA has led to the identification of the underlying genetic defect. Thus, we and others have identified nonsense mutations in the FAM161a gene in RP-28-associated recessive retinitis pigmentosa (Langmann et al., 2010; Bandah-Rozenfeld et al., 2010). To better understand the role of FAM161A in the degenerative process, our current projects study the retina-specific expression and functional consequences of Fam161a knockdown in the mouse retina. We have identified two isoforms of Fam161a that are prominently expressed in retina and arise by alternative splicing of exon 3a. Luciferase reporter assays and in vitro electroporation of mouse retinas with dsRed reporter plasmids identified a critical Crx-bound intronic region required for retina-specific expression of Fam161a. To model RP28-related loss of function in the mouse, short hairpin vectors targeting Fam161a mRNA were electroporated in the murine retina in vivo. Histological analyses of the retinal structure at different time point and expression profiles of retinal marker genes will be presented.

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## P-MonoG-107

### Tissue-specific effects of novel splice donor and acceptor site mutations in VCAN, leading to Wagner disease

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Purpose: Identification of novel mutations in Wagner patients with vitreoretinopathy and analysis of their effects on accumulation of splice variants in different tissues.

Methods: Genomic DNA from members of two families (French and British) with Wagner disease, whose clinical features had been described previously, was subjected to sequence analysis of VCAN exons and intron boundaries. Splice variants of VCAN were quantitatively analyzed from fibroblasts and blood. RNA analysis was also performed on tissues obtained from the original Wagner family with the mutation c.9265+1G>A.

Results: The French family segregates a novel heterozygous alteration in the conserved splice acceptor site of exon 8 c.4004-1G>C. In the British family, affected individuals carry a novel heterozygous mutation c.9265+2T>A in the conserved splice donor site of exon 8. Levels of splice variants V2 and V3 are elevated depending on the mutation and the tissue. The highest increase (V3: 700 fold) was measured in fibroblasts carrying mutation c.9265+1G>A. In blood, this mutation c.9265+1G>A showed a modest increase of 25 fold, while the mutation immediately next to it, c.9265+2T>A showed substantially higher levels (150 fold).

Conclusion: The two new mutations support the observations that all other known mutations causing Wagner vitreoretinopathy map to conserved splice acceptor and donor sites of exon 8. The amount of

normally occurring splice variants V2 and V3 is elevated to varying degrees. Specific requirements of the splicing machinery may be responsible for the mutation- and tissue-specific quantitative effects.

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## **P-MonoG-108**

### **Nf1 is required for normal skeletal muscle development.**

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Neurofibromatosis type I is a multi system disease caused by mutations in Nf1 gene. Nf1 encodes a Ras-GAP protein, neurofibromin, which negatively regulates Ras signalling. Nf1 diagnostic criteria relate to presence of neuroectodermal origin manifestations: café au lait spots, cutaneous or plexiform neurofibromas, axillary freckling, Lisch nodules, optic gliomas. Additionally, skeletal system is often affected, scoliosis and long bone dysplasias being main cause of considerable morbidity. Reductions of muscle strength and size have been reported, suggesting musculoskeletal involvement. We and others have previously shown using conditional Nf1 gene inactivation in various mouse models that neurofibromin is required for normal bone development. Specifically, Nf1 was shown to be involved in the regulation of proliferation and differentiation of chondrocytes, osteoblast as well as hyperactivation of osteoclasts. We have reported that, Nf1 has also an important role in the process of joint development and maintenance as mutant mice exhibit hip joint fusion, misshaped elbow and knee joints and diminished size of the menisci. Analysis of embryonic development revealed that the fusion of the hip joint occurs at an early developmental stage E13.5, and is caused by failure of the joint cavity formation. Muscle force is required for the joint cavitation. In search for the possible cause of hip joint fusion we investigated muscle development in Nf1Prx1 mice. The measurements of muscle force reveal a dramatic reduction of the average load and maximum load in the mutant mice. Histological analysis of the fore- and hind-limb musculature revealed reduction of the total muscle area, and a concomitant increase of the collagen rich connective tissue area. To further characterise the muscle developmental defect we performed whole mount in situ hybridization with muscle specific probes. Whereas there were no differences in the level of Pax3 and Lbx1 expression (markers of the migrating muscle progenitor cells), we observed reduced expression of the differentiation markers Myogenin and MyoD in the mutant embryos as compared to controls.

Presented data stress an important function of the Nf1 gene for the muscle development. We postulate that the hip joint fusion phenotype is indirectly caused by the defect in the associated muscle group development. The data suggest a possible role of muscle development defects in the genesis of the congenital scoliosis in NF1.

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## **P-MonoG-109**

### **A peculiar recurrent stop mutation in the N-terminal part of the dystrophin gene**

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Duchenne muscle dystrophy is normally caused by out of frame deletions/duplications or stop point mutations in the dystrophin gene. Histologically a dystrophic pattern is seen in the muscle biopsy, and there is an almost complete loss of dystrophin immunohistochemically. In the Westernblot dystrophin expression is strongly reduced or lacking.

We report on 2 families harbouring the same stop mutation c.9G>A/p.Trp3X in exon 1 of the dystrophin gene and an almost normal dystrophin expression in muscle tissue using the routine dystrophin antibodies. Index patient in the first family was a 3 years old boy having a motor retardation; CK value was extremely raised (6000 U/l). The muscle biopsy showed dystrophic changes, in immunohistochemistry, using the routine antibodies, dystrophin was only slightly reduced. Westernblot results were unsuspecting. His maternal cousin (2 y.) has a congenital pulmonary stenosis and his muscle strength was mildly affected, whereas CK value was very high (7000 U/l). Because of the obvious X-chromosomal inheritance, a MLPA deletion/duplication analysis in the dystrophin gene was performed followed by a point mutation search, resulting in a stop mutation c.9G>A/p.Trp3X of exon 1.

A similar situation was observed in the second family with patients in three generations. Muscle biopsy in the index patient showed reduced dystrophin expression, Phenotype could be best described as Becker

muscle dystrophy; there was an adult patient in this family aged 56 years affected by a mild muscle dystrophy.

We observed the stop mutation p.Trp3X in at least 6 independent cases, which makes it one of the recurrent point mutations in the dystrophin gene. In a report from 2009, Gurvich et al. gave a molecular mechanism for this stop mutation in exon 1 causing a mild phenotype: there is a restart of translation at two AUG codons in exon 6. Lack of dystrophin in muscle could only be realized using a special newly developed antibody against the domain encoded by the first five exons.

As a consequence, patients with a Becker- or LGMD-like phenotype and almost normal dystrophin expression in a muscle biopsy should be tested for mutations in the first five exons of dystrophin gene.

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## **P-MonoG-110**

### **Targeted disruption of Fam134b in mice as model for hereditary sensory and autonomic neuropathy type 2B (HSAN2B)**

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We recently identified loss-of-function mutations in FAM134B as causative for severe autosomal-recessive sensory loss in patients, classified as hereditary sensory and autonomic neuropathy type 2 (HSAN2B, OMIM #613115). Loss of touch, temperature and pain perception in the affected individuals causes mutilations of hands and feet and often necessitates surgical intervention. FAM134B belongs to a gene family with three members and is predominantly expressed in sensory ganglia neurons. Functional studies indicated a role for the protein in the shaping of membranes of the Golgi apparatus. To further elucidate the function of Fam134b in the pathogenesis of HSAN, we knocked out the gene in mice by targeted disruption. Initial characterization suggests that Fam134b deficient mice are a valuable model for HSAN2. Further analysis of the KO-mice will focus on the degeneration of sensory ganglia neurons and structural alterations of the ER/Golgi compartment in these cells. Since the Golgi apparatus is the central organelle in the processing and transport of proteins and lipids, Fam134b impairment potentially interferes with the known players in hereditary sensory and autonomic neuropathies. Thus, we will investigate the role of Fam134b in sphingolipid metabolism (HSAN1), endosomal transport (RAB7A associated HSN / CMT2B), WNK1 signaling (HSAN2A) and neurotrophin signaling (HSAN4 and 5). Beside its expression in sensory ganglia neurons, Fam134b is found in Schwann cells. Cell specific ablation of the gene in mice will allow us to assess neuron specific effects of Fam134b loss and the role of Schwann cells in the pathogenesis of HSAN2B.

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## **P-MonoG-111**

### **Investigation of a four-generation pedigree with dyslexia reveals a novel locus on chr4 and points to an involvement of a brain expressed gene in the aetiology of this disability.**

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Developmental Dyslexia is a specific reading and writing disability, affecting about 5 % of the general population. The phenotypic manifestation is characterized by difficulties with phonological awareness, phonological decoding, orthographic coding, auditory short-term memory, and/or rapid naming. There is a compelling body of evidence that developmental dyslexia runs in families and seems to be highly inheritable. Up to now, at least nine dyslexia susceptibility loci have been identified on chromosomes 1, 2, 3, 6, 11, 13, 15 and 18. In addition, six candidate genes (KIAA0319, DYX1C1, DCDC2, ROBO1, MRPL19 and C2ORF3) may play a role in the aetiology of dyslexia. In this study, we investigated a four-generation pedigree where dyslexia follows an autosomal dominant inheritance pattern. The clinical assessment included literacy and cognitive skills as well as records concerning the family history of reading and writing abilities. Genomewide SNP genotyping and parametric linkage analysis in this family revealed a single prominent linkage interval on chromosome 4, containing a putative novel locus for familial dyslexia. All protein coding exons and exon-intron boundaries within this interval were sequenced in one affected and one unaffected family member. Sequence analysis revealed a nucleotide change in the 3' UTR of a brain expressed gene in the dyslexic family member that was not found in the unaffected individual. The DNA change might affect the binding

efficiency of miRNAs and thus have an influence on the expression of the detected gene product. In addition we screened this gene in 96 individuals from a cohort of dyslexic individuals and found a heterozygous change (Y29C) in one proband and not in his unaffected sister, which underlines the likelihood of an involvement of this gene in the aetiology of dyslexia and thus points to a role of this protein in the relevant cognitive processes.

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## P-MonoG-112

### A large mutation screen in patients with Kabuki syndrome

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Kabuki syndrome is one of the classical, clinically well known multiple congenital anomalies/mental retardation syndromes. Patients with Kabuki syndrome have a very characteristic facial appearance and in addition to the craniofacial features, a wide range of congenital anomalies can be associated, e. g. short stature and skeletal anomalies, congenital heart disease, urogenital tract anomalies, persistence of fingerpads, and hearing loss. Mild to moderate mental retardation is common in patients.

Very recently, a whole-exome sequencing strategy identified de novo dominant mutations in the MLL2 gene, encoding a histone methyltransferase, as major molecular cause of Kabuki syndrome. MLL2 is located on chromosome 12q13 and the protein consists of 5537 amino acids and is encoded by 54 exons.

In our study, we sequenced all 54 coding exons of MLL2 in 36 patients with Kabuki syndrome and Kabuki-like syndrome. We identified seventeen distinct mutations in eighteen patients. The mutation spectrum included six missense mutations (p.P647Q, p.V1192M, p.H1453R, p.A1718V, p.R5153Q, p.S5498F), two splice-site mutations (c.954+1G>T, c.13531-1G>T), three nonsense mutations (p.G1320X, p.Q2811X, p.R2915X) and six small deletions or insertions (c.1483\_1486delTCTC, c.1512\_1513delTC, c.5269insG, c.6595delT, c.7290insT, c.9460delC). Parental DNA was available from twelve patients and in all of these cases a de novo occurrence of the mutation was shown. Only one missense mutation, p.R5153Q, was recurrent and found in two patients. Sixteen of the mutations were novel and only the c.6595delT mutation has been recently described in one patient. Interestingly, the mutations were located throughout the MLL2 gene without a clustering in specific exons of the gene or domains of the protein. Genotype-phenotype comparison indicated that MLL2 mutations are present in nearly 80% of patients with classical Kabuki syndrome, while the detection rate was much lower in Kabuki-like cases.

Mutation negative patients were subsequently tested for mutations in 14 functional candidate genes (such as MLL1, ASC2, ASH2, WDR82, DPY30). The results of this screen will be presented. Our results indicate that MLL2 is the major gene for Kabuki syndrome with a wide spectrum of de novo mutations and provide evidence for further genetic heterogeneity.

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## P-MonoG-113

### Exome Sequencing in a Family with Rapid-onset Dystonia-Parkinsonism

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Background: Rapid-onset Dystonia-Parkinsonism (RDP) is a rare movement disorder characterized by a combination of dystonia (sustained muscle contractions causing twisting and repetitive movements or abnormal postures) and parkinsonism (bradykinesia and rigidity). It follows an autosomal dominant transmission pattern with reduced penetrance. Different mutations in the Na/K-ATPase alpha 3 subunit gene (ATP1A3) have been associated with the disease. However, a number of families without a known genetic cause suggest that additional genes may be associated with RDP.

Methods: In order to identify a new gene causing RDP, we investigated a four-generation German family with four living affected members. Mutations in ATP1A3 were previously excluded. We conducted a genome-wide linkage analysis using microsatellite markers. Next, exome sequencing was performed in two affected members. Novel variants that were localized in regions showing probable linkage and found in both individuals underwent several evaluation steps. First, we sequenced the respective variants in all four affected and in an additional 16 family members using Sanger sequencing to confirm the variant and to test

for segregation. Second, frequencies of segregating variants were determined in German healthy control subjects by direct sequencing.

Results: The genome-wide linkage analysis resulted in seven chromosomal regions with probable linkage (LOD-Score > 1). Exome sequencing revealed 183 undescribed variants (missense n=26, untranslated regions n=8, silent n=35, intronic n=114) common to both patients. No splice site or nonsense variant or small exonic insertion or deletion was shared in the linked regions. Assuming that one of the detected missense variants causes pathogenicity, we began with these variants for re-sequencing. Twelve of the 26 missense variants were excluded because they were not confirmed by Sanger sequencing (n=6) or did not occur in all affected family members (n=6). Another six variants were excluded by segregation analysis in the unaffected family members because at least one homozygous carrier was detected. The remaining eight variants appeared in the heterozygous state in all affected and in three or fewer presumably unaffected family members and were further investigated according to possible reduced penetrance. Four of these variants had allele frequencies in controls of more than 1% and were thus excluded, leaving four for further exploration.

Conclusions: We have currently identified four novel missense variants as possible cause for RDP in a German family. Further analysis of unaffected controls and other patients with RDP will likely result in the identification of a novel RDP gene.

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## **P-MonoG-114**

### **ARID1B: a candidate gene for intellectual disability?**

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#### Introduction

Intellectual disability (ID), former known as mental retardation (MR), describes certain limitations in mental functioning and in skills such as communicating, taking care of him or herself, and social skills. A possibility to discover novel intellectual disability genes is the study of disease associated balanced translocations, which occur six- to seven- fold more often in populations of intellectual disabled than in healthy individuals. We report on a boy with ID and an apparently balanced translocation t(5;6)(q11;q25) disrupting the ARID1B gene.

#### Materials and Methods

Our patient displayed intellectual disability and statomotoric developmental delay. His weight, height and head circumference were at the third percentile. Also cryptorchidism and mouth ranulas were reported. Karyograms of lymphocyte cultures and a SNP 6.0 genomic array were performed for the patient and his parents. FISH experiments with labeled BAC probes were used to narrow down the translocation breakpoint.

#### Results

Conventional cytogenetics revealed a de novo balanced t(5;6)(q11;q25). The SNP 6.0 array showed no genomic gains or losses which could be causative for the disorder. The parents had normal karyotypes and array findings. By FISH the breakpoint on chromosome 6 was fixed within a single BAC probe (RP11-11M7) in an area of about 160kb within the ARID1B gene, which is interrupted by the translocation.

ARID1B is the DNA binding subunit of several SWI/SNF like chromatin remodeling complexes and part of an E3 ubiquitin ligase complex, which mediates monoubiquitination of histone H2B and transcriptional activation in vitro. It is implicated in maintenance of pluripotency and the self-renewal of embryonic stem (ES) cells and is part of the chromatin remodeling complex in neural progenitors (npBAF). No studies about the ARID1B- function in npBAF have been performed. Other subunits of this complex have a dosage sensitive non-redundant role in neural development. In accordance with its role in the ES cells, ARID1B could also be important for the maintenance of pluripotency of neural progenitors. If so, ARID1B insufficiency would lead to exhaustion of the stem cell reservoir before development of sufficient mature neurons, which could explain the patient's phenotype.

#### Conclusion

We have identified a gene that could play a role in development of ID. Our findings correspond to the gene function being part of the npBAF, involved in neural cell differentiation. Screening for ARID1B mutations in other patients with similar phenotypes will be necessary to confirm our findings.

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## P-MonoG-115

### Rubinstein-Taybi syndrome caused by a novel mutation in CREBBP

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Rubinstein-Taybi syndrome (RTS) is a congenital disorder, clinically characterised by psychomotor development delay, growth retardation and certain dysmorphic features. RTS can be caused by mutations of the CREBBP-gene. The gene encodes a cAMP-“Response Element-Binding Protein”.

CREBBP acts as a coactivator in cAMP regulated gene expression and possesses histone acetyltransferase activity on lysine residues of histones and nonhistone proteins. The gene is located on chromosome 16p13.3. Point mutations as well as large deletions and duplications are detected in patients presenting signs and symptoms of RTS.

Here we report the case of an eight year old girl with clinical features that directed to the suspicion of RTS as a diagnosis. Karyotype and array-CGH analysis revealed no pathological findings. The molecular genetic analysis of CREBBP revealed a so far unpublished point mutation located in the region encoding for histone acetyltransferase (HAT) in exon 18 at position c. 3541 (c.3541C>T). This mutation results in the amino acid substitution leucine to phenylalanine at position 1181 of the protein sequence (p.Leu1181Phe). The amino acid leucine at position 1161 is highly conserved in a broad range of species.

Clinical features seen in the affected patient are discussed in the context of our molecular findings.

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## P-MonoG-116

### Modifying effect of SUN-mutations on expression of clinical features in Emery-Dreifuss muscular dystrophy?

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Emery-Dreifuss muscular dystrophy (EDMD) is a rare neuromuscular disease characterized by early contractures, slowly progressive muscular weakness and life-threatening cardiac arrhythmia that can turn to cardiomyopathy. EDMD is genetically heterogeneous and patients can have LMNA, STA (EMD), FHL1, SYNE1 or SYNE2 mutations. Onset, course and severity of EDMD can vary remarkably, even among patients with mutations in the same gene and sometimes between patients carrying the same mutation. Modifiers are proposed to produce this clinical variability.

Recently, lamin A/C, emerin and nesprins were shown to interact with SUN proteins (SUN1 and SUN2), resulting in a complex termed the LINC complex, that connects the nucleoskeleton with the actin cytoskeleton via the nuclear envelope. Thus, the SUN1 and SUN2 genes have been considered as candidates for such modifiers.

We analyzed a cohort of 70 EDMD patients with defined mutations in LMNA, STA (EMD), SYNE1 or SYNE2 for mutations in the SUN1 and SUN2 genes. The complete coding region including intron/exon boundaries of SUN1 and SUN2 was amplified and used for direct Sanger sequencing.

As a result, mutations in SUN1 or SUN2 have been found in four EDMD patients in combination with mutations in LMNA (p.R453W, p.T528K, p.R98P) or STA (p.L84PfsX6). Co-segregation analysis in the families of the affected index cases provides preliminary evidence for a modifying effect of SUN1 mutations p.A203V and p.W377C as well as SUN2 mutations p.V378I and p.A56P. This results in an increase of the severity of clinical features and indicates that SUN1 and SUN2 are involved in the same cellular processes as the other proteins of the LINC complex.

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## P-MonoG-117

### MODY type 1 caused by a splice site mutation of the HNF4A gene within an European family

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Introduction:

Maturity-onset diabetes of the young (MODY) is a monogenic, autosomal dominant subtype of early-onset diabetes mellitus due to defective insulin secretion by the pancreatic beta cells. Defects in several

different genes have been described as cause of MODY. Mutations in the Hepatic-Nuclear-Factor 4 alpha (HNF4A) gene are correlated with MODY type 1 and are less common than mutations in the glucokinase (GCK) gene or Hepatic-Nuclear-Factor 1 alpha (HNF1A) gene.

**Patients:**

The index case is a 17-year-old female patient from Germany with familiar history of diabetes and asthma. Her mother, grandparents as well as an uncle suffered from diabetes. The father and older brother of the patient are unaffected, whereas the younger brother is effected by asthma.

**Methods:**

Genomic DNA was extracted from peripheral blood leukocytes. The analysis encompasses direct sequencing of PCR products of all coding exons of the HNF4A gene including corresponding exon-intron boundaries. In addition Multiplex Ligation-Dependent Probe Amplification (MLPA) was performed using the SALSA P241-B1 probemix by MRC-Holland for detection of deletions and duplications within the HNF4A gene.

**Results:**

Sequence analysis of the HNF4A gene of the girl revealed a splice site mutation in intron 4, 4 nucleotides upstream of exon 5 (IVS4-4G>A). Deletions or duplications of the HNF4A gene were excluded by MLPA. Disease-causing mutations of the HNF1A gene (MODY type 3) have been excluded previously.

The same splice site mutation was detected in the effected mother of the patient.

**Conclusion:**

This splice site mutation has been previously reported in the literature in a Philippino family in addition to another single-nucleotide substitution in intron 4 (IVS4-20C>T) and a missense mutation in exon 5 (p.Val199Ile; Gragnoli C et al. 2004).

Whereas in the report the missense mutation was expected to cause the phenotype, in our two cases no other mutation in the HNF4A gene was detected. Due to this fact we here describe for the first time an European family effected by MODY type 1 caused by a single splice site mutation in intron 4 of the HNF4A gene. Our findings suggest that this mutation (IVS4-4G>A) alone is able to cause MODY type 1.

We recommend to screen for mutations in the HNF4A gene in all patients suffering from a severe type of MODY, if disease-causing mutations within the HNF1A gene were excluded, to provide the basis for therapeutic decisions.

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**P-MonoG-118**

**The mutation spectrum of the ALPL gene among patients from 43 families with the clinical diagnosis of hypophosphatasia**

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Hypophosphatasia is a rare inborn error of metabolism that affects the development of bones and teeth. Clinical symptoms are highly variable, ranging from a profound lack of mineralization of bone leading to stillbirth up to pathologic fractures of the lower extremities developing only late in adulthood. In the mildest clinical form, odontohypophosphatasia, an early loss of teeth in adulthood is the only sign. The diagnosis can be suggested in individuals in whom serum ALP activity is subnormal and urinary phosphoethanolamine level is increased. The incidence of severe cases is about 1 in 100000 whereas the prevalence of mild forms is expected to be much higher. The disease is due to mutations in the liver/bone/kidney alkaline phosphatase gene (ALPL; OMIM #171760). Currently more than 220 different mutations have been reported in the ALPL gene ([http://www.sesep.uvsq.fr/03\\_hypo\\_mutations.php](http://www.sesep.uvsq.fr/03_hypo_mutations.php)).

Sequencing of genomic exonic DNA, including flanking intron sequences, allows the detection of about 95% of mutations in the more severe forms. In milder forms, the detection rate seems to be lower. In a series of patients affected by various forms of hypophosphatasia from 43 families and various origins we found 22 distinct mutations, five of them not previously reported: a small deletion, c.1412\_1429del18, three missense mutations, p.D458G, p.L276V, and p.R428L, and one mutation affecting the start codon, p.Met?. All other mutations were missense mutations that have been previously published. The most prevalent mutations were p.E191K (13 families), p.G334D (6), p.A179T (3), and p.R71H (3). In seven adults in whom a mild form of hypophosphatasia was suspected no mutation in the ALPL gene could be detected. Our results suggest the spectrum of mutations in our patients with hypophosphatasia to be similar to that of patients from other European countries.

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## P-MonoG-121

### **Hereditary spastic paraparesis type SPG4 and SPG3 – diagnostic DNA testing in Czech patients revealed novel mutations.**

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Hereditary spastic paraparesis (HSP) is a genetically very heterogeneous group of neurological movement disorders, clinically characterised by progressive lower limb spasticity. Pure and complicated forms are traditionally classified and all types of inheritance are known in this group. Mutations in the SPAST gene are the most common known cause of HSP named as SPG4 representing about 40% of pure autosomal dominant (AD) families. Mutations in the ATL1 gene are responsible for type SPG3A and are found more frequently in patients with childhood onset of HSP.

We report results from four years DNA diagnostic testing of SPAST and ATL1 genes in Czech patients suspected to have HSP.

In the time period 2006-2010 we sequenced the SPAST gene in 132 individuals from 112 unrelated families from Czech Republic send for diagnostics for clinically suspected HSP. Pathogenic mutations were found in 26 (19,7 %) individuals from 18 different families (14,8 %). In 105 patients/individuals no pathogenic mutation was found. We detected in total 16 different pathogenic mutations (c.29 A>C (K10T), c.80\_98dup19(p.P34fs), c. 943del A, c.963delCA, IVS 6+1 G>C, p. Glu150fsStop160, IVS 15-2 G>A, c.1051 G>A (R309H), c.1196 C>T (S399L), c.1291 C>T (R431X), c.1303 C>A (P435T), c.1378 C>T (R460C), c.1495C>T (R499C), c.1610T>C (L537P), c.1687+2 del TAGGT, c.1688-2 A>G) and further 2 variants known as possible modifier (S44L and P45Q) and one silent variant (P293P). Pathogenic mutations in SPAST are much more probable in familiar AD cases than in sporadic cases.

ATL1 gene was sequenced in the same time period in total in 47 patients from 46 unrelated families. Most of these patients were previously tested for mutations in SPAST gene. Pathogenic mutation namely c.311 A-G (N104S) was found in only one patient (2,1 %). Frequent silent variants P28 P and E117E were found in 11 and 17 patients respectively.

Testing for SPAST mutations seems to be much more useful than for ATL1 mutations in Czech patients. Testing for larger deletions in SPAST gene by MLPA in patients with clinically definitive HSP or sequencing the REEP1 gene in patients with childhood onset HSP may clarify the cause of HSP in further fraction of patients.

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## P-MonoG-122

### **Idiopathic short stature (ISS): a new variant detected in the SHOX gene as a putative cause of the phenotype**

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Short stature is a developmental, multifactorial condition with a strong genetic component. Approximately three per cent of the population worldwide is affected. Short stature can have many different causes; one of the most prevalent was shown to derive from a defect in the SHOX gene (short stature homeobox gene; MIM 312865) on the X chromosome. Defects in the coding region of SHOX causes a wide spectrum of short stature phenotypes including Leri-Weill, Langer and Turner syndrome, but mutations are also found in patients with idiopathic short stature (ISS).

Here we present a 12 year old girl with ISS. Karyotype analysis from lymphocytes revealed a normal female karyotype (46, XX). A molecular analysis of the SHOX-gene was performed using MLPA (multiplex ligation-dependent probe amplification) and no aberrations were detected with this method. Sequencing of the complete coding region of the SHOX-gene presented a so far unpublished alteration in the 5' UTR of exon 2 at position c.164C>T.

We report the family history as well as clinical signs and symptoms in detail and discuss further investigations to elucidate the relevance of the identified aberration.

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## P-MonoG-123

### Towards identification of the genetic defect underlying North Carolina Macular Dystrophy (NCMD)

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#### Objectives

NCMD is a highly penetrant, autosomal dominant macular dystrophy with congenital or infantile onset and a wide range of variation in clinical phenotypes. The NCMD gene locus maps to chromosome 6q16. An ancestral core haplotype has been identified in three German families with NCMD that defined a 2.4 Mbp candidate interval. The aim of this study was to conduct further genetic mapping in NCMD families and to perform rigorous analyses of candidate genes with a putative role in NCMD pathology.

#### Material and Methods

A total of 60 individuals from the three mapped and 14 novel families with clinical symptoms of NCMD were genotyped with up to 17 microsatellite markers located within the critical NCMD interval. The coding sequences of ten annotated genes (POU3F2, FBXL4, C6ORF168, COQ3, SFRS18, USP45, LOC100130890, CCNC, PRDM13, MCHR2) were screened for mutations by direct sequencing. Expression analysis of selected genes was performed by RT-PCR. Southern blot was used to screen for larger chromosomal abnormalities.

#### Results

The NCMD interval was further refined to a 1.8 Mbp region between newly identified STR markers Tetra4 and Di22. Besides the three mapped NCMD pedigrees, four additional families and one simplex case diagnosed with NCMD were shown to carry the ancestral NCMD haplotype. No disease causing mutation was found in the coding sequences of the known annotated genes located in the NCMD candidate region. The genes C6ORF168 and SFRS18 harbour conserved binding sites for the retina-specific transcription factor CRX. PRDM13 was shown to be preferentially expressed in the developing eye and adult neuronal tissue of mouse. Thus, these three genes represent prime candidates for further studies.

#### Conclusions

Most, if not all seemingly unrelated NCMD families worldwide share a consensus haplotype suggesting an early common founder. The failure to detect the genetic defect underlying NCMD in the coding sequence by direct sequencing suggests a novel gene or exonic sequence that is not represented in public databases to be mutated in NCMD. Alternatively, the NCMD mutation may affect regulatory regions possibly as a consequence of so far undetected genomic rearrangement in a known gene of the candidate interval.

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## P-MonoG-124

### Novel mutation in the COMP-gene as a molecular cause of pseudoachondroplasia

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Mutations in the COMP-gene are associated with two clinical phenotypes: pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). Both entities are dominantly inherited chondrodysplasias characterized by postnatal short-limb dwarfism, knock-knees or bowed legs and early-onset osteoarthritis. PSACH is the clinically more severe form with marked vertebral changes, deformity of lower limbs as well as brachydactyly. PSACH is one of the more frequent bone dysplasias with a frequency of about 1 : 30 000.

Here, we report the results of the molecular genetic analysis of 5 year old dizygotic twins. Because of diminished linear growth, disproportionately long trunk and waddling gait, appearing at about 2-3 years, they were admitted for endocrinological investigation. Radiographic and clinical features suggested pseudoachondroplasia. Pedigree analysis revealed that their father, grandfather and great-grandmother suffer from the same type of short stature.

The molecular genetic analysis of the COMP-gene revealed the nucleotide change c.983G>A in a heterozygous state. This mutation results in the amino acid substitution cysteine to tyrosine at position 328 of the protein sequence (p.Cys328Tyr). The amino acid cysteine at position 328 is highly conserved in a wide range of species. This affects a highly conserved residue in the second calmodulin-like repeat of COMP.

Clinical features and pathogenesis of pseudoachondroplasia are discussed in the context of our molecular findings.

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## P-MonoG-125

### Identification and luciferase activities of four different mutations in the promoter region of the factor VIII gene

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#### Background

Haemophilia A (HA) is the most severe inherited bleeding disorder caused by genetic defects in the factor VIII gene (F8) on chromosome Xq28. The mutation spectrum of this disease comprises inversions of introns 22 and 1, respectively, missense and nonsense mutations, small and large deletions or duplications, insertions, and splice site mutations. In about 3 % of HA cases, no mutation is found in the coding regions of the F8 gene. Recently, three different single base substitutions in the promoter region of F8 were identified and suspected to account for haemophilia A. The causality of one mutation was proven using a luciferase assay.

#### Methods

As described before the main promoter region of F8 spans approximately 300 bp upstream of the start codon. Therefore, we amplified this 5'UTR region in 77 HA patients in which no F8 mutations had been detected by standard diagnostic methods and sequenced the fragments obtained on an ABI 3130 sequencer.

The detected mutations and two previously published promoter mutations were assayed by the Dual-Luciferase Reporter System (Promega) for promoter activity. The wild type and mutated F8 promoter sequences were cloned next to the 5'-end of the luciferase gene and the constructs were transfected into HEK293 cells. After expression, the cells were lysed and luminescence was measured by a luminometer.

#### Results and Discussion

Screening of the 77 HA patients revealed two different promoter mutations, one published earlier (-219C>T) and a new one (-255A>G). Beside these, two further F8 promoter substitutions were reported previously (-112G>A and -257T>G). Altogether, four mutations in the main promoter region of F8 are known.

So far, only the -219C>T mutation was studied by a luciferase assay. To investigate their promoter activity we performed luciferase assays of all four promoter mutations and compared their activity to the wild type. The -219C>T mutation shows the greatest influence on promoter activity which is reduced to approximately 0.5 % of wild type. Although the mutations -255A>G and -257T>G are very close to each other they show different effects on F8 promoter activity (about 4 % and 40 %, respectively). Furthermore, the -112G>A substitution results only in a slight decrease of wild type promoter activity (about 80 %).

In conclusion, our data suggest that three out of four investigated F8 promoter mutations are likely causative for haemophilia A.

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## P-COMPLEX GENETICS / COMPLEX DISEASES

### P-Compl-126

#### Characterization of a small deletion within the Epidermal Differentiation Complex showed no evidence for association with psoriatic arthritis or psoriasis vulgaris.

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Psoriatic arthritis (PsA) is an immunologically mediated joint disease frequently appearing in patients with the most frequent skin manifestation in psoriasis, psoriasis vulgaris (PsV). Complex genetics and environmental influences lead to the manifestation of both disease entities. Even after very recent identifications of several further risk alleles, the heritability of PsA and PsV cannot be explained by the combination of known genetic risk factors. Recently, a common CNP was described as a risk factor for PsV: a 32 kb deletion located within the Epidermal Differentiation Complex (EDC, chromosome 1q) comprising two late cornified envelope (LCE) genes - LCE3B and LCE3C - has been described to be associated with PsV. In order to find more disease associated copy number loci in the EDC, we investigated this region in PsA patients.

We genotyped 609 PsA patients on Affymetrix Genome-wide Human SNPArray 6.0. Copy number states were called using the Birdsuite algorithm (Birdseye, Fawkes). We detected a common deletion which comprises coding parts of LCE1E and LCE1D. Based on array results, the size of the deletion was minimally 5.2 kb and maximally 11.98 kb. Although these two genes of the LCE1 family show a homology of up to 97% in exons, we were able to establish several specific PCRs spanning the deletion. Our PCR experiments could exactly detect the three different genotypes. The copy number state was also verified by Multiplex Ligation-dependent Probe Amplification (MLPA) with a probe located inside the deleted region. In order to screen larger groups of individuals, a Taqman real-time PCR targeting the deletion was established. We genotyped a cohort of 561 control probands as well as another patient group of 562 PsV cases for the LCE1E1D\_del using the Taqman and PCR-based methods. The copy number calling on an independently dataset of more than 1,200 POPGEN controls was performed analogously to the PsA samples by Birdsuite, 909 POPGEN fulfilled quality criteria.

In a quality adjusted group of 490 PsA patients allele frequency of the deletion was 35.2 %, in 1,470 combined control individuals 36.0 %, indicating no association with PsA. There was also no evidence for association with 562 PsV which showed an allele frequency of 37.3 %.

Our study shows the CNP in the LCE1 cluster is not relevant for the two psoriasis entities, but might be an interesting candidate for other skin diseases, such as atopic dermatitis.

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## P-Compl-127

### Resequencing of Neurexin 1 (NRXN1) and Follow-up in Schizophrenia

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Schizophrenia is a chronic, severely disabling neuropsychiatric disorder with a high a burden on patient, his relatives and also in terms of healthcare costs. The disorder has a complex genetic architecture with several common risk variants of low penetrance and rare risk variants of high penetrance known up to date. Among the rare variants are large copy number variations (CNVs). The majority of these CNVs span several genes making it difficult to pinpoint the real schizophrenia risk genes. An exception to this is rare microdeletions of the neurexin 1 (NRXN1) gene on chromosome 2p16.3 which were demonstrated to be associated with schizophrenia in independent studies. Array based data has shown no association of common variants in NRXN1 with schizophrenia. The remaining question is whether NRXN1 harbours rare variations other than CNVs such as single nucleotide exchanges or small insertion/deletions which also confer susceptibility to schizophrenia. We had therefore initiated a re-sequencing study of all exons and splice sites of the NRXN1 isoforms alpha1, alpha2 and beta in 94 schizophrenia patients and 94 sex-matched controls, all of German ancestry. Results of this study were presented at the GfH meeting in 2010. The identified variants with a minor allele frequency (MAF) of < 3% were then classified to be potentially functional or non-functional and assessed by various bioinformatical tools. As a consequence of the limited sample size, none of the rare variants annotated to be 'potentially functional' reached statistical significance in the re-sequencing sample. To follow-up on a potential involvement in the etiology of schizophrenia, we are now genotyping these variants in 1100 patients and 1000 controls by a Sequenom iPLEX genotyping assay. Results of this follow-up will be presented.

F.B. Basmanav and A.J. Forstner contributed equally to this work.

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**P-Compl-128****Genome-wide Meta-analysis on Dyslexia**

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Developmental dyslexia is one of the most common learning disorders affecting about 5% of school-aged children. Based on twin and family studies it is known that genetic factors are involved in the development of dyslexia.

We performed a meta-analysis comprising two genome-wide association studies (GWAS) in order to identify the genetic factors underlying dyslexia: one GWAS was of UK/German origin (cases > 580), the second GWAS was from Iceland (cases > 800). In total, we identified 109 SNPs located in 36 different chromosomal regions which showed association with the same risk alleles in both single GWAS (best hit:  $P_{meta} = 3.3 \times 10^{-6}$ ). These 36 regions are likely to contain genes contributing to dyslexia.

We genotyped 38 out of the 109 SNPs in an independent replication sample of European descent (cases > 800) and analyzed the most promising markers in a third dyslexia sample from the US (cases from > 300 families).

We expect that the increased power of our study will lead to the identification of associated risk markers, which might help to better understand the genetic architecture underlying dyslexia.

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**P-Compl-129****Functional profiling of lipid GWAS loci identifies genes with putative role in cholesterol trafficking and metabolism**

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Elevated serum lipid levels are a major risk factor for atherosclerosis and coronary heart disease. To this date 21 genome-wide association studies (GWAS) involving >100,000 individuals have identified 124 loci associated with alterations in serum LDL-c, HDL-c, cholesterol and triglyceride levels or cardiovascular disease. Typically, however, GWAS fall short to identify mechanisms how variants at these loci lead to the observed trait and if and how this involves disease-relevant genes. With the aim to better characterize basic biological processes contributing to the pathogenesis of cardiovascular disease we have applied a functional genomics strategy that relies on RNA-interference (RNAi) of multiple genes in parallel and functional analysis in a cell culture model using high-content screening microscopy. Profiling of 110 genes in 56 lipid trait-associated loci enabled us to identify a number of genes with putative roles in regulating the uptake of low density lipoproteins (LDL) into cells. Multi-parametric analysis of image data as well as of LDL receptor (LDLR) mRNA and protein levels allowed us to generate hypotheses if and how these genes may affect cellular lipid regulatory mechanisms. Taken together we introduce RNAi as a promising strategy to functionally profile genes within lipid trait-associated loci and as a powerful tool to prioritize candidate genes identified by GWAS for subsequent detailed characterization.

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**P-Compl-130****Genome-wide Association Study (GWAS) for Albuminuria in Individuals of African and European Descent With and Without Diabetes**

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**Background**

Albuminuria is an independent predictor of chronic kidney disease progression and of cardiovascular morbidity and mortality in the general population, but little is known about the role of common genetic variants on urinary albumin excretion.

**Methods**

We performed a meta-analysis of genome-wide association study data from 63,153 individuals from 28 cohorts of the general population of European ancestry (CKDGen consortium) and from a large candidate gene study (CARE consortium) to identify susceptibility loci for the quantitative trait urinary albumin-to-creatinine ratio (UACR) and the dichotomous trait microalbuminuria (MA). We verified findings in 6,981 participants of African ancestry in the CARE Consortium, and in a prospective study of 1304 patients with type I diabetes (DCCT/EDIC).

**Results**

We identified a nonsynonymous SNP in the CUBN gene in association with UACR ( $p=1.1 \times 10^{-11}$ ) and MA ( $p=0.001$ ) in individuals of European descent. Similar and significant associations were observed between the SNP and UACR as well as MA in CARE African Americans, and also in individuals of European ancestry irrespective of diabetes and hypertension status. Finally, the SNP was associated with the development of persistent MA over 20 years of follow-up in the DCCT/EDIC study (hazard ratio=1.41 per copy of the minor C allele,  $p=0.02$ ). Cubilin, the protein encoded by CUBN, is an integral component of the complex responsible for receptor-mediated endocytotic reabsorption of albumin and low molecular weight proteins in the kidney's proximal tubule. Rare variants in CUBN cause Imlerslund-Gräsbeck-Syndrome (OMIM #261100), a syndrome of vitamin B12 deficiency and low-grade proteinuria.

**Conclusion**

These findings identify a nonsynonymous SNP in the CUBN gene that is associated with albuminuria in both the general population and individuals with diabetes, thus highlighting the potential role of shared genetic susceptibility for albuminuria in diverse clinical settings.

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**P-Compl-131****Localization of RAD51C in nuclear DNA repair foci**

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DNA-double strand lesions are highly toxic since unrepaired transcription and replication become stalled. Interstrand crosslinks (ICLs) are among the most complicated injuries for DNA damage response networks to eliminate because there is no matrix strand left. This is remedied by a pathway designated as homologous recombination (HR). In somatic cells, HR serves replication-dependent DNA repair. In contrast to two related error-prone pathways, non-homologous end-joining and translesion synthesis, HR is the single error-free mechanism to warrant DNA fidelity and maintain genomic stability. This is possible as HR is a process that invokes the second allele for sequence reconstitution of the first. The Fanconi anemia (FA)/BRCA pathway recognizes and signals ICLs, initiates their removal and leads into HR, executed by RAD51 recombinase and five RAD51 paralogs that are all highly conserved in evolution. RAD51C is required for checkpoint activation and cell cycle arrest. It accumulates at sites of DNA damage where it is retained, for unknown reasons, after disassembly of RAD51 nucleoprotein filaments. It plays an important role in Holliday junction processing. In addition to being part of the HR machinery, RAD51C has recently become a newly recognized member of the FA/BRCA pathway, termed FANCO. A major limitation in defining more precisely cellular functions of RAD51C in the past was a lack of monospecific antibodies sensitive enough to visualize this low abundant

protein and a need for RAD51C-deficient human cells. Here we report initial results of studies where we transduced fibroblasts (SH2038-F) harboring the homozygous RAD51C-inactivating missense mutation c.773G>A (p.R258H) with N-terminally GFP-tagged RAD51C (Vaz et al, 2010). When SH2038-F fibroblasts were transduced with GFP as a control and exposed to mitomycin C they proved FANCD2 foci-positive (>47% of cells), but RAD51 (4.7%) and RAD51C (11%) foci-negative. The small fraction of cells with RAD51C foci indicated the hypomorphic nature of the R258H mutation. Only RAD51C foci-positive cells were RAD51 foci-positive. Positive cells had only few (<10) and rarely colocalizing RAD51 and RAD51C foci. Expression of wild-type RAD51C converted SH2038-F cells to RAD51C (53%) and RAD51 (44.1%) foci-positive. Again, only RAD51C foci-positive cells were RAD51 foci-positive, whereas the presence of FANCD2 foci was independent of RAD51 foci. RAD51C and RAD51 foci frequently colocalized but less so did FANCD2 foci. Vector-induced overexpression of R258H-mutant RAD51C revealed intermediate numbers of RAD51C and RAD51 foci-positive cells. By further experiments we seek to elucidate the time course of how RAD51C directs RAD51 to repair foci and which other proteins are involved.

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## P-Compl-132

### Resequencing and association analysis of the schizophrenia-associated microdeletion region on 1q21.1

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Schizophrenia is a complex neuropsychiatric disorder with a lifetime prevalence of about 1%. The disease is characterized by hallucinations, delusions and disorganized speech and shows a high heritability of up to 80 %. In large genome-wide surveys we and others have recently identified rare recurrent microdeletions on chromosome 1q21.1 as a strong genetic risk factor for schizophrenia (odds ratio ~ 10). The discovered microdeletion spans 1.35 megabases and contains several genes. In the present study, we aimed to identify rare small-sized susceptibility variants at the microdeletion region (single base exchanges and small deletions/duplications) that were below the resolution of previously applied array-based technologies. We had therefore performed systematic resequencing covering seven RefSeq genes in the microdeletion region aiming to detect additional rare variants that possibly contribute to the disease development at this particular locus. 94 DSM-IV-diagnosed schizophrenia patients and 94 sex-matched controls had been investigated. Resequencing was focused on coding exons and splice sites since these regions are more likely to carry variants with potential functional consequences. We had amplified 67 target regions, resulting in 28.4 kb of genomic sequence per individual and had discovered a numerical overrepresentation of rare sequence variants in schizophrenia patients in comparison to controls (n= 30 in 94 patients to n= 20 in 94 controls, minor allele frequency <1%). Twelve of the rare variants detected in patients represented previously undescribed non-synonymous amino acid changes with a predicted effect on protein structure, one led to a premature stop codon. The relevance of the single genes was further evaluated by expression analyses in pre-mortem human hippocampus tissue and mouse brain.

Based on the sequencing and expression results, we now selected four of the genes for an extension of our study to additional 96 patients. We discovered five additional rare and potentially functional variants including a nonsense mutation and two splice site changes that were observed in a single gene. In accordance with complementary analysis of biological function these results implicate two brain-expressed genes in the region as possible novel candidate genes for schizophrenia.

As a preliminary conclusion, we hypothesize that such rare and potentially functional sequence variants contribute to the schizophrenia disease allele spectrum at this particular locus in addition to the recently discovered microdeletions. All rare non-synonymous variants identified in patients are currently being followed up by genotyping in a large cohort of 1,400 schizophrenia patients and 1,100 controls. Results of these analyses will be presented.

A.J.Forstner and F.B.Basmanav contributed equally to this work.

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**P-Compl-133****Regulation and Functional Analysis of a Strong Candidate Gene for Coronary Artery Disease (CAD) - MRAS**

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**Background:** Our recent genome-wide association study (GWAS) identified the MRas gene on human chromosome 3q22 with genome-wide significance for CAD. M-Ras is a member of the Ras superfamily of small GTPases; many of which function as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes. M-Ras has been implicated in the regulation of TNF  $\alpha$  -stimulated LFA-1 activation and integrin-mediated leukocyte adhesion downstream of various inflammatory cytokines.

**Aim of the study:** We aimed to perform a functional analysis of M-Ras related to atherosclerosis in mice to further understand the pathogenesis of CAD and also perform functional analysis of MRas 3'UTR related to a possible regulation element located in this region.

**Methods and Results:** First, we confirmed the association of the MRas locus in the CARDIOGRAM data set involving 20.000 cases and 60.000 controls ( $p=7,4 \times 10^{-13}$ ). Second, we determined tissue expression of MRas/M-Ras and found abundant mRNA levels and immunofluorescence in mouse and human aorta and heart. Third, we obtained an MRas-KO mice and started adhesion and enzyme-linked immunosorbent assays with macrophages, monocytes, B- and T-cells to study the influence of the MRas on adhesion and migration as a potential pathomechanisms of atherosclerosis. We found that leukocyte adhesion, especially B-cell adhesion, in MRas-KO mice is about 20% reduced in comparison with leukocytes of WT mice ( $p<0.01$ ). Fourth, we start crossbreeding the MRas-KO mice into the ApoE-KO background for atherosclerosis studies by feeding these mice with a special diet. Additionally we see in first preliminary data a reduction of atherosclerosis in MRas/ApoE-KO mouse. In vitro data also show after LPS stimulation increased TNF- $\alpha$  secretion of MRas-KO macrophages in comparison with WT macrophages ( $p<0.05$ ) and in vivo we observed obesity after feeding MRas-KO mice with "western" diet ( $p<0.05$ ).

To study the regulatory relevance of the 3'UTR we performed luciferase assays. We cloned four regions out of the MRas 3'UTR, one spanning the position of lead SNP rs9818870, three other fragments covering conserved regions within the 3'UTR. Preliminary data show a clear downregulation of the luciferase activity due to all four constructs.

**Conclusion:** Our experiments confirm association of MRAS locus with CAD and show that MRas has functional relevance in leukocytes and macrophages. These cells could be responsible for the reduction of the atherosclerosis observed in the MRas/ApoE double KO mice. In our preliminary luciferase assays we show that the 3'UTR of MRas has potential functional relevance for gene regulation.

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**P-Compl-134****Towards a Genetic Risk Model for Age-Related Macular Degeneration (AMD)**

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**Introduction:** Age-related macular degeneration (AMD) is the leading cause of blindness in the Western World and affects an estimated 50 million people worldwide. It is a multifactorial trait involving both genetic and environmental effects although the precise aetiology of AMD still remains elusive. Age, smoking, and to a lesser extent, diet and sunlight exposure are among the most commonly reported risk factors for disease onset. A genetic contribution to AMD is well established by familial aggregation analyses and twin studies. Recently, variations in the complement factor H (CFH) gene on 1q32 and in the ARMS2/HTRA1 gene locus on 10q26 have revealed a strong association with AMD likely explaining over 50% of the disease load. Further associations with AMD were demonstrated in other genes of the complement pathway including complement factor B (CFB), complement Factor I (CFI) and complement component 3 (C3). So far, there is no comprehensive risk model for AMD taking into consideration both known genetic variations and environmental factors.

**Methods:** Genotyping of patients was performed by various methods including i-plexing, Taqman assays, and sequence analysis. Risk model computation and assessment were performed in R, a scripting language used in statistics and epidemiology. Classification was performed by logistic regression and Support Vector Machines (SVM).

**Results:** 1,166 AMD patients and 796 matched controls all recruited from the Lower Franconian area were used as a training sample. Interaction and conditional analysis suggested 11 high performing SNPs (classifiers) in 7 known AMD risk loci that were used for model generation. Logistic Regression was used to

order patients by their risk score and to classify them by decreasing the threshold, resulting in an Area Under the Curve (AUC) of 0.81. Ninety-six percent of all samples in the highest risk decile were cases, 19% of all samples in the lowest risk decile were cases. An overall classification performance was estimated with support vector machine to be around 72%. Classification performed in the top and bottom deciles, quintiles and terciles of genetic risk resulted in an accuracy of 88%, 84% and 80% respectively.

Conclusions: Previous studies showed high AUC and high prediction accuracy in the highest deciles of genetic risk. In contrast, our data suggest that a simple logistic model with genetic factors alone might not suffice to accurately predict disease risk. However, classification performed by SVMs in the top and bottom percentiles can yield decent classification accuracies. Furthermore, additional genetic markers might increase the predictive power of the model.

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#### **P-Compl-135**

### **Set based analysis of monoaminergic genes implicated in neuropsychiatric disorders**

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Major depressive disorder, bipolar disorder and schizophrenia are common, genetically complex neuropsychiatric disorders. Besides a so far unknown number of liability genes, environmental factors and gene-environment interactions are thought to be involved in their etiology. Recent genome-wide association studies (GWAS) have successfully identified the first genetic risk factors for each of these disorders. One major problem, however, of GWAS are the necessary rigid correction procedures for multiple testing. Given the small genetic effects that seem to characterize common risk variants (Odds Ratios <1.2), many of these risk variants will not surpass the required significance thresholds. One possibility to reduce the complexity of GWAS and enhance the sensitivity for small genetic effects are set-based analyses using a subset of SNPs that have an a priori higher chance of being involved in disease etiology. Although our knowledge of the biological pathways underlying pathophysiological mechanisms of neuropsychiatric disorders are limited there are widely accepted hypotheses regarding the etiology, partly based on the mechanism of action of effective drugs. Those hypotheses include the "dopamine hypothesis" of schizophrenia and the "monoaminergic hypothesis" of depression. In the present study, we defined SNP subsets for dopaminergic and serotonergic genes from existing GWAS data for major depressive disorder, bipolar disorder and schizophrenia and analyzed them using set-based tests. Results of these analyses will be presented.

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#### **P-Compl-136**

### **Identification of common structural variation in genes of the serotonergic system yields two candidate deletion loci for neuropsychiatric disorders**

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Background & Aims. Genomic structural variants (SVs) are hypothesized to account for a fraction of psychiatric disease heritability not captured in SNP-chip based genome wide association studies (GWAS) (1). Common SVs which contributing to disease etiology can be found associated in large enough case-control cohorts and thereby facilitate the identification of disease genes and pathways. The major goal of our study was the identification and frequency estimation of common SVs in the coding region of genes of the serotonergic pathway, which has previously been implicated in psychiatric and neurogastrointestinal disorders. Therefore, we selected genes involved in serotonin metabolism and transport and serotonin

receptors as well as associated factors based on literature research, and analyzed them with respect to common structural variation. For this purpose, we applied in silico analysis of genes involved in the serotonergic pathway in individuals from the "1000 Genomes Project" (2).

Methods. SVs were identified applying the paired-end mapping approach (3) on sequence data derived from 146 individuals from the "1000 Genomes Project" (50 individuals of European ancestry from Utah, 30 of Han ancestry from Beijing (CHB), 20 of Japanese ancestry from Tokyo, and 46 Yoruba from Nigeria) (2). Putative SVs were then validated by PCR, and expression analysis of affected genes was performed in 29 different human tissues.

Results. Two common deletions in the coding regions of the serotonin type 5A receptor gene HTR5A and the tryptophan hydroxylase gene TPH2 were identified by PEM. Both variants were frequent, the SV in HTR5A was detected in 21, and the TPH2 SV in 81 of the 146 analysed individuals. The deletion in HTR5A spans 3.6 kbp and affects the 3' UTR from position c.\*800 on downstream. Subsequent expression analysis revealed HTR5A to be predominantly expressed in the brain, including structures of the limbic system. The TPH2 deletion comprises 1.7 kbp and resides in intron 5 of the canonical TPH2 isoform. However, it disrupts an exon of the respective alternative splice variant AK094614. Interestingly, this isoform was found to be expressed exclusively in amygdala and hippocampus, while the canonical isoform was present in all tested brain tissues.

Conclusion. We have identified two common deletion variants that represent promising candidates for association studies in psychiatric disorders, since both genes HTR5A and TPH2 have been implicated in the pathoetiology of bipolar affective disorder (BPAD) and other psychiatric phenotypes. Therefore, 2000 patients suffering from BPAD along with 2000 healthy controls are currently being genotyped. The HTR5A 3'UTR deletion may result in an altered regulation on post-transcriptional level by microRNAs or transcript stability, whereas the TPH2 variant potentially affects the structure and function of the hippocampus and amygdala specific isoform.

(1) Maher, B. (2008). Personal genomes: The case of the missing heritability. *Nature*, 456(7218), 18-21.

(2) 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing (2010). *Nature*, 467(7319), 1061-73

(3) Korbelt, J. O., Urban, A. E., Affourtit, J. P., Godwin, B., Grubert, F., Simons, J. F., et al. (2007). Paired-end mapping reveals extensive structural variation in the human genome. *Science*, 318(5849), 420-426.

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## P-Compl-137

### From clinics to genetics: A novel stop mutation in exon 2 of the SALL4 gene causing clinical Townes-Brocks or atypical Okihiro syndrome

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#### Introduction

Okihiro syndrome (Duane Radial Ray syndrome) is an autosomal dominant disease, which is caused by mutations of the SALL4 gene on chromosome 20q13.13-q13.2. The main clinical features are radial ray defects and Duane anomaly, but the clinical presentation is very variable. Eyes, upper and lower extremities, kidneys, internal ear and hearing, heart, gastrointestinal tract, face, spine and pituitary gland (growth hormone deficiency) may be affected. Here, we report a patient carrying a stop mutation c.1900A>T, p.K634X, in exon 2 of the SALL4 gene which, according to the human gene mutation database (HGMD professional 2010), has not been described before. In some cases, SALL4 mutations are associated with a clinical phenotype of Townes-Brocks syndrome (TBS) rather than Okihiro syndrome.

#### Case report

A newborn boy presented with facial dysmorphism including ear dysplasia, crying-face syndrome showing unilateral ptosis and downturned corner of the mouth. In addition, duplication of the right thumb and triphalangism of the left thumb was noted. Cardiac examination revealed a ventricular and an atrial septal defect. In the first month of life he suffered from anal stenosis necessitating several hospital admissions for morphological (contrast enema) and functional investigations (anomanometry) and for symptomatic treatment by repeated anorectal dilatations. The initial clinical diagnosis was TBS based upon presence of thumb, ear and anal malformations. After excluding a SALL1 mutation as the typical cause of TBS, the molecular analysis of the SALL4 gene showed a heterozygous mutation in exon 2, c.1900A>T, p.K634X, confirming a SALL4 defect as the cause for the phenotype in this patient. The family history revealed that further family members are also affected. His sister has a history of anorectal malformation with rectovaginal fistula and an atrial septal defect. The mother has a partial duplication of the thumb and the maternal uncle

has a thumb hypoplasia. Molecular testing of the known SALL4 gene mutation was offered to the affected family members.

#### Conclusion

This is the second proven case of clinical Townes-Brocks syndrome due to SALL4 defects. Both Okhiro syndrome and TBS overlap clinically, but Okhiro syndrome clearly differs from TBS in the presence of limb defects including shortening of the radius which is not associated with SALL1 defects. The mutation of exon 2 c.1900A>T, p.K634X, is causing rather a TBS phenotype here but may also cause clear Okhiro syndrome in other patients. The finding of a SALL4 mutation instead of a SALL1 defects means that the prognosis is better with respect to renal function and psychomotor development, since SALL4 point mutations are not typically associated with renal failure, delayed psychomotor development, or behavioural abnormalities commonly observed with SALL1 mutations.

Besides the genetic examination of our patient we also aim at a full family testing to shed more light on this exceptional condition.

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### P-Compl-138

#### **Meta-analysis identified 13 novel loci for waist-hip ratio and reveals a powerful gene-by-sex interaction: Do we gain from sex-stratified analyses?**

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Introduction: Waist-hip ratio (WHR) is a measure of body fat distribution and a predictor of metabolic consequences independent of overall adiposity. WHR is heritable, but few genetic variants influencing this trait have been identified. Differences in the waist-hip-ratio (WHR) between men and women are well-known, which raises several questions: (1) Are there genetic effects that are different between men and women? (2) If yes, are these (i) apparent in both sexes, but stronger in one than in the other (consistent effect direction, CED), (ii) or are there directionally opposing signals in men and women (opposite effect direction, OED)? (3) When do we gain from sex-stratified analyses?

Material and methods: We collected results of study-specific genome-wide association analyses based on ~2.8M genotyped and imputed SNPs with WHR. We conducted sex-combined meta-analysis of 32 genome-wide association studies for WHR adjusted for body-mass-index (up to 77,167 participants), following up 16 loci in an additional 29 studies (up to 113,636 subjects).

We then performed the sex-stratified analyses. We pursued two parallel strategies: (i) We scanned sex-specific p-values controlling for false discovery rate at 5% to pinpoint signals with CED. (ii) We scanned the p-values testing for sex difference to grasp signals with OED.

Results: In the sex-combined analyses, we identified 13 novel loci in or near RSPO3, VEGFA, TBX15-WARS2, NFE2L3, GRB14, DNM3-PIGC, ITPR2-SSPN, LY86, HOXC13, ADAMTS9, ZNRF3-KREMEN1, NISCH-STAB1, and CPEB4 ( $P$   $1.9 \times 10^{-9}$  to  $1.8 \times 10^{-40}$ ), and confirmed the known signal at LYPLAL1. When stratifying the analyses of these 14 loci by sex, we found seven of these loci with marked sexual dimorphism, all with a stronger effect on WHR in women than men ( $P$  for sex-difference  $1.9 \times 10^{-3}$  to  $1.2 \times 10^{-13}$ ) including the only previously identified women-specific association near the LYPLAL1 gene on chromosome 1 ( $P$  for association among men =0.36, among women = $4.9 \times 10^{-33}$ ,  $P$  for heterogeneity = $1.8 \times 10^{-13}$ ).

In the sex-stratified analysis on the genome-wide scale, our list of hits included promising candidates with CED implicating fat metabolism (ADIPOQ) or the regulation of adipocyte differentiation (PPARG), which we put forward to a replication stage. There was no clear indication for signals with OED.

Conclusion: Our findings provide evidence for multiple loci that modulate body-fat distribution, independent from overall obesity, and reveal a powerful gene-by-sex interaction.

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### P-Compl-139

#### **rare variations in patients with combined congenital thyroid and heart defects**

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Two to three percent of the children are born with a major birth defect, the majority being sporadic cases. Congenital hypothyroidism from thyroid dysgenesis (CHTD) occurs at an incidence of 1:3000-4000 in newborns. The co-occurrence of CHTD and congenital heart defects (CHD) is statistically overrepresented and predominantly arises on a sporadic manner. Over the last years, several genomic variants including coding mutations as well as large Copy number variants (CNV) have been implicated in the pathogenesis of

both diseases and their combination. Therefore, we hypothesized that rare inherited and rare de novo variants (mutations and/or CNVs) contribute to the co-occurrence of CHD and CHTD.

We accessed a clinic database at the CHU Sainte-Justine childrens hospital (Montreal, Canada) to identify patients with CHTD and hemodynamically relevant CHD. For this pilot study we selected three patients and their parents and studied at high resolution their CNV distribution and sequenced critical genes known to play a role in CHTD and CHD pathogenesis. Due to concerns of identifying truly rare CNVs we used a fully-phenotyped and matched control cohort (N = 466) to identify CNVs absent among French-Canadians. In addition we excluded common copy number polymorphisms using public available Databases. Further validation was performed using quantitative PCR and caryotyping for the identified CNVs.

Several rare variants have been detected. One individual showed a low penetrance allele in NKX2.5 (R26C). In the second case, we found a rare inherited deletion on chromosome 16p12.3 encompassing SYT17. The third patient showed a complex 47, XXY karyotype, a rare de novo deletion on chromosome 17p13.1 intersecting NTN1 as well as an atypical de novo 22q11deletion (CRKL deleted but intact TBX1).

We have strong evidence that rare variants contribute to the combined phenotype of CHTD and CHD. In silico analysis of the genes identified as well as preliminary studies in animal models point to a role in disease pathogenesis. Our approach holds great promise for the further genetic dissection of CHTD and CHD as a complex trait caused by rare variants with variable effects.

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## P-Compl-140

### Association study of 17 different pain-relevant genes with Complex Regional Pain Syndrome (CRPS)

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Introduction: Complex Regional Pain Syndrome (CRPS) is a complex pain disease evoked after noxious events and characterized by neurogenic inflammation, edema and trophic changes of the skin. In most cases CRPS is induced by an inciting local trauma followed by continuing pain disproportionate to the inciting event, hyperalgesia, skin temperature and/or sweating asymmetry and edema. The etiology of CRPS remains unclear, however, multiple studies strongly suggested that genetic factors contribute to the disease. Several attempts have been made to associate genes involved in pain perception with this disorder but to date without success. We therefore performed a genetic case-control association study by genotyping 32 SNPs representing 17 different genes previously found to be associated with pain perception and pain response. A cohort of 389 German CRPS patients were analysed which, to our knowledge, currently represents the biggest CRPS cohort available worldwide.

Methods: Genomic DNA from 389 CRPS cases and 428 gender-matched controls were tested by allelic discrimination for 32 SNPs using primer/probe sets on a high-throughput genotyping platform (TaqMan OpenArray, Applied Biosystems). The software Haploview was used to do association testing on all 32 SNPs and six haplotype blocks. Five of the 32 SNPs revealed no unequivocal genotyping results and had to be reanalysed by Sanger sequencing. Genotype and allele frequencies were compared between cases and controls using a chi square test for independence. Statistical significance was considered if the p value was below 0.05 after application of 10000 permutation tests.

Results: High-throughput genotyping revealed no significant association between the tested SNPs or haplotypes and the whole cohort of CRPS patients. Also analysis of clinical subgroups comprising CRPS I and CRPS II or CRPS of 'primarily warm' or 'primarily cold' type revealed no significant association. An association (p=0.007) was detected for a SNP in the gene CARD15/NOD2, which has been shown to be strongly associated with Crohn's disease. However, CRPS would be associated with the 'protecting' allele. We conclude that this significant p-value was most likely due to the very low frequency of the minor allele (MAF, 0.04) which was significantly more frequent in controls and does therefore not represent a true association.

Conclusion: No significant association between well known polymorphisms in genes involved in pain perception/pain response and CRPS was observed. Genome-wide association studies in combination with additional CRPS cohorts will be necessary to reveal the genetic factors contributing to CRPS and are currently under way.

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## P-Compl-141

### Increased genomic copy number of $\beta$ -defensins on 8p23.1 and its influence on psoriasis

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Previously, we reported association of an increased genomic copy number of a  $\beta$ -defensin cluster on 8p23.1 with psoriasis in Dutch and German case control cohorts (Hollox et al. Nat Genet 2008). In the meantime, our findings have neither been replicated nor rejected by independent studies, probably due to technical difficulties of differentiating copy number states of more than four copies.

In order to check the relevance of the association finding for psoriasis, we performed extensive methodological comparisons and an independent replication study. All DNAs of patients and control probands - previously genotyped and novel ones- were purified with Qiagen columns. Furthermore for validation of copy number values previously determined by paralog ratio test (PRT) based on pseudogene HSPD5.8 (= classical PRT), we established two independent methods: MLPA and a new PRT method (HSPD21). We observed satisfying correlation coefficients between the three methods:  $R^2 = 0.92$  for MLPA and classical PRT ( $n=159$ ),  $R^2 = 0.93$  for classical and new PRT ( $n=68$ ). Furthermore, copy numbers obtained by a high resolution SNP array (Affymetrix 6.0) were compared with PRT copy numbers in 600 psoriasis patients. No correlation was observed indicating that the array technique is not a suitable method to detect smaller copy number differences at loci with highly variable copy numbers such as the  $\beta$ -defensin cluster. We concluded that the classical PRT method on purified DNA is a suitable method to determine copy number at this locus.

We then re-evaluated findings in the German case control cohort as published in Hollox et al. (Nat Genet 2008) and confirmed the association with a  $p = 1.22E-05$  (Mann-Whitney-test for unrounded copy number data). For the replication study we genotyped 1315 psoriasis patients and 593 control individuals with the classical PRT method. Statistical analyses of unrounded copy number data replicated the association with higher copy number in psoriasis ( $p = 3.46E-03$ ). A combination of previous and replication datasets (obtained with purified DNAs only) resulted in a  $p$ -value of  $9.94E-07$ . Overall, our study confirms association of psoriasis with increased copy number of  $\beta$ -defensin cluster on 8p23.1.

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## P-Compl-142

### Genetic variants within the promoter region of CNTNAP2 – A possible cause of autism spectrum disorder

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#### Introduction

Autism spectrum disorders (ASD) are predominantly genetically determined with an onset in early childhood and a high heritability of around 90% (Freitag, 2007). A core feature of this genetically complex neurodevelopmental syndrome is language deficit. Previous association analyses suggested the involvement of CNTNAP2 (Arking et al., 2008; Alarcon et al., 2008), located in one of the best replicated linkage regions for ASD on 7q35. This gene is coding for the 180-kD Neurexin Caspr2, a cell adhesion molecule concentrated at juxtaparanode. Several single nucleotide polymorphisms (SNPs) and a short tandem repeat (STR) have been identified in the promoter region of CNTNAP2, but not yet been assessed as risk factors for ASD. The aim of the present study was to confirm an association of common variants within the promoter region of CNTNAP2 with ASD.

#### Methods

DNA of 172 parent child trios and 51 parent child duos and 14 single children with the index child carrying an ASD diagnoses according to ADI-R and ADOS was extracted from blood cells. The genomic region of interest was amplified by PCR and analysed by sequencing. Heterozygous STRs were verified by RFLP-analysis. Sequencing was accomplished according to standard methods. Family based association test were done by UNPHASED, quantitative analyses by SAS.

#### Results

In our sample eight SNPs were found which are currently not included in any database. In contrast seven known SNPs (rs13225855, rs13225866, rs13225872, rs13226652, rs13225963, rs13225979, rs13226843) could not be replicated. rs34712024 and the trimeric STR rs71781329 were nominally associated with ASD and the number of STR inserts could be verified by RFLP-analysis. Nominal association of the detected SNP at position 145813047 (GRCh37) was observed with age of first sentence in months (ADI-R) in the subsample of ASD children with the respective data.

#### Conclusion

Our findings suggest an association of the known CNTNAP2 promoter variants rs34712024 and rs71781329 with ASD, and of the new variant at position 145813047 (GRCh37) with language delay in ASD. These variants are currently genotyped in a larger sample with increased power.

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### P-Compl-143

#### Genome-wide association study of pseudoexfoliation syndrome in German population

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Pseudoexfoliation (PEX) syndrome represents a complex, late-onset, generalized disease of the extracellular matrix characterized by the progressive, stable deposition of abnormal fibrillar aggregates in various intra- and extraocular tissues. It is the most common identifiable cause of open-angle glaucoma accounting for about 25% of all open-angle glaucoma worldwide. The etiology of PEX is poorly understood, but two single nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (LOXL1) gene have been recently identified as strong genetic risk factors for both PEX syndrome and PEX glaucoma (PEXG). In order to identify further genetic variants underlying its etiology, we performed a genome-wide association study (GWAS) on German patients with PEX and PEXG using the Affymetrix Genome-wide Human SNP Array 6.0.

After quality filtering and imputation, 1,564,797 SNPs were tested for association to pseudoexfoliation in a sample of 771 cases and 899 population controls of German descent from the KORA study. Association analysis yielded no genome-wide significant loci except the known susceptibility locus LOXL1, which was confirmed with an odds ratio of 2.98 at the SNP rs2165241 ( $p=1.43E-36$ ). Subsequently, we selected 32 SNPs in 15 chromosomal regions showing nominally significant differences in allele frequencies ( $p \geq 2.64E-05$ ) and genotyped these 32 SNPs in further 544 German patients with PEX and PEXG, using the TaqMan OpenArray platform. Genotypes of 1,190 individuals from the PopGen study were used as additional control sample. After quality control, 28 SNPs in 13 loci remained associated with nominal significance. In the combined data set of all 1,315 cases and 2,089 control subjects, the most significant p-value was observed at SNP rs8064995 ( $p=7.69E-06$ , OR=1.33) on chromosome 17, about 20 kb upstream of PRKCA gene locus.

None of the 13 loci showed genome-wide significant association, suggesting that like in many other complex traits, most genetic factors in PEX must have small effect sizes requiring very large studies to be detected in GWA studies. Our study confirms the strong association of the LOXL1 locus with PEX/PEXG and identifies further candidates which have to be corroborated in larger cohorts.

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**P-Compl-144****Investigation of three susceptibility loci for non-syndromic cleft lip with or without cleft palate in Mesoamerican and Baltic populations**

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Cleft lip with or without cleft palate (CL/P) is one of the most common congenital malformations worldwide. It may either occur as part of a complex malformation syndrome, or as an isolated, non-syndromic anomaly (NSCL/P) representing about 70% of all CL/P cases. NSCL/P is considered to be of multifactorial etiology with both, genetic and environmental factors, respectively, contributing to disease susceptibility.

In European populations, NSCL/P has a prevalence of about 1 in 1,000. Prevalence, however, varies between different ethnicities. In recent genome-wide association studies (GWAS) performed in German cases and controls, our group has identified three chromosomal regions (Birnbaum et al. 2009, Mangold et al. 2010). Besides variants in IRF6, these loci are the only regions that have been conclusively implemented in the etiology of NSCL/P to date. To investigate whether these three regions might also contribute to NSCL/P in non-Western-European populations, we genotyped the same variants in two additional samples from Estonia (maximum 105 cases / 1,023 controls) and Mexico (149 cases, 303 controls).

In the Estonian population, single marker analysis identified a highly significant association for rs987525 at chromosome 8q24, with similar odds ratios but a lower population attributable risk as compared to the initial study. Also, a significant association was observed for the locus at 10q25, but not at 17q22. In the Mayan population, single marker analysis revealed a significant association between NSCL/P and risk in both, the 8q24 and 10q25 loci. In contrast to previous findings, here, the association at the 8q24 locus was driven solely by homozygote carriers of the risk allele. This suggests that this locus might act in a recessive manner in the Mayan population. Again, no evidence for association was found at the 17q22 locus.

Our results suggest that variants in chromosomal regions at 8q24 and 10q25 contribute to NSCL/P-development in both, European and Mesoamerican ethnicities.

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**P-Compl-145****Linkage analyses, genome-wide association studies and next generation sequencing in an extended family with myocardial infarction**

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Introduction: Our group established a large collection of myocardial infarction (MI)-families with 2-5 affected first-degree relatives. Based on a genome-wide linkage analysis with 805 microsatellites, we found a peak in one of the families on chromosome 8q24 spanning approximately 12 Mb.

Aim: Our Aim was to confirm and narrow down the linkage signal by genotyping the Affymetrix 10K array and to further study the corresponding region in CARDIoGRAM (1). Furthermore, whole exome sequencing of 2 affected cousins may identify a deleterious mutation in this family.

Methods: 305 single nucleotide polymorphisms (SNPs) from the 10K array in the same region on chromosome 8 were used for fine-mapping. The linkage analysis based on 43 microsatellites of 15 family members and the analysis based on 305 SNPs of 23 family members were model-based assuming an autosomal-dominant inheritance pattern with a disease frequency of 1% and incomplete penetrance allowing

for phenocopies. The results were then compared with those from a large meta-analysis of genome-wide association (GWA) studies on coronary artery disease in the CARDIoGRAM consortium. This consortium includes 22,233 patients with coronary artery disease (CAD) and 64,762 controls mostly from population-based studies. Whole exome sequencing in two affected cousins is ongoing.

**Results and Discussion:** On account of the linkage analysis with 305 SNPs the linkage region on 8q24 could be narrowed down to a 8 Mb haplotype cosegregating with the disease in the family. Corroborating the linkage findings, several SNPs in the region were found to be associated in the CARDIoGRAM data set. The lead SNP rs11993784 in this region showed association with CAD at  $p_{FE}=1.26 \cdot 10^{-6}$  in the fixed effects (FE) model. Specifically, these SNPs are located within an intergenic region. This region includes regulatory elements that are currently being sequenced.

**Conclusion:** A comprehensive genetic analysis using linkage, GWA studies and next-generation sequencing allowed to narrow down a locus for MI in an extended family to a 8 Mb region. Further sequencing, especially of an intergenic region at 0.1 Mb is of greatest interest, because of an association signal in the CARDIoGRAM data that hints at an underlying causal mutation.

(1) M. Preuss et al., Design of the Coronary ARtery Disease Genome-wide Replication And Meta-Analysis (CARDIoGRAM) Study. *Circ Cardiovasc Genet*, 2010 Oct 1; 3(5): 475-83

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## **P-Compl-146**

### **Heat shock protein HSPA4 is required for progression of spermatogenesis**

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HSP110 family members act as molecular exchange factors (NEF) of mammalian and yeast HSP70 chaperones during the ATP-hydrolysis cycle. We describe here the expression and physiological function of murine HSPA4, a member of the HSP110 family. HSPA4 is ubiquitously expressed, but is highly enriched in both male and female germ stem cells during prenatal development of gonad. Analyses of Hspa4-deficient mice revealed that all null mice on hybrid C57BL/6J × 129/Sv genetic background were apparently healthy. Although Hspa4 is expressed as early as E13.5 in male gonad, a lack of histological differences between Hspa4<sup>-/-</sup> and control littermates suggests that Hspa4 deficiency does not impair the gonocytes or their development to spermatogonia. However, the majority of the Hspa4-deficient male mice displayed impaired fertility, whereas females were fertile. Number of spermatozoa and their motility were found to be drastically reduced as compared to wild-type littermates. Most pachytene spermatocytes in juvenile Hspa4-mutant mice failed to complete the first meiotic prophase and became apoptotic. Furthermore, the down-regulation of transcription levels of genes known to be expressed in spermatocytes at late stages of prophase I and post-meiotic spermatids lead to suggest that spermatogenesis is arrested at late stages of meiotic prophase I. These results provide evidence that HSPA4 is required for normal spermatogenesis.

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**P-Compl-147****Genome-wide SNP-SNP Interaction Analysis in Bipolar Disorder**

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In recent years, genome-wide association studies (GWAS) have identified many common single-nucleotide polymorphisms (SNPs) that contribute to complex traits. However, there is still missing heritability in genetically-complex traits, including bipolar disorder (BD) and other common neuropsychiatric diseases. Although this might be explained by overestimation of heritability, it could be explained in part by yet unidentified common variants with very small genetic effect sizes, or by epistatic effects (interaction) between genetic variants. Due to the high number of possible SNP-SNP interaction pairs arising from typical microarray-based GWAS datasets (about 500K SNPs), genome-wide interaction analysis (GWIA) are computationally challenging (about 1.0E11 SNP pairs).

In the present study, we applied a novel approach for GWIA to a dataset of 473,227 SNPs from 1,158 patients with a DSM-IV diagnosis of BD and 2,172 population-based controls, all of German ancestry. This approach has been implemented in the latest version of the program INTERSNP (Herold et al. 2009). Given the genome-wide significance at the 0.05 level for genotypic interaction and a marker set with 500K SNPs ( $P=1.0E-12$ ; Becker et al. 2010), we found a genome-wide significant interaction between a SNP in the gene B3GALTL on chromosome 13q12 and an intergenic SNP on chromosome 11q24 ( $P=9.49E-13$ ). The B3GALTL marker also participates in the second most significant pair by interacting with another SNP on chromosome 11q24, which is in strong linkage disequilibrium with the 11q24 SNP of the top finding ( $r\text{-squared}>0.8$ ).

This supportive result suggest that there is a true-positive interaction between the loci 13q12 and 11q24 in bipolar disorder. Our main GWIA finding, however, requires evidence for replication. For this purpose, we are currently following-up our top results in large independent samples of bipolar disorder. The results will be presented at the conference.

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**P-Compl-148****Identification of risk genes for age-related macular degeneration and drusen formation in rhesus macaques as animal model**

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Age-related macular degeneration (AMD) is a multifactorial, degenerative disorder of the macula and the leading cause of blindness in industrialized countries. Heritability is estimated up to 71%. Since 2005, two major and numerous moderate AMD susceptibility loci have been identified. Several variants in the region 10q26, harboring the genes ARMS2 and HTRA1, are strongly associated with AMD in humans. Both genes are promising candidates for being involved in the pathogenesis. However, the associated variants are located in a region of strong linkage disequilibrium (LD) and, thus far, in humans neither statistical nor functional approaches could determine the actually causative gene.

The aim of this study is to analyze the LD structure of the 10q26 homologous genomic region in the rhesus monkey and to uncover whether variants in ARMS2 or HTRA1 are causative for an association.

Rhesus monkeys – like humans – possess a macula and suffer from drusen, one major hallmark of AMD. Studies have shown that humans and rhesus macaques share important candidate genes for AMD. Moreover, several genetically homogenous groups exist, offering an excellent opportunity for the identification of risk genes. We studied such a genetically homogeneous cohort of 91 rhesus monkeys descending from the island Cayo Santiago that are currently housed in the German Primate Centre in Göttingen. Within this group, ophthalmoscopic examinations revealed a high drusen prevalence of about 55% in monkeys >5 years.

We genotyped 56 polymorphisms in a 20Mb region around ARMS2 and HTRA1 and created a LD-plot using Haploview. Furthermore, a test for allelic association was performed with PLINK.

Only one of these polymorphisms deviates from Hardy-Weinberg-Equilibrium. The LD-plot resembles those of humans: the two exons of ARMS2 as well as exon 1 of HTRA1 are in linkage disequilibrium. An adjacent block starts halfway in intron 1 of HTRA1. The test for allelic association revealed a significantly associated variant(-558T>G) in the promoter of HTRA1 ( $p=0.0225$ ).

These preliminary results indicate that – as in humans - a determination of the risk gene with statistical methods alone is not possible in rhesus monkeys. However, the conserved LD-plot could point to a functional connection between ARMS2 and HTRA1. To finally resolve whether ARMS2 or HTRA1 is the responsible risk gene, further studies including HTRA1 promoter assays and a more detailed statistical analysis considering covariates and haplotypes are needed.

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## P-Compl-149

### Investigation of variants of the AR/EDA2R and 20p11 locus and diverse hormone receptor genes in women with female pattern hair loss

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Androgenetic alopecia (AGA) is a common hair loss disorder with a complex mode of inheritance, occurring in both men and women. In men this condition is known as male-pattern baldness, in women as female pattern baldness (FPHL).

Affected women typically present with a rather diffuse, mostly progressive thinning of hair at the crown. The etiopathogenesis of female pattern baldness is incompletely understood. However, the familial occurrence of AGA in females and in males suggests that a shared pathological reaction pathway is involved in the development of AGA in both sexes. On the basis of this assumption, we firstly examined in a FPHL collective the role of the androgen receptor (AR), the ectodysplasin A2 receptor (EDA2R) and the 20p11 locus which are the major genetic susceptibility loci for male pattern baldness.

In a sample of 145 unrelated white English and 53 German FPHL patients and 179 English, respectively 150 German controls, we genotyped 31 SNPs including the three most significant markers rs12558842, rs2497938 and rs1998076 that had been reported for the human AR/EDA2R locus in male pattern baldness. Our results do not show any significant association, neither in the English or German sample, subgroup analyses nor in a meta-analysis.

Assuming that an androgen-independent pathway might play a role in the development of FPHL, we investigated in a second step an additional 32 variants of several hormone receptor genes, including the aromatase-gene (CYP19A1), the progesterone receptor (PGR), the steroid-5-alpha-reductase alpha polypeptide 1 and 2 (SRD5A1, SRD5A2) and the estrogen receptors 1 and 2 (ESR1, ESR2). However, none of the genotyped variants showed any significant association in the overall samples, the subgroup or meta-analyses. In future, further enlargement of the collective as well as a genomewide association study might help to identify genes contributing to the development of FPHL.

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## P-Compl-150

### Analysis of skewed X-chromosome inactivation in British patients with systemic sclerosis

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Introduction: Systemic sclerosis (SSc) is an autoimmune disorder characterized by excessive fibrosis, vascular abnormalities and immune system dysfunction that can affect several organs or tissues (mainly skin, lungs and kidneys). The disease is the end result of a complex interaction of genetic factors and unknown environmental influences. Three subgroups can be distinguished, morphea, limited and diffuse SSc. SSc is 3 (diffuse SSc) to 9 (limited SSc) times more frequent in females than in males. Uz et al. (2008) found significantly elevated ratios of skewed X-chromosome inactivation (XCI) in SSc patients. To verify these findings in an independent patient population and also enable epigenotype-phenotype correlations with regard to different clinical subgroups, we investigated the XCI patterns in a British cohort of 95 patients with diffuse SSc and 175 patients with limited SSc.

Method: The XCI status was analysed using the androgen receptor (AR) gene (HUMARA) assay. This assay is based on indirect determination of the methylated state of CCGG sites adjacent to the highly polymorphic CAG repeat in the AR gene, using digestion with the methylation sensitive restriction endonuclease HpaII, followed by amplification of the undigested methylated DNA using PCR and size fractionation as well as quantification of fluorescence-labelled PCR products employing capillary electrophoresis. Random X inactivation was assumed as a 50:50 ratio. Skewed XCI patterns were further subdivided into moderately skewed (>70:30 to <80:20), skewed (>80:20 to <90:10), and extremely skewed (>90:10) patterns.

Results: 240 patients were heterozygous for the CAG polymorphism and thus could be compared to a cohort of phenotypically unaffected females with regard to their XCI pattern (Amos-Landgraf et al., 2006). The frequency of skewed XCI was much higher in patients (40.8%) than in controls (25%,  $p=0.0000113$ ). Extremely skewed XCI, skewed XCI, and moderately skewed XCI, respectively, all were observed more frequently in patients than in controls, at 10% vs. 3.6% ( $p=0.00068$ ), 15% vs. 10.6% ( $p=0.025$ ), and 15.8% vs. 10.8% ( $p=0.018$ ). Further, the frequency of moderately skewed XCI was significantly elevated ( $p=0.038$ ) in the limited SSc (18%, 29/161) compared to the diffuse SSc (11.4%, 9/79) group, whereas no significant difference between both subgroups was observed with regard to the incidence of extremely skewed XCI and skewed XCI. In addition, homozygosity for the CAG polymorphism (rate of heterozygosity is 88%) was detected in 30 patients (12.5%); the frequency of homozygous was higher in patients with diffuse SSc (19%, 16/95) than in patients with limited SSc (8%, 14/175) ( $p=0.015$ ).

Discussion: Our data confirm the findings of recently published studies that suggest skewed XCI as a predisposition factor for SSc. In addition, they demonstrate for the first time differences in the frequency of skewed XCI between the diffuse and the limited SSc subgroups. Skewed XCI may compensate for the presence of an X chromosome with a deleterious mutation that would provide a growth disadvantage to the affected blood cells. Further, it may be speculated that the increased rate of homozygosity for the CAG polymorphism in diffuse SSc is indicative for a microdeletion of one AR allele.

#### References:

- Amos-Landgraf JM et al (2006) *Am J Hum Genet* 79:493-499  
Uz E et al (2008) *Clin Rev Allergy Immunol* 34:352-355

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## P-Compl-151

### Proteomic Analysis of the Cellular Response to Ionizing Radiation in ATM-Mutation Carriers

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Annually 57,000 women are diagnosed with breast cancer in Germany, 5-10% of the cases being familial. Beside mutations in BRCA1/2, ATM mutations confer an increased breast cancer risk. This holds in particular for missense mutations in the C-terminal FAT, kinase and FATC domains of the ATM gene, while heterozygous protein truncating and splice junction variants confer only a marginal risk (1).

The present proteomic study on the effect of compound heterozygous nonsense and heterozygous missense ATM mutations on the cellular response to ionizing radiation (IR) complements an earlier study that employed gene expression profiling (2). EBV-transformed lymphoblastoid cell lines (LCLs) of breast cancer patients heterozygous for 7271T>G, compound heterozygous A-T patients, and control patients were gamma-irradiated with 3 Gy and harvested after 6 hours of incubation. After nuclei separation the

corresponding proteins were purified and purity was monitored by immunoblotting against several compartment-specific proteins.

The levels of ATM and pATM (Ser1981) protein were determined by immunoblotting. In comparison to the control cell lines the phosphorylation of ATM was significantly decreased in A-T patients ( $P \leq 0.001$ ). Afterwards, the nuclear proteomes of the irradiated and non-irradiated cells were compared using differential in-gel electrophoresis (DIGE). The comparison of the irradiated and non-irradiated nuclear proteomes of compound heterozygous A-T patients, heterozygous breast cancer patients, and control patients showed 134 regulated spots with  $P$ -values  $\leq 0.05$  and a statistical power  $\geq 0.8$ . These significantly regulated spots were picked, digested and analyzed by nano-LC-QTOF-MS/MS. We identified proteins such as SMC1A, Mre11 and Rad23B, which play a role in DNA repair and/or are known downstream targets of ATM. The SMC1A-containing spot was 1.6-fold down-regulated in controls ( $P \leq 0.008$ ), while the Mre11-containing spot was about two-fold down-regulated in both controls and A-T patients ( $P \leq 0.014$ ). These down-regulations occur presumably upon posttranslational modifications. In addition, the proteins RED, KHSRP and NONO were identified. In large-scale proteome analysis these proteins were already shown to be phosphorylated in response to DNA damage on consensus sites recognized by ATM (3). Currently, regulated proteins are validated by immunoblotting.

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### **P-Compl-152**

#### **Evidence for a male-specific association between the mitochondrial tRNA-threonine variant 15928G>A and Alzheimer's disease**

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Alzheimer's disease (AD) is a complex neurodegenerative disorder that is characterized by progressive loss of memory and cognitive abilities. As a common disease, AD severely affects the quality of life in the ageing population and constitutes the most common cause of dementia in the elderly (age 60+) and affects an estimated ~40% of the population aged 90+. Mutations in several human genes are known to play a causative role in the pathogenesis of familial (early-onset) AD, but sporadic (late-onset) AD constitutes a complex, multi-factorial disease with numerous causes of genetic, environmental, and sociological origin. While dysfunctional mitochondrial energy metabolism constitutes a well-established risk factor in numerous late-onset degenerative diseases of ageing including AD, the importance of mitochondrial DNA (mtDNA) variation as genetic risk factor in AD still remains an issue of controversy.

Here, we studied the role of mtDNA variation in the pathogenesis of late-onset AD in a large, longitudinal, prospective cohort of ageing subjects, matched by age (75+), sex and geographic origin from the Vienna-Transdanube-Aging (VITA) Study. By sequencing large stretches of the mtDNA in a total of  $n=83$  subjects selected from the cohort, we observed that carriers of the minor European haplogroups J and T were significantly overrepresented in the AD group. When we subsequently analyzed specifically the m.4216T>C variant within the NADH dehydrogenase subunit 1 (ND1) gene (which is a marker for the J and T haplogroups) and the m.15928G>A variant in the tRNAThr gene (haplogroup T marker) in the total cohort ( $n=470$ ), we found a statistically significant association of the tRNAThr m.15928G>A variant with AD (14.6% vs. 6.9%,  $P=0.023$ , two group Yates' continuity corrected Chi-Square test). Remarkably, the association was (i) not identified in carriers of the apolipoprotein E (APOE) APOE\*4 AD-risk allele (7.4% vs. 9.9%,  $P=1$ ), and (ii) much stronger in male subjects (22.7% vs. 5.6%,  $P=0.027$ , odds ratio 5 [CI: 1.4-18.2]) compared to females (16.3% vs. 6.7%,  $P=0.064$ ). We further found that this association is statistically significant only in subjects developing AD between age 75 and 80, but not in subjects developing AD after the age of 80.

Notably, our results greatly support a possible role of the mitochondrial tRNAThr m.15928G>A polymorphism, a T-haplogroup marker, in the pathogenesis of AD. Owing to the great advantage of a large, longitudinal cohort of ageing subjects in a prospective study, we have ultimately identified a novel genetic risk factor for AD, which is probably male-specific and accounts for a non APOE\*4-related disease subgroup.

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### **P-Compl-153**

#### **Functional role of Optineurin (OPTN) in the morphology of the Golgi apparatus and in endocytosis of neurotrophic factors**

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Purpose:

It has been shown that mutations in the OPTN gene can cause hereditary forms of open-angle glaucoma whereas most of the patients suffer from normal-tension glaucoma (Rezaie et al., 2002). Optineurin is a 74 kDa protein implicated in several cellular processes like signal transduction of the tumor necrosis factor pathway (Li et al., 1998), vesicular trafficking (Sahlender et al., 2002) and regulation of transcription (Moreland et al., 2000). In the present study we sought to determine the subcellular function of Optineurin at the Golgi apparatus and in secretion of neurotrophic factors in the retinal ganglion cell line RGC-5 after knock-down of the endogenous protein by RNA-interference.

#### Methods:

The expression of the OPTN gene was silenced in the RGC-5 cell line applying a shRNA expression vector to generate a stable knock-down as well as transient transfection of siRNA. The localization of the endogenous protein and the morphology of the Golgi apparatus was compared to cells treated with scrambled siRNA using a confocal microscope by immunostaining of Optineurin and the Golgi marker GM130. In addition, the ultrastructure of the Golgi apparatus was analyzed with a transmission electron microscope. To study the secretion of neurotrophic factors the concentrations of CNTF and Neurotrophin 3 were quantified after knock-down in the culture medium and expression levels of CNTF and Neurotrophin 3 were analyzed by RT-PCR. In order to observe the cell growth and volume of cells the CASY® Cell Counter and Analyser TT was used and apoptosis was quantified by an ELISA for the detection of cytoplasmic histone-associated DNA fragments. Cell cycle analyses were performed using a FACS.

#### Results:

In control cells Optineurin was located in diffuse distributed vesicles and in close proximity to the Golgi complex. After knock-down the cells showed a fragmentation of the Golgi complex and an increase in cells containing abnormal and multiple nuclei. This was also observed by electron microscopy. The Golgi stacks were dispersed and had swollen and irregular cisternae. Multinucleated cells exhibited an abnormal number of centrosomes.

In stable knock-down with decreasing amounts of Optineurin the cells became bigger in diameter and volume and showed a slower increase in cell number. After transient transfection, there was a dramatic decrease in cell numbers compared to cells treated with scrambled siRNA. In addition, a significant number of apoptotic cells was observed using an ELISA in Optineurin-deficient cells when compared to controls. In contrast no evidence was found for an arrest in mitosis by FACS analyses.

In culture media of Optineurin-deficient cells the amounts of Neurotrophin 3 and CNTF were smaller than in controls.

#### Conclusions:

Optineurin is critically required for maintenance of the Golgi apparatus and the secretion of neurotrophic factors in retinal ganglion cells. We conclude that a lack of neurotrophic factors leads to apoptotic cell death in Optineurin-deficient cells. A comparable scenario could account for RGC death in glaucoma patients with mutated Optineurin.

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## P-Compl-154

### Imaging genetics of FOXP2 in dyslexia

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Dyslexia is a developmental disorder, affecting about 5-12% of all school-aged children. It is characterised by extensive difficulties in the acquisition of reading or spelling skills. Genetic influence is estimated at 50-70%, however, the link between genetic variants and phenotypic deficits is largely unknown.

One of the most prominent genes influencing speech and language development is FOXP2. Our aim was to investigate a possible role of genetic variants of FOXP2 in dyslexia using imaging genetics. This technique can combine functional magnetic resonance imaging (fMRI) and genetics to investigate the relevance of certain genetic variants on brain activation. To our knowledge, this study represents the first usage of functional imaging genetics in the field of dyslexia.

Due to the size of the FOXP2 gene and the high number of genetic variants in that region, we initially applied a case / control study (N=245) for prioritisation of FOXP2 polymorphisms for later use in imaging genetics. Eight SNPs were selected by a tagging approach and a non-synonymously coding mutation reported to be relevant for verbal dyspraxia was also investigated.

Tagging SNP rs12533005 showed nominally significant association with dyslexia (genotype GG relative risk = 1.9 [95% confidence interval 1.1-3.4], p=0.025). Therefore, this variant was chosen to study the influence of carriage of the rs12533005-G risk variant on brain activity by imaging genetics. In fMRI, the contrast of a rhyming task vs. fixation revealed a significant main effect for the factor "genetic risk" in a temporo-parietal area known for its role in phonological processing as well as a significant interaction effect

between the factors “disorder” and “genetic risk” in activation of inferior frontal brain areas. In addition, this variant was shown to alter expression of FOXP2 transcripts in silico as well as in vivo in human hippocampus tissue. Hence, our data support a role of FOXP2 genetic variants in the processing of language and phonology relevant for the development of dyslexia and demonstrate a possible framework for the application of imaging genetics in dyslexia research.

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## **P-Compl-155a**

### **Epigenetic analysis of inflammatory bowel disease**

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Ulcerative colitis (UC) and Crohn’s disease (CD) are the two main types of inflammatory bowel disease (IBD). CD can affect the whole bowel wall of each part of the gastrointestinal tract, while UC is restricted to the mucosa of the colon and the rectum. Both diseases are caused by different genetic, environmental and microbial factors, which lead to a loss of intestinal mucosa integrity. In this context, an impact of epigenetic mechanisms on IBD development must be assumed but has not been thoroughly studied up to now. To investigate the involvement of epigenetic factors in IBD pathogenesis, we aim at analyzing gene-specific [by bisulphite pyrosequencing of IBD candidate genes such as Aiolos, E2A, IRF8, OBF1, PAX5, E2A, GATA3, Ikaros, RUNX1, TCF/LEF1, members of the NOTCH gene and the MUC (Mucin) gene family] and genome-wide [using the Infinium Methylation 27k Bead Chip interrogating 27.000 CpGs that cover the promoter regions of approximately 14.000 genes] DNA methylation in affected and non-affected ileal and colonic mucosal biopsies from IBD patients as well as ileal and colonic mucosal biopsies from non-IBD patients. First experiments detected a marked decrease of methylation at the MUC2 and MUC6 promoters in affected biopsies which correlates very well with already published data on upregulation of Mucin gene expression with increasing inflammation. Methylation analysis of the other candidate genes and genome-wide methylation analysis is still in progress or pending. Our preliminary data complement current concepts that involve aberrant regulation of protective factors for intestinal epithelial barrier function in IBD pathogenesis. A comprehensive understanding of the epigenetic mechanisms contributing to IBD will likely enable development of new therapeutic agents and strategies targeting epigenetically dysregulated genes.

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## **P-Compl-155b**

### **Genetic variants in genes of the postsynaptic FMRP signalling pathway may be risk factors for autism**

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Introduction: Autistic spectrum disorders (ASD) are characterised by qualitative impairment in social interaction and communication, and restricted repetitive and stereotyped patterns of behaviour, interests, and activities. The prevalence of ASD is estimated to around 0.5 – 1%. ASD are predominantly genetically determined with a heritability of around 90% (1). However, only a few mutations and common variants have been replicated as risk factors for ASD. Fragile X syndrome is one monogenic disease which goes along with autism. Recently published data report a microduplication of chromosome 15q11.2, including CYFIP1, co-segregating with autism in a Dutch family (2). Interaction of Fragile X Syndrome Protein FMRP with CYFIP1 and cap-binding factor eIF4E, may hypothesize an involvement of CYFIP1 and eIF4E in autism (3). Thus, the aim of this study was to assess common variants within CYFIP1 and eIF4E as risk factors for ASD. Methods: Three Single nucleotide polymorphisms (SNPs) in the genes CYFIP1 and EIF4E with a MAF >5% were selected, respectively. DNA of 160 families with the index child carrying an AD diagnosis according to ADI-R and ADOS was genotyped. Genomic DNA was extracted from blood cells and genomic regions surrounding the SNPs of interest were amplified by PCR and analysed by Real-time PCR or RFLP-analysis. Family based association tests were done by UNPHASED.

Results: We observed a nominal association of CYFIP1 SNP rs7170637 with ASD. The haplotype main effect of all three analysed CYFIP1 SNPs was also tested and we observed a protective effect for haplotype CAT in the haplotype block rs2289818-rs7170637-rs3693 towards ASD.

Conclusion: Our results point towards the relevance of the postsynaptic FMRP pathway in the etiology of ASD. Common variants in CYFIP1 may be risk factors for ASD while variants in EIF4E seem to play no major role. The findings are currently replicated in a second sample. References: (1) Freitag C.M. et al.

(2010). *Eur Child Adolesc Psychiatry* 19, 169-178. (2) van der Zwaag B. et al. (2009). *Am J Med Genet B Neuropsychiatr Genet* 153B, 960-6. (3) Napoli I. et al. (2008). *Cell* 134, 1042-1054.

## P-BASIC MOLECULAR MECHANISMS

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### P-Basic-156

#### **Overexpression of HMGA2 in acute aortic dissection may lead to EndMT of vasa vasorum endothelial cells**

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**Introduction:** Acute aortic dissection (AAD) is a lethal injury of the aorta. We studied the expression of the high mobility group AT-hook 2 gene (HMGA2) in AAD tissue, since the HMGA2 protein is known to participate in epithelial mesenchymal transition, a process during which epithelial cells are reorganized to migratory mesenchymal cells. The transition of endothelial cells is a special form of EMT called endothelial mesenchymal transition (EndMT).

**Methods and Results:** Aortic tissue samples of 19 patients with AAD (mean age 59.6±16.4 years) and 32 control patients (comprised of 26 patients with aortic aneurysm, four aortic valve samples and two samples from patients with marfan syndrome, mean age 56.9±14.5 years) were collected during surgery. Quantitative PCR revealed a significantly higher HMGA2 expression in AAD compared to the control group (193.1 vs. 8.1 fold normalized to calibrator,  $P < 0.001$ ). Immunohistochemical analyses detected HMGA2 and the EMT marker SNAI1 mainly in the vasa vasorum of AAD patients.

**Conclusion:** These data suggest an upregulation of the HMGA2 gene in patients with AAD. Since the HMGA2 protein is known to participate in the regulation of EndMT and the EndMT marker SNAI1 is also expressed, the transition of aortic vasa vasorum endothelial cells to mesenchymal cells during AAD seems possible. Further studies have to clarify whether this process contributes to the development of dissections or presents a reaction to the injury.

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### P-Basic-157

#### **Fibulin 1 as a potential biomarker in acute aortic dissection**

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**Introduction:** Fibulin 1 is an extracellular matrix protein and an essential component of the aorta. Acute aortic dissection (AAD) is a catastrophic event of cardiovascular disease and requires an urgent clinical intervention. Although there are several biomarkers for the detection of AAD available, none of them is completely reliable. In an attempt to create a more reliable biomarker profile for AAD, we analyzed the serum amount of Fibulin 1 protein in AAD.

**Methods and Results:** For this study 33 serum samples were collected from patients with AAD type A (mean age 58.13±16.73 years), while 8 sera from other patients without AAD served as controls (mean age 63.75±1.39 years). Whole protein amounts were determined for each sample and 30 µg of protein were used for the quantification of the Fibulin 1 concentration in western blot. Patients with AAD featured a significantly higher Fibulin 1 serum concentration than patients without (146.32±52.4 vs. 104.82±52.24,  $p = 0.04$ ).

**Conclusion:** The overabundance of Fibulin 1 in sera obtained from patients with AAD can be explained by the fact, that during aortic dissection a tear splits the different layers of the aorta, which could possibly lead to a release of proteins into the blood. This finding warrant further investigation to define the usability of Fibulin 1 as part of a biomarker profil for acute aortic dissections.

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**P-Basic-158****Functional analysis of the obesity associated SNPs within intron 1 of the FTO gene**

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As shown by genome-wide association studies, single-nucleotide polymorphisms (SNPs) within intron 1 of the human fat mass and obesity associated gene (FTO) are associated with the body mass index and type II diabetes. Recently we have shown the SNPs affect the expression of FTO, but not the closely linked RPGRIP1L and RBL2 genes, and that the FTO transcripts made from the risk allele are more abundant than those from the non-risk allele in many different cell types. Our findings suggest that increased expression of FTO is associated with increased body weight. Nevertheless, the exact molecular mechanism by which the SNPs modulate FTO expression remains unclear. Owing to the strong LD relations ( $r^2 > 0.8$ ) between the obesity associated SNPs in European populations, the identification of the functionally relevant variation(s) is difficult. Based on association studies in populations of African ancestry (reference), open chromatin maps (UCSC genome browser) and our own in silico analyses of potential transcription factor binding sites, we have narrowed down the list of the potentially causative variations to rs3751812, rs9941349, rs9936385, rs9923233, rs17817964, rs8050136 and rs9939609. For experimental validation, we use electrophoretic mobility shift assays (EMSA) with fluorescently labelled double stranded oligonucleotides spanning the SNP of interest. So far, we have tested rs9941349 and rs3751812 with nuclear extracts from lymphoblastoid cells. For rs9941349 we observed a strong band with the risk allele, but not with the non-risk allele, suggesting that the risk allele binds a transcriptional activator. These and other EMSA results will be presented.

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**P-Basic-159****A genome-wide search for novel imprinted genes**

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Imprinting is an epigenetic process leading to parent-of-origin specific DNA methylation and gene expression. Monoallelic expression of imprinted genes is often regulated by allele-specific DNA methylation. Because of experimental limitations, the identification of imprinted genes in humans is challenging. We had the opportunity to study a patient with aberrant methylation at multiple imprinted loci. Based on the data of a genome-wide CpG methylation analysis (Infinium® HumanMethylation27 BeadChip, Illumina) in DNA from blood of this patient and appropriate controls, hypo- or hypermethylation at maternally and paternally imprinted loci was identified in the patient which was confirmed by other techniques. In addition, we observed hypo- or hypermethylation at genes not known to be imprinted. One of these genes - RB1 - was recently shown to be imprinted. In order to identify additional novel imprinted genes a group of 22 candidate genes that showed DNA methylation levels in the range of hemimethylation in the patient's parents and normal controls but hypomethylation ( $\beta$ -values  $< 0,25$ ) in the patient was chosen for allele-specific methylation and expression analysis. For five of these genes (TRPC3, LAMA3, ALX4, SLC22A8 and SLC4A11) we have completed detailed DNA methylation analysis. By bisulfite cloning and sequencing we obtained for all five genes methylated, unmethylated und partially methylated sequences for both alleles, thus methylation is random and not allele-specific.

From these data we conclude that the patient has a more general methylation defect at imprinted and non-imprinted loci.

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**P-Basic-160****Novel intrachromosomal breakpoint mechanism in AZFb region of human Y chromosome causing Oligo-Asthenozoospermia (OAT syndrome)**

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Knowledge of the complete genomic sequence of the human Y chromosome has revealed that intrachromosomal non-allelic homologous recombination (NAHR) events between large repetitive sequence blocks (called amplicons) in middle and distal Yq11 have caused genomic microdeletions in these Y regions causing infertility. They were designated AZF deletions because Tiepolo and Zuffardi mapped an

“Azoospermia Factor (AZF)” here by cytogenetic analyses of Yq11 aberrations in some men with azoospermia, 35 years ago.

The association of three of these AZF microdeletions with distinct testicular histologies (AZFa: Sertoli-cell-only-syndrome, SCO; AZFb: meiotic germ cell arrest; MA); AZFc: severe hypospermatogenesis/SCO) then confirmed, not only the early prediction of a functional AZF locus in Yq11, but also the functional contribution of some encoded AZF proteins to different phases of human spermatogenesis. Therefore, AZF is now functionally subdivided in three distinct spermatogenesis loci: AZFa, AZFb, and AZFc, respectively.

Sequence analyses have shown that AZFb and AZFc are overlapping and bordered by distinct Y-specific amplicons between 115 kb and 678 kb in length located in middle to distal Yq11. Most interesting, the amplicons are structurally assembled in five palindrome structures (P1-P5). It has therefore been thought that all AZFb and AZFc microdeletions might have been created by the same basic molecular mechanism, namely intrachromosomal NAHR events. Consequently, simple PCR assays with some internal non-polymorphic STS marker were developed which should then be able to detect them and a detailed analysis of individual genomic breakpoint locations in each infertile patient with a putative AZFb or AZFc deletion was omitted.

However, this seems only to be the tip of the ice-berg. Analysing the genomic breakpoints of an infertile man with OAT (Oligo-Asthenzo-Teratozoospermia) syndrome and a 3.45 Mb long AZFb deletion we were able to reveal a novel molecular mechanism for the creation of an AZFb deletion. Sequence analyses identified the breakpoint-fusion event proximal in the tip of P4, outside the flanking amplicons and distal in the t1 amplicon. It is probably based on the testis specific transcriptional activity which we found in the associated proximal and distal AZFb border regions.

Since more patients with putative AZFb deletions and distinct testicular pathologies have been reported in the literature, we speculate that testis-specifically transcribed sequence domains in AZFb located outside the protein encoded Y genes domains might have generally the potential to break preferentially, but only in the male germline. If this holds true, we would have found also another explanation for the frequent occurrence of the dicentric so called iso-Yp chromosomes visible in the microscope as non-fluorescent Y chromosome (Ynf) with breakpoint- and fusion-points mainly in the genomic AZFb region.

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## P-Basic-161

### **Lack of Wdr36 leads to preimplantation embryonic lethality in mice and delays the formation of small subunit ribosomal RNA in human cells in vitro**

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#### Introduction

Mutations in WDR36 (WD repeat domain 36) play a causative role in some forms of primary open-angle glaucoma (POAG), a leading cause of blindness worldwide (Monemi et al., HMG 2005). WDR36 encodes for a 951 aa protein that is characterized by the presence of multiple WD40 repeats and shows homology to Utp21, an essential nucleolar ribonucleoprotein in the yeast *Saccharomyces cerevisiae* and part of the small subunit (SSU) processome required for maturation of 18S rRNA (Footz et al., HMG 2009).

#### Materials and methods

To clarify the functional role of Wdr36 in the mammalian organism, we generated mutant mice with a targeted deletion of Wdr36. The phenotype of heterozygous (+/-) and homozygous (-/-) embryos was analyzed throughout development. To investigate early stages of development, zygotes were isolated and cultivated up to the blastocyst stage. In parallel experiments, we used RNA interference to deplete Wdr36 mRNA in wild-type mouse embryos. Specific siRNA was also used to knock down the expression of WDR36 in a human trabecular meshwork cell line (HTM-N). The effects of siRNA experiments were analyzed by Northern blot hybridization and experiments involving metabolic labeling (pulse-chase). Subcellular localization of WDR36 was investigated by immunocytochemical stainings.

#### Results

Deletion of Wdr36 in the mouse caused preimplantation embryonic lethality, and essentially similar effects were observed when Wdr36 mRNA was depleted in wild-type mouse embryos by RNA interference. Further, depletion of WDR36 mRNA in HTM-N cells caused apoptotic cell death and up-regulation of mRNA for BAX, TP53, and CDKN1A. By immunocytochemistry, staining for WDR36 was observed in the nucleolus of cells, which co-localized with that of nucleolar proteins such as nucleophosmin and PWP2. In addition, recombinant and epitope-tagged Wdr36 localized to the nucleolus of HTM-N cells. By Northern blot analysis, a substantial decrease of 21S rRNA, the direct precursor of 18S rRNA, was observed following knock down of WDR36. In addition, metabolic labeling experiments consistently showed a delay of 18S rRNA maturation in WDR36 depleted cells.

#### Conclusion and outlook

Our results provide evidence that Wdr36 is an essential protein in mammalian cells which is involved in the nucleolar processing of SSU 18S rRNA. In ongoing studies, genetic rescue of the WDR36 knock out phenotype by an ubiquitous expression of a mutant WDR36 variant is currently investigated.

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#### P-Basic-162

### **Partial colocalization of retinoschisin (RS1) and the Na<sup>+</sup>/K<sup>+</sup>-ATPase subunit in membrane rafts and their role in signaling**

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#### Purpose

Previous studies have shown that Retinoschisin (RS1) binds to the Na<sup>+</sup>/K<sup>+</sup>-ATPase within the retina. In other cell types like cardiac myocytes, the Na<sup>+</sup>/K<sup>+</sup>-ATPase is located in detergent resistant membranes (DRMs), which represent rafts floating on the cell surface. These membrane domains can serve as platforms for receptor-ligand interactions and intracellular signaling. We hypothesized that RS1 binding to the  $\beta$ 2-subunit of the  $\alpha$ 3 $\beta$ 2 Na<sup>+</sup>/K<sup>+</sup>-ATPase occurs in retinal membrane rafts and thereby may regulate signaling functions.

#### Methods

To isolate DRMs from WERI-RB1 cells and mouse retinas, cells were pelleted and lysed after treatment with 2% Lubrol or Triton-X 100 in TNE buffer, respectively at 4°C. A discontinuous density gradient centrifugation was then performed with OptiPrep® solution. After 4 hours of centrifugation at 100.000x g, six fractions were collected from top to bottom. The proteins were precipitated using methanol/chloroform before resuspension in buffer containing 1% SDS. The samples were then analyzed by Western Blot using antibodies against the  $\beta$ 2 subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase, RS1 and flotillin as a DRM-marker. For signaling approaches retinal lysates or cryo-sections were stained with antibodies against phospho-ERK1/2 and p38 MAPK.

#### Results

We could successfully implement a raft isolation procedure from WERI cells and mouse retinas. DRMs were floating up in the low density fractions as demonstrated by strong staining with the marker flotillin. Staining for the  $\beta$ 2 subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase also showed high signals in raft fractions from WERI-RB1 cells. In retinal samples, staining for the  $\beta$ 2 subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase was strong in the raft fraction, but was also present in high density fractions. We could further show that RS1 was also partially present in DRMs of WERI cells and wild-type retinas. Our results also suggest that ERK1/2 and p38 MAPK are activated in Retinoschisin-deficient and ATP1b2 knock out mice at postnatal days 14 and 10, respectively.

#### Conclusions

Our experiments demonstrate that the  $\alpha$ 3 $\beta$ 2 subunits of Na<sup>+</sup>/K<sup>+</sup>-ATPase and RS1 partially colocalize in retinal rafts. This distribution of RS1 implicates that it has a dual function. On the one hand, RS1 may serve as a secreted adhesion molecule and on the other hand it may trigger intracellular signaling by interaction with the Na<sup>+</sup>/K<sup>+</sup>-ATPase in rafts as signaling platforms.

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#### P-Basic-163

### **Molecular characterization of two new mutations in the NKX2.5 gene and in the PAX8 promoter that cause congenital hypothyroidism in a girl with thyroid dysgenesis.**

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A girl was found to be hypothyroid on neonatal screening. An ultrasound of the neck suggested athyreosis. Her parents, a brother and grandparents had normal thyroid function tests and normally located thyroid glands. No mutation in genes involved in congenital hypothyroidism, including thyroid-stimulating hormone receptor (TSHR), thyroid transcription factor 1 (TTF1) and thyroid transcription factor 2 (TTF2) were detected. However, further investigation revealed a new heterozygous NK2 homeobox 5 (NKX2.5) mutation which was inherited from the father and a new heterozygous mutation in the paired box 8 (PAX8) promoter region that was transmitted from the mother. Both mutations were not found in hundred normal alleles.

In vitro studies were performed to unravel the underlying pathogenic mechanisms by which these two mutations might cause congenital hypothyroidism in our patient.

Immunofluorescence microscopy exhibited a correct nuclear localisation of the wild-type (WT) and the mutant NKX2.5 proteins. Electromobility shift assays (EMSA) demonstrated that the mutant NKX2.5 binds to the NKE\_2 and its target promoters deiodinase, iodothyronine, type II (DIO2), thyroid peroxidase (TPO) and thyroglobulin (TG) promoter elements equally well as the WT protein. However, in transient transfection studies the mutant NKX2.5 protein showed a 30–40% reduced transactivation of the thyroglobulin and the thyroid peroxidase promoters. Moreover, in presence and also in absence of TTF1, a dominant negative effect of the mutant NKX2.5 was observed.

EMSA studies of WT and mutant PAX8 promoter sequences incubated with nuclear extracts isolated from HeLa cells exhibited a severely reduced protein binding capacity of the mutated promoter. In addition, the mutant PAX8 promoter showed a significantly reduced transcriptional activation of a luciferase reporter gene in vitro. Thus, this promoter mutation is expected to lead to a reduced PAX8 expression.

In summary, we identified two new heterozygous mutations in both NKX2.5 and PAX8 in a girl with congenital hypothyroidism due to thyroid dysgenesis. These defects in two different transcription factors which both play essential roles in early thyroid development might be sufficient to explain the phenotype.

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## **P-Basic-164**

### **ES cell differentiation as model for genomic imprinting of Ube3a and Rb1**

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Murine embryonic stem (ES) cells are pluripotent cells derived from the 3.5dpc mouse blastocyst which can be modified by gene targeting and differentiated in vitro. We will make use of both features in studying the genomic imprinting process of the genes Ube3a and Rb1.

UBE3A is the causative gene for Angelman Syndrome, an imprinting disorder caused by genetic and epigenetic defects at the PWS/AS imprinted gene cluster on human chromosome 15q11q13 (conserved on mouse chromosome 7C). UBE3A shows maternal only expression in brain. It is hypothesized that expression of UBE3A on the paternal allele is silenced in cis by transcription of a long non-coding antisense RNA, UBE3A-ATS. To elucidate the function of this putative antisense RNA in imprinting of Ube3a, we differentiated ES cells into neurons and determined the expression of neuronal marker genes like Nestin,  $\beta$ -tubulin, Tau, GFAP and synaptophysin and of Ube3a by quantitative real-time PCR. We observe an upregulation of Ube3a during neuronal differentiation, although we expect one allele to be silenced due to expression of Ube3a-ATS. Expression of Ube3a-ATS was indeed induced during the neuronal differentiation of ES cells.

In addition, we will use the ES cell differentiation system to study the dependency of Rb1 imprinted expression on the pseudogene KIAA0649P. In humans but not in mice, KIAA0649P is inserted into intron 2 of the RB1 gene, driving expression of an alternative RB1 transcript (transcript 2B). As a CpG island in KIAA0649P is methylated on the maternal allele only, we hypothesize that expression of transcript 2B from the paternal allele causes skewing of allelic expression of RB1. To test this, we first introduced a single nucleotide polymorphism (SNP) into exon 3 of Rb1 to be able to distinguish between the expression levels of the two alleles. In a consecutive round of gene targeting, the human KIAA0649P will be introduced into the analogous position in intron 2 of the mouse Rb1 gene. Having generated this new ES cell line we will determine if KIAA0649P is sufficient to serve as promoter of transcript 2B and if its expression leads to skewed expression of Rb1.

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## **P-Basic-165**

### **Specific loss and gain of IGF2R methylation in different growth retardation subgroups**

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Imprinted genes are of fundamental importance in normal human growth and development. Meanwhile several imprinted loci have been reported to be specifically involved in the etiology of congenital growth disturbance disorders, e.g. Silver-Russell (SRS) and Beckwith-Wiedemann syndrome. However, in a growing number of imprinting disorders multilocus hypomethylation (MLH) has been identified, i.e. further imprinted loci are hypomethylated in addition to the disease specific ones. A relatively frequently affected locus in MLH is the imprinted IGF2R gene in 6q26. We therefore screened 52 SRS patients carrying a 11p15 hypomethylation, 32 idiopathic SRS cases and 24 probands with isolated primordial growth retardation for

aberrant IGF2R methylation. Whereas we could not detect any IGF2R loss of methylation (LOM) in the idiopathic group, we found LOM in 3/52 SRS patients with 11p15 hypomethylation. Two patients with isolated growth retardation carried a gain of methylation (GOM). This finding is consistent with a previous study describing LOM and GOM in a subset of 11p15 hypomethylation carriers and GOM in patients with isolated growth retardation. Functionally, hypermethylation might result in an increased IGF2R expression and thus indirectly promote growth retardation, but for the IGF2R hypomethylation the functional relevance is not obvious. It is therefore rather conceivable that the aberrant IGF2R methylation patterns is a unspecific indicator of a general problem to maintain the methylation status than that it is pathophysiologically relevant.

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#### **P-Basic-166**

### **Mechanism and control of iron transport through the blood testis barrier. The role of the iron regulatory protein 2 in testicular iron homeostasis.**

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The Blood Testis Barrier (BTB) within the seminiferous tubules of the testis consists of tight junctions created by adjacent Sertoli cells. The BTB regulates the nutrient transport to the developing germ cells and protects the male reproductive system from blood derived pathogens and nutritional imbalances. Sertoli cells also secrete hormones and nutrients essential for sperm development, which is regulated by the metabolic demands of the developing germ cells. We hypothesize that iron is an essential nutrient for the developing germ cells and that iron deficiency or excess could affect male fertility.

The iron metabolism is regulated, both at the systemic and the cellular level, by the peptide hormone hepcidin and the iron regulatory proteins (IRP) 1 and 2, respectively. IRP1 and IRP2 regulate proteins involved in iron metabolism such as the iron transport proteins transferrin receptor 1 (TfR), ferroportin, and the iron storage protein ferritin. Irf2 knock out mice exhibit higher ferritin expression levels and lower TfR expression in most tissues, develop anemia and a late onset progressive neurodegenerative disease.

This project focused on how testicular iron metabolism, spermatogenesis and spermiogenesis are affected by IRP2 deficiency. Especially an abundant expression of IRP2 in the mouse testis points to an important role of IRP2 in the regulation of iron homeostasis in this organ. We examined the testes and epididymides of 4 and 11 months old IRP2 deficient mice compared to age-matched C57BL/6J controls and observed that spermatogenesis is not affected by IRP2 deficiency. Irf2<sup>-/-</sup> males of 4 month and 11 months did not significantly differ in the rates of apoptotic and proliferative testicular cells in comparison to age-matched controls. Although there were no significant differences in epididymal sperm numbers in 4 months old Irf2<sup>-/-</sup> deficient mice and age-matched controls, sperm velocity (VAP, VSL, VCL) and lateral sperm head displacement (ALH) were significantly decreased in Irf2<sup>-/-</sup> mice compared to control males. Our findings point to a role of IRP2 in sperm motility. Whether this effect is mediated by structural abnormalities of the sperm flagellum or due to a functional blockade in a physiological process of sperm movement remains to be elucidated. We are currently performing low iron diet experiments with Irf2<sup>-/-</sup> deficient mice and wildtype controls in order to examine if this finding is increased by systemic iron deficiency and if iron deficiency could generally affect spermatogenesis and male fertility.

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#### **P-Basic-167**

### **Comparative analyses of the Fanconi anemia core complex genes reveal conserved transcription regulatory elements**

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The Fanconi anemia (FA) gene family is a recent addition to the complex network of proteins that respond to and repair certain types of DNA damage in the human genome. Since little is known about the regulation of this novel group of genes at the DNA level, we characterized the promoters of eight genes (FANCA, B, C, E, F, G, L and M) that assemble into the FA core complex. The promoters of these genes show characteristic attributes of housekeeping genes: high GC content, rich in CpG islands, and lack of TATA boxes. They function in a monodirectional way and are, in their most active portions, comparable in strength to the SV40 promoter in the reporter plasmids. They are marked by a distinctive transcriptional start site (TSS). In the 5' region of each promoter, we identified a region that, in isolation, is able to negatively regulate promoter activity in HeLa and HEK 293 cells. The central and 3' regions of the promoter sequences harbor binding sites for several common and rare transcription factors including STAT, SMAD, E2F, AP1 and YY1, indicating that there are cross-connections to several established regulatory pathways. Electrophoretic

mobility shift assays and siRNA experiments confirmed shared regulatory responses between prominent members of the TGF-beta; and JAK/STAT pathway and members of the FA core complex. These findings allow us to demonstrate a regulatory connection between the FA/BRCA pathway and a set of unexpected DNA-binding factors. Hence, we speculate that mutations in one of these upstream acting pathways may be pathogenic and lead to FA, explaining a couple of unclassified patients.

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## P-Basic-168

### Retinal degeneration and microglial activation in mouse models of neuronal ceroid lipofuscinoses

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Introduction: Neuronal ceroid lipofuscinoses (NCL) are early onset lysosomal storage disorders characterized by vision loss, mental and motor deficits, and spontaneous seizures. Patients remain in a vegetative state for several years before premature death occurs. Neuropathological analyses of human autopsy material and brain from NCL animal models revealed neuroimmune processes closely associated with neuronal degeneration. It is currently unclear whether this phenomenon is confined to the brain or also occurs in the retina. Therefore, the aim of our study was to characterize the relation between retinal degeneration and microglial activation in different mouse models of NCL.

Methods: Retinal degeneration of the NCL mutant mouse strains Cln3(ex7/8)KI and Cln6nclf was characterized by detailed structural analyses at different ages. Microglial morphology and migration was analyzed by immunohistochemistry. Visual acuity was determined by measuring the optokinetic response in an Optomotry system.

Results: Our data show that there is a migration of microglia from the plexiform layers to the nuclear layers in CLN6(nclf) and Cln3(ex7/8)KI retinas, which is consistent with an alerted state of microglia. Moreover, the cell shape of these cells changed from a ramified form to an amoeboid form. Histological analyses revealed that this microglial activity was accompanied by a prominent retinal degeneration. Optomotry tests showed that the CLN6(nclf) and Cln3(ex7/8)KI mice had a progressive decline in visual acuity as they aged.

Conclusion: Our results identified a coincidence of microglial activation, retinal degeneration, and vision loss in CLN6(nclf) and Cln3(ex7/8)KI mice. We therefore hypothesize that therapies aimed at modulating retinal microglia activation could be helpful to preserve vision in NCL patients.

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## P-Basic-169

### SPOC1 – a novel player in the epigenetic control of development and differentiation?

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SPOC1 (Survival time associated protein in ovarian cancer 1; PHF13) was originally identified as a gene whose expression is negatively correlated with the survival time in a cohort of patients with ovarian cancer. The gene encodes a protein containing a plant homeodomain (PHD) which is involved in protein-chromatin interaction. We showed that SPOC1 associates dynamically with chromatin in cell lines and plays a role in chromosome condensation and cell division. Recently, we demonstrated that male Spoc1<sup>-/-</sup> mice with a mixed (129/P2 x C57Bl/6) genetic background show a pronounced hypoplasia of the testis with a progressive loss of germ cells leading to a "Sertoli cell only" phenotype in older mice. The present data strongly indicate that Spoc1 is essential for the differentiation of spermatogonial stem cells. Despite the testis phenotype Spoc1<sup>-/-</sup> mice appear normal. However, intercrossings of heterozygous animals give rise to Spoc1<sup>-/-</sup> animals at a ratio of ~14 %, which clearly deviates from the expected Mendelian ratio of ~25 %. This indicates that SPOC1 has an essential function in mammalian embryonic development although there is considerable variability possibly caused by the mixed genetic background or underlying epigenetic differences.

In order to further investigate the loss of functional SPOC1 in early mouse development we generated a congenic (C57Bl/6) Spoc1<sup>-/-</sup> mouse strain. In this congenic background there was not only a more severe testis phenotype but also a further reduction of viable Spoc1<sup>-/-</sup> offspring to 9 %. This was due to pre- (1.) and perinatal (2.) lethality. 1. Dissection of dead newborns revealed absence or hypoplasia of the spleen. Nevertheless the viable and fertile animals showed a normal sized spleen with a normal morphology and histology. Remarkably, hemograms of these adult Spoc1<sup>-/-</sup> mice revealed an increase in the number of thrombocytes which is distinctive for spleen dysfunction. 2. Investigation of a prenatal developmental stage

(day 15.5) showed several *Spoc1*<sup>-/-</sup> animals with severe malformations, comprising growth retardation and defects in neuronal, craniofacial and eye development.

The fact that the observed developmental defects were present in the congenic genetic background (N12) strongly indicates that the variability of the phenotype is not due to the genetic background but possibly caused by epigenetic effects. We present preliminary data demonstrating that SPOC1 specifically interacts with trimethylated lysine 4 of histone 3 (H3K4me3), an epigenetic mark associated with active chromatin and gene expression. This, the PHD finger and the chromatin-binding capacity during chromosome condensation and mitosis strongly indicate that SPOC1 might act as an epigenetic reader. We propose that SPOC1 is a factor playing a role in epigenetic control of various differentiation processes during organ and germ cell development.

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## P-Basic-170

### The impact of eNOS inhibition on aortic endothelial cell apoptosis

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**Introduction:** The nitric oxide (NO)-producing enzyme endothelial nitric oxide synthase (eNOS) is known to be dysregulated in patients suffering from bicuspid aortic valve (BAV), the most common congenital cardiac defect. BAV patients exhibit a predisposition to aneurysm of the ascending aorta. Furthermore formation of aneurysm in BAV patients seems to be linked to apoptosis of medial vascular smooth muscle cells. We therefore hypothesized that a dysregulation of eNOS in BAV patients would lead to an increase in vascular cell apoptosis as a contribution to the dilation of the aorta.

**Methods and Results:** Human aortic endothelial cells (HAECs) were cultivated and grown to 80% confluence. Cells were then kept in serum-free medium for 24h and exposed to 1mM eNOS inhibitor L-NAME for 1h. Proteins were extracted from the cells using cell lysis buffer and concentration of nitrite/nitrate, the breakdown products of NO, was determined using a commercial nitrite/nitrate detection kit. The nitrite/nitrate amount was approximately 20% lower in HAECs treated with L-NAME as in untreated cells. Afterwards whole protein concentration was determined in the cell lysates and 200 µg of protein were used in a human apoptosis proteome profiler kit to compare the levels of 35 apoptosis-related proteins between treated HAECs and controls. Analysis of the protein expression profile revealed several apoptosis-related proteins to be dysregulated.

**Conclusion:** A dysregulation of eNOS effects apoptosis in multiple ways. Lowering of NO level seems to have a negative effect on the inhibition of caspases. This might contribute to the apoptosis of vascular smooth muscle cells in patients with BAV and therefore lead to the development of aortic aneurysm.

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## P-Basic-171

### Promoter methylation of learning/memory-related genes in anterior cingulate cortex of mice trained in the Morris Water Maze

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As neuronal plasticity is generally associated with de novo gene expression, we expected that learning of a specific task induces specific epigenetic changes in gene regulation. Cortical DNA methylation appears to be critical for remote (long-lasting) memory. To support this hypothesis, we trained mice for four days in the Morris Water Maze (MWM) test, followed by a probe trial at day 5. Mice were classified according to their performance in the MWM as good versus poor learners. Quantitative bisulfite pyrosequencing was used to study methylation of learning/memory-related genes in the anterior cingulate cortex (ACC) at several time points. The ACC is part of the medial prefrontal cortex as well as the prelimbic and infralimbic cortices. *Bdnf* (brain-derived neurotrophic factor), *Creb1* (cyclic AMP responsive element) and *Reln* (reelin) have been implicated in memory enhancement, whereas *CaN* (protein phosphatase 3, catalytic subunit) and *Ppp1cc* (serine/threonine-protein phosphatase PP1-gamma catalytic subunit) are involved in memory suppression.

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**P-Basic-172****The Pathophysiological Role of Bestrophin-1 (Best1) in Best Vitelliforme Macular Dystrophy: Phenotypic Characterization of Knock-in and Knock-out Mouse Lines**

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**Background:**

Bestrophin-1 encodes an integral membrane protein of the retinal pigment epithelium (RPE). Mutations in this gene are associated with Best vitelliforme macular dystrophy (BVMD), an autosomal dominant form of macular degeneration with highly variable expressivity and reduced penetrance. Key features of BVMD are a striking accumulation of lipofuscin-like deposits in the RPE, degeneration of photoreceptors, impairment of visual function and a reduced light-peak in the electrooculogram. Currently, the pathomechanism of the disease is not well understood. To gain insight into the function of BEST1 and its role in the pathophysiology of BVMD, we have generated two Best1-manipulated mouse models. Here, we present a detailed phenotypic characterization of the knock-in mouse carrying the BVMD-causing mutation Y227N (Best1N/N) and the knock-out mouse deficient in murine Best1 (Best1<sup>-/-</sup>).

**Methods:**

Murine Best1 exon 6 was targeted to introduce the Y227N mutation by site directed mutagenesis and to achieve Best1 deficiency by disruption. Histological examinations of retinal sections were done by light and electron microscopy. Retinal electrophysiology was determined by electroretinography (ERG) and visual acuity by using a virtual optomotor system (OptoMotry, CerebralMechanics). Retinal metabolism was assessed by measuring docosahexaenoic acid levels by GC-mass-spectrometry, RPE lipofuscin by HPLC analysis and RPE-phagocytosis by both immunofluorescence labeling of RPE flat mounts and quantitative RT-PCR for expression of genes involved in RPE-phagocytosis. Ca<sup>2+</sup> homeostasis of cultured RPE cells was analyzed using FURA-2 AM as fluorescent Ca<sup>2+</sup> detector.

**Results:**

Transcription level of Best1 mRNA was normal in Best1N/N mice but with strongly reduced protein synthesis. As expected, Best1<sup>-/-</sup> mice showed complete Best1 protein deficiency. In both mouse models, histology revealed normal retinal structures when compared to wildtype littermates. ERG responses of Best1N/N mice showed regular amplitude and ERG waveform and both mouse lines showed normal visual acuity in optometry. Retinal metabolism was similar to wildtype animals and intracellular Ca<sup>2+</sup> imaging showed no obvious differences in basal intracellular Ca<sup>2+</sup> concentrations or in Ca<sup>2+</sup> transients.

**Conclusion:**

The molecular and histological phenotypes of both the knock-in and the knock-out Best1 mouse models revealed no obvious signs of impairment. Importantly, the Best1N/N line revealed a largely reduced expression of the mutant protein suggesting partial proteolysis likely via a process known as endoplasmic reticulum-associated degradation. Together, our findings demonstrate that absence of Best1 is tolerated in the mouse and a minor expression of mutant protein fails to result in a dominant-negative effect. Whether this reflects the situation in human BVMD patients remains to be elucidated.

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**P-Basic-173****Search for mutations and pathogenic haplotypes in hemizygous genomic regions of patients with intellectual disability (ID)**

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**Background:** Molecular Karyotyping in a large number of intellectually disabled children from healthy parents has revealed family-specific deletions which do not represent known CNVs. Such deletions could either be harmless familial variants or recessive mutations. If the latter is true, the affected child should carry an additional mutation on the homologous chromosome. Since autosomal recessive inheritance is supposed to account for nearly a quarter of all individuals with non-syndromic ID, the likelihood of finding a second mutation in children with an inherited deletion is substantial.

Experimental approach: Sanger-sequencing of coding exons of selected candidate genes. Criteria for gene selection: complete or partial deletion of one allele plus expression in the mammalian brain. Selected genes: SYT11, NRXN1, ARPP-21, HIST1H2AG, HIST1H2AH, HIST1H2BJ, HIST1H2BK, HIST1H4I, ANLN, EXOC4, LINGO2, VCL, ALDOA, DOC2A, ASPHD1, KCTD13, RAB37, and SULT4A1.

Results: Besides several known SNPs in various candidate genes we have identified three unclassified sequence variants in LINGO2 (p.Asp135Asp, p.Arg369Gln, p.Asn522Tyr) in three unrelated children with ID. Since all of these children have a partial LINGO2-deletion as well, the observed sequence variants may represent recessive mutations.

Future work: LINGO2 belongs to a family of neuronal growth modulating proteins. Furthermore, down-regulation of the closely related LINGO1 in cell culture and knockout mice has been shown to be critical for CNS myelination. Thus, LINGO2 may also be critical for mammalian brain development. As a first step to elucidate a clinical relevance of our LINGO2 variations, we are going to quantify LINGO2 expression on cDNA level in our ID-patients with a LINGO2 deletion, as well as in the corresponding healthy family members. Furthermore, we will determine the allele frequency of the LINGO2 variants mentioned above by genotyping 500 healthy individuals at these loci.

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## P-Basic-174

### WT1 is a stem cell factor in early haematopoiesis

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The Wilms tumour protein 1 (WT1) has many surprising faces. Discovered early in 1990, it has since then been shown to be both tumour suppressor and oncogene. In normal development, the WT1 transcription factor acts as a master switch in the formation of mesodermal tissues and organs, like the kidney, the gonads, and the spleen. Recently, growing evidence implicated WT1 in normal haematopoiesis, and vasculogenesis of heart, lung, and gut. Furthermore, WT1 was shown to drive tumour angiogenesis, acute myeloid leukaemias, and may have a role in the generation of cancer stem cells. We therefore concentrated on the developmental role of WT1 in early haematopoiesis and stem cell formation, focussing on a potential function in the haemogenic endothelium of the dorsal aorta, the aorta-gonad-mesonephros region (AGM).

To this end, we generated two different conditional mouse models, both based on an inducible Wt1 tTA knockin effector line carrying a tetracycline-dependant activator (tTA) in place of the first exon of the endogenous murine Wt1 locus. In the absence of tetracycline, the tTA effector is active in all cells expressing Wt1, and thereby faithfully recapitulates the expression domain of endogenous Wt1. In double transgenic animals bearing a tetracycline-responsive responder construct for both EGFP, and lacZ, our first model system, Wt1-positive cells can be traced by monitoring EGFP fluorescence and histochemical staining for beta-galactosidase. In the second mouse model, triple transgenic animals bearing a tetracycline-responsive transgene for Cre recombinase, and the Cre reporter line ROSA26 lacZ (and EYFP, respectively), the fate of Wt1-positive cells was followed in vivo by tetracycline administration at different embryonal time points, again using beta-galactosidase and EYFP fluorescence to identify cells and tissues derived from Wt1-positive precursors at later developmental stages.

Analysis of embryos from day 9.5 to 12.5 post coitum (d.p.c.) by histochemical staining demonstrated earliest Wt1 expression at day 10.5 d.p.c in the AGM. When the fate of these Wt1-positive cells was analysed in later embryonal, and adult blood cells, all haematopoietic lineages were marked, indicating that Wt1 is active in multipotent haematopoietic stem cells. To further characterize the Wt1-positive progenitor cells in the dorsal aorta, we performed extensive co-immuno fluorescence studies with established markers for endothelial and stem cell populations. Our results suggest a central role of WT1 in the formation of the haemogenic endothelium and in early haematopoietic differentiation. Furthermore, tetracycline-regulated knockout and overexpression studies with our conditional model systems provide an experimental tool to unravel the mystery of haematopoietic stem cell development.

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**P-Basic-175****Mouse CCDC33 is a germ cell-specific peroxisomal protein, which interacts with peroxisomal testis specific protein 1 (PXT1).**

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Peroxisomes are indispensable for normal function of eukaryotic cells. We have characterised a novel male germ cell specific gene, which encodes for a peroxisomal protein (Pxt1). Using yeast-two-hybrid system, we identified mouse CCDC33 as an interaction partner of PXT1. The CCDC33 protein was previously reported as cancer/testis (CT) antigen. Recently, we ascertained that mouse Ccdc33 is predominantly expressed in the testis and its expression was found to be restricted to the germ cells. We could show that the putative protein encoded by Ccdc33 gene contains two peroxisomal targeting signal type 2 (PTS2) motifs. By introducing mutations into these motifs, we could demonstrate that only the second PTS2 is responsible for the peroxisomal targeting of the dsRED-CCDC33 fusion protein in the transiently transfected cells. The expression of Ccdc33 and Pxt1 genes starts at the primary spermatocyte stage of spermatogenesis, which suggests that the interaction between these two proteins might play an important role in male germ cell development. We confirmed the CCDC33-PXT1 interaction in mammalian cells by bi-fluorescence complementation assay and by colocalization of dsRED-CCDC33 and EGFP-PXT1 fusion proteins in the HeLa cells. In the cytoplasm, both proteins showed a punctate pattern of co-localization, which is a typical hallmark of peroxisomes. Deletion or introduction of mutations in the respective interacting domains of PXT1 and CCDC33 abolished the interaction. Also the mutant EGFP-PXT1 and mutant dsRED-CCDC33 showed aberrant intracellular distribution.

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**P-Basic-176****The NF1 gene contains a hotspot region for L1 endonuclease-dependent de novo insertion**

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Long interspersed (L1) and Alu elements are still actively amplified in the human genome through retrotransposition of their RNA intermediates by the ~100 still retrotranspositionally competent L1 elements. L1 endonuclease (EN) cleaves the target DNA at the insertion site and L1 reverse transcriptase integrates the RNA intermediate into the genomic DNA. Retrotransposition can cause an inherited disease if an L1 or Alu element is de novo inserted near or within a functional gene. Using direct cDNA sequencing as the primary assay for comprehensive NF1 mutation analysis we uncovered in 16 index patients splicing alterations, mainly exon skipping events, which were explainable neither by an underlying point-mutation nor by a deletion of genomic sequences. Improved PCR protocols that avoided allelic drop-out of the mutated alleles uncovered de novo insertions of twelve Alu elements, three L1 elements, and one poly-(T) stretch most likely representing a severely truncated Alu or L1 element, as causes of these splicing defects. All 16 inserted sequences showed the characteristics of L1 EN-mediated retrotransposition: the integration sites match the reported L1 EN consensus cleavage site; the elements were flanked by 6- to 17-bp target site duplications and contained poly-(A) tails derived from their transcripts. Although integration sites were distributed over the entire NF1 gene, six different insertions clustered in a relatively small 1.5-kb region (NF1 exons 21-23) within the 280-kb NF1 gene. Furthermore, this cluster region contained a specific integration site that was used twice. In two unrelated patients an AluYa5 element was inserted at exactly the same position in exon 21. A second site in NF1 exon 33 was also used twice to insert an AluYa5 and an AluYb8 element, respectively. Taken together, the 16 pathogenic L1 EN-mediated de novo insertions represent the largest number of this type of mutations characterized in a single human gene and substantially add to the so far published ~60 mutations of this type. Our findings show that retrotransposon insertions account for 0.4% of all NF1 mutations which is two to four times higher than anticipated for any genetic disorder. Since altered splicing was the main effect of the inserted elements, the high frequency of this type of mutation in the NF1 gene may be at least partly explained by the use of improved detection by the RNA-based mutation analysis protocols applied in our laboratories. Clustering of 6 of the 16 identified insertions within a small 1.5-kb region as well as the identification of two loci, one located within the cluster region, that each served twice as integration site for independent retrotransposition events, supports the notion of non-random de novo insertion of retrotransposons in the human genome. Currently, little is known which features make sites particularly vulnerable to L1 EN-mediated insertions. The here identified integration sites may serve to elucidate these features in future studies.

## P-CYTOGENETICS / CNVS

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### P-CytoG-177

#### Array CGH: BAC chips and oligo chips, an overview of more than 200 cases

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In recent years, array comparative genomic hybridisation (array CGH) has evolved to a common technique for the diagnosis of imbalances not detectable via conventional cytogenetics such as chromosome-banding and karyotyping. It is the tool of choice for identification of submicroscopic imbalances as a main genetic cause for mental retardation.

During the last three years, we have performed array CGH in more than 200 cases in our laboratory.

Starting with BAC chips (Cytochip V2 and V3, BlueGnome, UK), we examined 96 patients. In addition eight parents were analysed to clarify the previous results of their children. 53 of these cases gave possible pathogenic results that had to be verified by additional techniques: Fluorescence in situ hybridisation (FISH) (probes by Abbott/Vysis and BlueFish by BlueGnome) and/or oligo array CGH (CytoChip Oligo, BlueGnome).

10 cases of these 53 were confirmed as real pathogenic. In four cases the BAC array CGH result was confirmed but the clinical significance of the detected imbalances still remained questionable as parental analysis was not possible. In 39 cases (41% of all samples) the detected imbalances were not confirmed (false-positive results) and therefore the results were reported as normal.

15 months ago, we switched to oligo array CGH (4x44K CytoChip Oligo, BlueGnome). 95 patients and 19 parent examinations were performed. Normal results were obtained in 71 patients. 24 cases gave certain pathogenic (9) or possible pathogenic (15) results with the necessity of further confirmation. Large but submicroscopic microdeletions or duplications were found in seven of these nine pathogenic cases that could have been identified via BAC CGH as well. However, the identification of two positive cases detected in Oligo array CGH would have failed using BAC array CGH analysis or FISH due to the small size of imbalance.

Oligo array CGH compared to BAC array CGH did not increase the hit rate in our laboratory (equal for both platforms with 10% each) but gave more reliable results due to the higher resolution. It minimizes the number of false-positive results that need to be further analysed (6% instead of 41%) while reducing substantially the duration of analysis, workload and costs.

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### P-CytoG-178

#### Small losses and gains in molecular karyotyping using SNP Array: The Upper Austrian experience

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##### Introduction

High-resolution molecular karyotyping in patients with developmental delay or congenital anomalies in addition to conventional karyotyping is an integral part of diagnosis and is discussed even as first-line test. However, small losses and gains in CNV regions or outside variant regions represent an unsolved problem in the diagnostic setting and results have to be interpreted carefully using diverse data bases. A better understanding of small "aberrations" is urgently needed.

##### Materials and Methods

We analyzed 132 patients (77 females / 55 males) with diverse clinical presentations by SNP 6.0 array (Affymetrix). All patients also underwent conventional karyotyping which showed no microscopic visible aberrations. Small (Submicroscopic) gains and losses >300 kbp using ChAS Software (Affymetrix) were selected and evaluated regarding chromosomal region, CNVs, gene count, marker count, copy number state and frequency.

##### Results

Small losses and gains were found on most chromosomes (autosomes), the median was 8,5 gains and 1,2 losses per analyzed genome. Interestingly, chromosome 11, 12, 18, 19, 20 and 21 showed no losses, chromosome 6 and chromosome 13 neither losses nor gains. There was no significant correlation between any change of copy number and gene density in chromosomes. Copy number state for losses was 1 (100%) and for gains 3 (79%) and 4 (21%). The maximal size of gains and losses of unclear phenotype correlation

was up to 5000 kbp. The size of the most frequently losses varied: <500 kbp (59,5%), 501-1000 kbp (25,5%) and from 1001-2000 kbp (15%), for gains <500 kbp (47%), 501-1000 kbp (39,1%), 1001-2000 kbp (12,1%), 2001-5000 kbp (1,8%). 3 of 155 losses and 7 of 1145 gains were unique findings, the remaining presented from at least twice up to 143 in our population with variations of starting- and endpoints.

94,6 % of these changes were covered by CNV regions, but we also detected 23 copy number changes in regions outside annotated CNVs. Of these 3,9% were losses and 1,5% were gains. Gene counts ranged from 1 to 15.

#### Conclusion

In our population, copy number variations between 300 kbp and 2 (losses) respectively 5 (gains) Mbp were detected in all samples. After matching our results with known CNVs, 5,4% of them were located in non-polymorphic regions. These included up to 15 genes. A clear genotype-phenotype correlation was difficult to prove because detailed data for gene function are often lacking and even triplet testing not always provides clear clinical information. Collecting and analyzing data of copy number variations in different populations is therefore important to reveal clinical relevant findings in the future.

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## P-CytoG-179

### Microduplications 22q11.21 frequently associated with classic bladder exstrophy

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Classic exstrophy of the bladder (CBE) is part of the rare exstrophy-epispadias complex (BEEC), a spectrum of urogenital anomalies in which part or all of the distal urinary tract fails to close. Previously, four CBE cases with underlying microduplication 22q11.2 in a cohort of 102 BEEC patients have been reported (Lundin et al., 2010; Draaken et al., 2010).

Screening of additional 250 patients with CBE (223) as well as epispadias (9) and exstrophy of the cloaca (18) using multiplex ligation-dependent probe amplification (MLPA) analysis. This identified additional five CBE patients with a microduplication 22q11.2. Paternity was confirmed in all families. Among the nine cases the duplication had been inherited three times from an unaffected parent (two mothers, one father). All duplications differ in size ranging from about 0.07 Mb to 2.71 Mb. MLPA results were confirmed using Illumina's Beadchips thereby specifying the maximum region from SNPs rs2543958 to rs12484186. According to hg18 this region on chromosome 22 (nt:17,257,787-19,965,324) comprises 2.71 Mb, harboring 47 genes as well as the sequence information of at least four microRNAs.

Given these observations, we were able to confirm the association of causally underlying microduplications 22q11.2 in cases of isolated CBE and hypothesize that a single phenocritical gene might reside in the smallest region of overlap.

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## P-CytoG-180

### Non-random formation of acrocentric dicentric chromosomes in a human cell culture model that enriches de novo dicentric chromosomes

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Genome rearrangement often produces chromosomes with two centromeres (dicentrics) that are inherently unstable because of bridge formation and breakage during cell division. However, mammalian dicentrics, and particularly those in humans, can be quite stable, because one centromere is functionally silenced. In order to investigate the timeline of dicentric chromosomal rearrangements we performed multicolor karyotyping using 24-color fluorescence in situ hybridization on metaphase chromosomes obtained at three different time points (36 hours, 3 days, 5 days) from a cell culture model that enriches dicentric chromosomes due to transient disruption of telomere structure.

A cell culture system was used in which the expression of a tetracycline/ doxycycline-responsive dominant-negative truncation mutant of the telomere protein TRF2 (TRF2DBDM, hereafter called dnTRF2) interferes with the accumulation of endogenous TRF2 at chromosome ends. Metaphase chromosomes were

isolated from multiple independent inductions of dnTRF2 expression in two cell lines derived from parental fibrosarcoma cells known to be hypertetraploid. The same cell lines without dnTRF2 induction were used as controls.

M-FISH results showed in 30% of the uninduced control cells two or more fusions. In the TRF2 induced cells more than 60% contained two or more fusions after 36 hours of induction. Roughly 80% of these fusions involved two acrocentric chromosomes and predominantly the p-arms. Longer induction time of three and five days produced even more chromosome fusions with an increasing number also of non-acrocentric chromosome. Although complex rearrangements involving 3-7 chromosomes, ring chromosomes, chromatid fusions as well as chromosome deletions were also observed, the number of acrocentric-acrocentric fusions did not decrease and consistently represented the largest proportion of all chromosome interactions.

Dicentric chromosomes, while unstable in many organisms, are quite stable in human cells, although a selection bias for the most stable dicentrics may contribute to this perception. Thus, our investigation revealed parallels between the identity of prevalent naturally-occurring human translocations and experimentally-produced rearrangements. The prevalence of induced Robertsonian translocations suggests an inherent property of acrocentric chromosomes that predisposes them to rearrangement and fusion.

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## P-CytoG-181

### The first genome-wide survey of copy number variants (CNVs) in androgenetic alopecia suggests an involvement of specific CNV-regions in its etiology

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Male-pattern baldness (androgenetic alopecia, AGA) is the most common form of hair loss in humans. The hair loss proceeds progressively and follows a characteristic pattern. The genetic predisposition and the individual's hormonal situation are considered as established etiological factors contributing to the development of AGA. Studies aiming at the identification of the causative genetic factors have identified single nucleotide polymorphisms (SNPs) at the X-chromosomal androgen receptor (AR)/ectodysplasin A2 receptor (EDA2R) locus and a further locus on chromosome (chr) 20p11 to be strongly associated with AGA. Although the causative genetic variants at these two loci have not yet been characterised, it is assumed that a significant fraction of the overall genetic risk is explained by these two loci. However another fraction of the genetic risk still remains to be identified and further systematic genetic studies aiming at the identification of additional risk loci are warranted. In addition to SNPs, structural genomic variations, such as copy number variants (CNVs), have been recognized as another important source of genetic variability contributing to the development of common disease. We therefore systematically investigated for a potential contribution of CNVs to the development of AGA. So far, this is the first study to analyse for CNVs in AGA on a genome-wide level. To screen for CNVs we used SNP-array data of a well characterised German case-control sample comprising 401 unrelated AGA-affected individuals and 527 controls. After applying stringent filter criteria, the detected CNVs were analysed for a potential association with AGA both, on the genome-wide and the individual level. Although the genome-wide burden analyses did not show any significant association, the analyses for association of specific CNV-regions suggest a role of six autosomal CNV-regions in the development of AGA. We found evidence for one rare (chr6p26) and three common microduplications (chr11q25, chr12q13 & chr22q11) as well as one common microdeletion on chr4q28 that showed nominal-significant association. A second common microdeletion on chr14q11 showed genome-wide significant association. All six CNV-regions could be confirmed via real-time quantitative PCR. As CNVs are a well known source of genetic variability they might affect gene expression by altering the copy number of genes or their regulatory elements. Further analyses, such as expression studies for the genes that lay within or in close vicinity to these newly identified regions might help to clarify the role of the detected CNVs in the development of AGA.

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## P-CytoG-182

### Microdeletion 4q34.1 encompassing HAND2 in a patient with CHD and hand malformations

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**Introduction:** Congenital heart defects (CHD) represent the most common congenital malformation in humans. Multiple genes involved in the occurrence of CHD have been identified. For other genes the contribution to heart malformation has been postulated but could not be confirmed so far. Here we present a patient with a microdeletion encompassing the HAND2 gene who was affected by heart and hand malformations.

**Case report:** We report on a boy who was diagnosed with a complex CHD, consisting of valvular pulmonary stenosis, persistent ductus arteriosus, dysplastic tricuspidal valvula and arterial septum defect. Furthermore he was affected by postaxial hexadactyly on the right hand resembling a mirror hand, clinodactyly and stiff digit V on the left hand and single palmar crease on both hands. Aside from naevus flammeus of forehead and both eyelids no other malformations or dysmorphisms were found. The psychomotor development was within the normal range at the age of 20 months.

**Results:** Chromosomal analysis revealed a normal male karyotype (46,XY). Analysis of SNP oligonucleotide array discovered a de novo 570b deletion in chromosomal region 4q34.1 (chr4:174,425,438-174,998,288, hg18; cnvi0012414-rs4695888) indicated by decrease of intensity values across 93 SNPs. This copy number variation encompassed seven RefSeq genes: GALNT7, HMGB2, SAP30, SCR1, HAND2, NBLA00301, and MORF4. The deletion could be confirmed by fluorescence in situ hybridization (FISH). All further detected CNVs have been described as known polymorphisms in the Database of Genomic Variance.

**Discussion:** To our current knowledge HAND2 is the most likely gene contributing to the complex phenotype CHD and hand malformation in the boy reported here. HAND2 encodes for a basic helix-loop-helix transcription factor which is asymmetrically expressed in the developing ventricular chambers. Different animal studies have shown that HAND2 is playing an essential role in the formation of the right ventricle and aortic arch as well as in limb development, especially in the establishing of the anterior-posterior polarization. Surprisingly, HAND2 mutations could not be identified in children with CHD to date. Nevertheless the involvement of this gene in heart and limb development and the results from animal models indicates that HAND2 variations contribute to heart and hand malformation in humans as well.

**Conclusion:** Mikrodeletion 4q34.1 encompassing the HAND2 gene in a boy with CHD and hand malformation suggest that haploinsufficiency of HAND2 is contributing to this phenotype.

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## P-CytoG-183

### Familial inheritance of NF1 microduplication and 14q23.3 microdeletion: correlation of genotype and phenotype

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We report on a 10-year-old boy with mental retardation, developmental delay, muscular hypotonia, adipositas and minor dysmorphic features. He is the only child of a 23-year-old mother and a 25-year-old father. Both parents are healthy and non-consanguineous. Standard cytogenetic analysis (GTG banding) revealed a normal karyotype, 46,XY. Molecular investigations for several potential diagnoses i.e. fragile-X syndrome, Prader-Willi syndrome, gave normal results. Using array-based comparative genomic hybridization (aCGH) with the 180K oligonucleotide microarray (Agilent, Santa Clara, CA, USA) revealed a duplication of 1.35Mb (position 26,035,863-27,386,667 on hg18 assembly) on 17q11.2. The site of duplication corresponds to the NF1 microdeletion region. The duplication was confirmed by FISH (MD NF1(17q11)/MPO (17q22), Kreatech). Investigations of both parents and further family members showed that the microduplication 17q11.2 segregated on the paternal side of the family through three generations. However, all other carriers of the 17q11.2 duplication in the family were normal without any specific phenotype. Our patient furthermore showed a microdeletion on chromosome 14q23.3, which comprises the GPHN gene. The results were confirmed by qPCR. Gephyrin, which is encoded by the GPNH gene, plays a role in neuronal signal transduction by glycine and GABA receptors. The resulting metabolic deficiency might explain the distinctive clinical features in our patient. In conclusion we suggest that the microduplication on 17q11.2 is unlikely to be linked to specific phenotypic anomalies. This is in agreement with earlier discussion of similar cases, which were inconclusive regarding a definite phenotype for the NF1

microduplication. The clinical findings in the only affected boy in this family are most probably linked to the microdeletion 14q23.3.

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## **P-CytoG-184**

### **The detection of PAX8/PPARgamma fusion transcripts in follicular-patterned thyroid tumours**

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A subgroup of follicular neoplasias of the thyroid gland harbours the chromosomal translocation t(2;3)(q13;p25), which leads to a fusion of the genes encoding for the thyroid-specific paired box 8 transcription factor (PAX8) and the peroxisome proliferator-activated receptor gamma (PPARgamma). While the chromosomal rearrangement was initially reported in a few cases of thyroid adenomas, PAX8/PPARgamma fusion transcripts were first detected in follicular carcinomas and considered as a marker for malignancy until their presence was reported in follicular adenomas as well. Although the chromosomal translocation has been described in only five cases of follicular adenomas, the prevalence of PAX8/PPARgamma fusion transcripts in adenomas varies between 0% and 54.5% according to different studies with a mean of 8.2%. Herein, a series of 192 thyroid adenomas was karyotyped, and the chromosomal translocation t(2;3)(q13;p25) was detected in only two cases (1%). This result has also been confirmed by RT-PCR based detection of the PAX8/PPARgamma fusion transcripts. Thus, the prevalence of the gene fusion in adenomas is much lower in the present series than reported in several previous studies.

In addition, formalin-fixed, paraffin-embedded (FFPE) samples of follicular carcinomas (n=21) and follicular variants of papillary carcinomas (n=7) were analysed for the presence of the fusion transcripts. These cases have not been karyotyped or analysed by fluorescence in situ hybridization previously and therefore it was unknown whether or not the tumours carry the chromosomal translocation. When RT-PCR is performed on formalin-fixed samples, the degradation of the RNA templates has to be considered. Recently, a method for the detection of PAX8/PPARgamma fusion transcripts by RT-PCR in FFPE samples was reported. However, since primers labeled with fluorescent dyes were used, a fluorescence detection system is required to analyse the PCR products. We performed a conventional RT-PCR and separated the products on agarose gels. As a positive control, a short (125 bp) fragment of exons 5 and 6 of the PAX8 mRNA was amplified, and a distinct band was visible after gel electrophoresis in all samples under investigation (n=28) indicating a success rate of 100%. PAX8/PPARgamma fusion transcripts were detected in approximately one third of the follicular carcinoma samples. It has been shown that conventional RT-PCR in combination with gel electrophoretic separation of the products is well suitable for the detection of PAX8/PPARgamma fusion transcripts in archival FFPE samples.

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## **P-CytoG-185**

### **Recurrent aberrations identified by array-CGH in patients with Mayer-Rokitansky-Küster-Hauser syndrome**

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Mayer-Rokitansky-Küster-Hauser syndrome (MRKH) is a congenital anomaly of the female genital tract characterized by agenesis of the upper vagina and rudimentary or absent uterus. Typically, the external genitalia as well as the ovaries are normal and the karyotype is 46,XX. A variety of different defects, especially renal anomalies, can be associated. To date in most of the cases the cause remains unclear. Therefore, we performed array-CGH analysis in a group of 48 MRKH patients by using high-resolution Agilent oligonucleotide arrays and detected 21 losses and gains being potentially causative. Of these regions detected in our MRKH patient collective we could delineate three definitively relevant regions (1q21.1, 17q12, and 22q11.21), which have been repeatedly observed in our and other MRKH patients. Furthermore, sequential analysis of LHX1 und HNF1B, being located in candidate region 17q12, revealed in one MRKH patient a missense mutation in LHX1 gene, making LHX1 a strong candidate gene for MRKH. However, HNF1B is another candidate gene in the 17q12 region because mutations of this gene have been observed in patients with renal cysts, diabetes, and Müllerian aplasia.

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## P-CytoG-186

### Four patients with overlapping microdeletions in 9q33.3-q34.1 associated with developmental delay and micro- / brachycephaly

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We report four patients with overlapping microdeletions of chromosome 9q identified by molecular karyotyping using various array platforms. All four patients had some common clinical features, including developmental delay of varying degrees and micro- and/or brachycephaly. Additional shared features between patients 1, 2, and 4 include strabismus and delayed or absent speech. Clinical information of patient 3 is forthcoming and a comparison of the phenotypes of all four patients will be presented.

In patients 1 through 3, de novo deletions of 1.76 Mb, 1.3 Mb, and 2.8 Mb, respectively, encompassing chromosomal bands 9q33.3-q34.11 have been detected. Patient 4 had a smaller deletion of approximately 432 kb spanning 9q33.3. Her parents have not been analyzed yet for the occurrence of the deletion and her mother is reported to have mild intellectual disability.

Interestingly, the three larger deletions of patients 1, 2, and 3 had 27 genes in common including the STXBP1 gene, which is predominantly expressed in brain and plays a role in synaptic transmission. In numerous studies, loss-of-function point mutations and deletions of STXBP1 have been shown to be associated with early infantile epileptic encephalopathy (EIEE, or Ohtahara syndrome), and also identified in patients with both severe to profound intellectual disability and non-syndromic epilepsy. However, neither patient 1 nor patient 2 presented with a seizure phenotype, although an EEG of patient 2 gave pathological results. Thus, the penetrance of the seizure phenotype in deletions of STXBP1 appears to be incomplete. Notably, patient 4 presented with epilepsy, and although her deletion does not include STXBP1, deletion of long range regulatory elements cannot be ruled out. Depending on the origin of the deletion of patient 4, it may be possible to define a critical region including only three genes for our patients' common clinical features.

We suggest that deletions of this region on chromosome 9q may cause, besides possibly epilepsy of incomplete penetrance, a wider clinical spectrum including developmental delay concerning especially speech, micro- and / or brachycephaly and mild dysmorphisms.

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## P-CytoG-187

### Presence of harmless small supernumerary marker chromosomes hampers molecular genetic diagnosis: a case report

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Mental retardation is correlated in approximately 0.4% of cases with the presence of a small supernumerary marker chromosomes (sSMC). However, here we report a case of a carrier of a heterochromatic harmless sSMC with fragile X syndrome (FraX). In approximately 2% of sSMC cases, similar heterochromatic sSMC were observed in a clinically abnormal carriers. In a subset of such cases, uniparental disomy (UPD) of the corresponding sister chromosomes was shown to be the causes of mental retardation. For the remainder of the cases, including the present one, the sSMC was just a random finding not related to the clinical phenotype. Thus, it is proposed to test patients with heterochromatic sSMC and mental retardation of unclear causes as follows: i) exclude UPD, ii) test for FraX as it is a major causes of inherited mental retardation, and iii) perform chip-based assays or tests for special genetic diseases to the

phenotype. In any case, the diagnosis of a cytogenetic aberration such as an sSMC should not automatically be considered the resolution of a clinical case.

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## **P-CytoG-188**

### **Molecular karyotyping in 130 patients with intellectual disability– the Münster experience**

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We present our results of high resolution molecular karyotyping (array CGH) in 130 patients with non-specific or syndromal mental retardation. The study was performed in conjunction with The German Mental Retardation Network (MRNET).

Fragile X syndrome and major chromosomal as well as subtelomeric rearrangements were excluded in 122/130 patients. Two patients demonstrated larger de novo balanced chromosomal translocations; in six patients de novo unbalanced subtelomeric rearrangements were detected by FISH analyses. In all 130 patients, array CGH using Agilent's 244A and 400K microarray platforms was performed. We compared our results to known polymorphisms listed in the Database of Genomic Variants. Aberrations not listed in this database were validated by fluorescence-in-situ-hybridisation (FISH) or quantitative PCR in the patients and in their parents to distinguish between inherited and de novo aberration.

In our cohort of 130 patients, we found de novo aberrations in 29 (22.3%) patients. In 21 patients, array CGH revealed 26 de novo losses or gains, including 16 deletions and 10 duplications. In six patients we were able to confirm the unbalanced subtelomeric rearrangements detected by FISH analyses before. The two patients with the cytogenetically balanced translocations did not show imbalances within the breakpoint regions. 24 patients exhibited only known copy number variations, predicted as polymorphisms. The remaining 77 patients showed inherited aberrations of unclear significance.

The de novo alterations varied in size from 53.0 Kb to 7.9 Mb, in which the median of the deletions size was 1.7 Mb, for the duplication size 0.342 Mb. The inherited alterations were significantly smaller (median: 0.12 Mb).

Aberrations not detected by standard cytogenetic and molecular cytogenetic methods can be identified by array CGH in patients with intellectual disability. Sequence analyses in patients with inherited microdeletions or microduplications not described as polymorphism, are in progress to detect possible mutations in the non-deleted or non-duplicated alleles.

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## **P-CytoG-189**

### **Elevated sperm aneuploidy levels in an infertile Robertsonian translocation t(21;21) carrier with possible interchromosomal effect**

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Somatic chromosomal abnormalities are frequently found in infertile men, particularly in those with low sperm count. These abnormalities mostly consist of numerical sex chromosome abnormalities and of Robertsonian or reciprocal translocations. Here we report on a sperm aneuploidy screening in an infertile patient showing low-frequency mosaicism of a balanced Robertsonian translocation involving both chromosomes 21. The 36 years old man was previously diagnosed of oligo-astheno-teratozoospermia. Somatic chromosome analysis revealed a low-frequent (4%) mosaic of a balanced Robertsonian translocation 45,XY,t(21;21)(q10;q10) in a normal 46,XY male background.

We screened a sperm sample of this patient for elevated disomy levels of all chromosomes (1-22, X and Y) by multicolour interphase fluorescence in situ hybridisation (sperm-FISH). Over 1000 sperm cells were scored per chromosome. An increase of the aneuploidy rate in the patient by over two standard deviations compared to the mean baseline level in healthy controls described in the literature was considered statistically significant ( $P < 5\%$ ). In the investigated sperm cells of the patient we found a significantly elevated level of disomy 21 compared with the mean baseline frequency. 23/2133 cell nuclei (1,08%) showed disomy 21. However, also chromosomes 2 (0,38%), 4 (0,29%), 6 (0,19%), 9 (0,29%), 15 (0,29%) and most notably chromosome 16 (0,87%) showed significantly increased frequencies. In contrast, no increased diploidy frequency was observed.

Our results demonstrate that even patients with low-frequency mosaicism concerning Robertsonian translocations may have increased aneuploidy rates in sperm cells, which can be associated with infertility,

leading to increased risk for miscarriages or fetal malformations. Moreover, these aneuploidies may also involve other chromosomes than the translocation products. The occurrence and impact of this so-called interchromosomal effect (ICE) is controversially discussed, but it might be connected with a general disturbance of meiotic processes caused by the translocation product. We conclude that analysis of sperm aneuploidy by FISH is helpful in a comprehensive clinical work-up and may increase the accuracy of a prognostic prediction.

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## P-CytoG-190

### Partial deletion of chromosome X and partial duplication of chromosome 5 in a child with a distinct morphological phenotype

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We report on a child with a derivative X-chromosome due to a maternal balanced translocation t(X;5). Amniocentesis was performed at 32 weeks of gestation of a 33 year old woman because of IUGR and ventricular septal defect. The first pregnancy of the couple was uneventful; the second pregnancy resulted in an abortion at 7 weeks. The prenatal cytogenetic and molecularcytogenetic analysis revealed a female karyotype with a derivative X-chromosome with terminal deletion of the long arm of the X-chromosome and a duplication of a part of the long arm of one chromosome 5 [46,X,t(X;5)(q28;q31.1)mat]. Parental karyotypes showed a maternal balanced translocation 46,X,t(X;5)(q28;q31.1).

The female child was born spontaneously at 36 weeks. The birthweight was 2585g (10th-25th percentile), the length 45 cm (3rd percentile). The newborn had a microcephaly with a head circumference of 29,5 cm (1,5 cm below 3rd percentile). An atrial septal defect II and a pulmonary stenosis were diagnosed. There were duplex kidneys on both sides and a dorsal dermal sinus. Ultrasound examination of the spine revealed no vertebral and no intraspinal anomalies. Sonography of the head showed a reduced cortical gyration at the age of one day, the re-examination at the age of 6 weeks showed normal findings. The face was characterised by a sloping forehead, a short nose with broad nasal bridge and depressed nasal tip, tented upper lip, thin lips and mandibular retrognathia.

Most of the dysmorphic features seen in our patient are likely to be attributed to partial duplication of chromosome 5 indicating that spreading of inactivation from the X-chromosome to the adjoining Chromosome 5 region is incomplete. This results in a milder manifestation of the symptoms than in cases of duplication without the involvement of the X-chromosome.

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## P-CytoG-191

### Systematic analysis of copy number variants in a large German patient-control sample of schizophrenia

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Schizophrenia is a severe, disabling psychiatric disorder with a prevalence of around 1% worldwide. Multiple recent studies showed association of copy number variants (CNVs) with schizophrenia. Here, we present data from a genome-wide screening for CNVs in an extended sample of 1,637 patients with schizophrenia or schizoaffective disorder and 1,627 controls. All individuals were of German descent and genotyped on Illumina's HumanHap550, Human610 or Human660W arrays, sharing about 550,000 markers. Identification of putative CNVs was achieved via two different bioinformatic tools: QuantiSNP and PennCNV.

Both algorithms use Hidden-Markov models to predict CNVs from SNP intensity and allele frequency data. To reduce the number of false-positive CNV calls, we developed a stringent quality protocol, requiring CNVs to have a minimum of 30 consecutive SNPs and a log Bayes Factor (QuantiSNP) or confidence value (PennCNV) of at least 30. These quality measures indicate the confidence of each predicted CNV, with higher values pointing towards better statistical reliability. The two datasets generated by QuantiSNP and PennCNV were analyzed independently. Using PLINK, we performed permutation-based tests for association of specific chromosomal regions with schizophrenia.

In chromosomal region 8p23 we identified a significant association of CNVs in patients compared to controls. Interestingly, a promising candidate gene, CSMD1, is located in the CNV region. Common variants in CSMD1 have previously shown evidence for association with neuropsychiatric phenotypes (major depression (Rietschel et al. 2010), schizophrenia (Ripke et al., WCPG 2010)). In addition, we monitored rare CNVs that have previously been associated with neuropsychiatric disorders. CNVs in 1q21, 2p16, 2q32, 7q34, 15q11, 15q13, 16p11, 17p12 were overrepresented in our patients compared to controls, but did not reach significance (probably due to the limited power of our sample to replicate these rare events at a nominally significant level). Although the finding on 8q23 is interesting, additional support from independent data sets is needed before final conclusions regarding the involvement of CNVs in this region in SCZ can be drawn.

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## P-CytoG-192

### **Multicystic kidney disease in a female patient with monosomy 1p36- a so far unreported coincidence**

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**Background.** The monosomy 1p36 deletion syndrome is characterized by loss of a variable part of the most distal 1p region. All patients demonstrate characteristic craniofacial features including deep-set eyes, straight eyebrows and pointed chin, developmental delay and a variable pattern of malformations. Renal abnormalities are rarely seen. We report on a coincidence of monosomy 1p36 with unilateral multicystic kidney disease in an otherwise attenuated phenotype.

**Case report.** The proband is the first born child of healthy unrelated parents, no history of renal abnormalities. Low birth weight, but normal OFC, no dysmorphic aspect was noticed. Right kidney inoperable to multicystic anomaly as the only malformation. Normal growth and weight gain, motor and particularly speech development were delayed. Unaided walking with 2 1/2 years. Markedly delayed closure of the widened fontanelles. At 3 years growth (Pc 42) and OFC (Pc 22) were still in the normal range, but no speech. Re-examination of the facial features raised eventually suspicion of monosomy 1p36.

**Lab diagnosis.** Despite normal karyotype the clinical suspicion was confirmed by FISH using a commercial 1p36 probe (D1S2217, Kreatech) and by subtelomer MLPA kits P036 and P070, MRC Holland. The parent's karyotype and FISH were normal.

**Conclusion.** Monosomy 1p36 is well known to be associated with a number of major congenital anomalies eg cardiomyopathy and visual abnormalities. This to our knowledge first observation of a completely inoperable multicystic disease in this subtelomeric anomaly raises the question if this coincidence has happened only by chance or due to a specific monogenic mutation. Array studies might be helpful to look for unexpected cryptic rearrangements. Other uncommon features of our case report are normal vision and only subtle dysmorphic aspects and the lack of microcephaly and growth retardation. This underlines the broad clinical spectrum of this condition.

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## P-CytoG-193

### **A rare chromosomal abnormality – duplication of chromosome 1p**

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The delineation of a recognizable phenotype of "pure" partial trisomy 1p is complicated by the rareness and the involvement of different chromosomal segments in the few described cases. The patients described so far appear to exhibit a quite variable clinical manifestation but the features often comprise short survival, impaired development and congenital malformations.

Here we present the clinical, cytogenetic and molecular characterisation of a patient with a duplication 1p31.1-p33.

Our patient, a 6 year and 10 month old girl is the first of three children of healthy non-consanguineous parents. She was born at 42nd weeks of gestation after an uneventful pregnancy. Body measurements were

in the lower norm and OFC was 32 cm (1cm <P3) at birth and 47,5 cm (1cm <3P) at our examination. She shows a cognitive ability according to the age of 4 years, hypotonia, mild facial dysmorphic features, and hypoplasia of one of the distal phalanges, but no organ or skeletal malformations.

By whole genome microarray analysis (Affymetrix 6.0) a duplication 1p31.1 to p33 was detected and the size was defined to be approximately 21 Mb.

By cytogenetic investigations of the patient and her parents we could characterise this intrachromosomal rearrangement being a de novo inverted duplication of the region 1p31.1 to p33.

To the best of our knowledge, there is no reported case of a partial trisomy 1p similar to the abnormality in our patient. Reviewing the literature, only two individuals possess a comparable but larger duplication 1p. One of these patients revealed craniosynostosis, plagiocephaly, and developmental delay, whereas the other one showed sex reversal, mid-face abnormalities, an eczema-like skin condition, severe growth and mental retardation.

According to the UCSC genome browser, the duplicated region in our patient encompasses approximately 125 genes.

In consideration of the size of the duplicated segment and the number of genes involved the given imbalance is associated with a comparatively mild phenotype.

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## **P-CytoG-194**

### **Microdeletion 1q42.12q42.2 in a boy with hypogenesis of the corpus callosum**

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**Introduction:** Agenesis of the corpus callosum (ACC) is common amongst brain malformations. It is characterized by complete or partial absence of the large bundle of fibres that connect the cerebral hemispheres. The underlying genetic defects of ACC are variable, reflecting the complexity of callosal development. ACC has been described in association with different microdeletions, often located at the chromosomal region 1q44. In two cases the microdeletion was located at 1q42 more than 10 Mb proximal of 1q44.

**Case report:** We report on a 3-year-old boy from Irak who is the first child of non-consanguineous parents. Family history and pregnancy were unremarkable. The postpartal period was characterized by feeding difficulties. At the age of 3 years a febrile seizure occurred. Speech development was delayed, behaviour was hyperactive and aggressive. He had mild facial dysmorphism with inverse epicanthic folds and two café-au-lait spots. Cranial MRI showed hypogenesis of the corpus callosum. Metabolic and ophthalmologic examinations did not detect any abnormality.

**Results:** SNP oligonucleotide array testing on Illumina Human 660W-Quad Bead Chip revealed a 3.6 Mb duplication in chromosomal region 1q25.1 (chr1:171,453,887-175,034,075, hg18) and a 4.2 Mb deletion in chromosomal region 1q42.12q42.2 (chr1:224,884,179-229,103,128, hg18). The duplication encompassed 30 RefSeq genes, none of them being an OMIM annotated disease gene. The deleted region harbors 40 RefSeq genes, five of them listed to be associated with diseases (PSEN2, CABCA1, GJC2, ACTA1, AGT).

**Discussion:** Our observation is in keeping with two previous reports on ACC patients with deletions of 1q42. This region is more than 10 Mb apart from 1q44 where deletions are known to cause ACC. The finding thus supports the notion of an independent ACC-associated region at 1q42. Some of the genes located in this region, e.g. WNT3A (OMIM \*603341) and RHOA (\*606366), have been discussed as candidates of ACC and neural tube defects. Behavioural abnormalities and speech delay in our patient may be explained as consequences of ACC. However a phenotypic effect of the duplication at 1q25.1 cannot be excluded.

**Conclusion:** This report underpins the role of 1q42 microdeletion as a potential cause of agenesis/hypogenesis of the corpus callosum.

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## P-CytoG-195

### Screening for submicroscopic chromosomal imbalances in idiopathic Silver-Russell syndrome patients

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Silver Russell syndrome (SRS) is a congenital imprinting disorder mainly characterized by severe intrauterine and postnatal growth retardation, relative macrocephaly, a triangular face and asymmetry. The clinical spectrum of the disease is broad and the diagnosis is quite subjective.

In about 50 % of SRS cases (epi)genetic alterations can be detected: >38 % show a hypomethylation of the imprinting control region 1 in 11p15, a further 10 % carry maternal uniparental disomy of chromosome 7 (upd(7)mat). In addition to conventional cytogenetic findings recently submicroscopic chromosomal imbalances have been reported in single cases and therefore significantly contribute to the mutational spectrum of the disease.

To determine the relevance of submicroscopic imbalances for the aetiology of the SRS, we performed molecular karyotyping of 26 patients referred as SRS without (epi)mutations in 11p15 and upd(7)mat using the Affymetrix Genome-Wide Human SNP Array 6.0. The detected imbalances were evaluated in respect to their overlap with registered copy number variations (CNVs), common microdeletion/microduplication syndromes and gene coverage by use of online databases (UCSC, DECIPHER, DGV).

In two patients chromosomal de-novo imbalances were found which are likely causative for their phenotype. One patient carried a 2.5 Mb microdeletion of chromosome 22q11 while the other showed a 9.1 Mb duplication of chromosome Xq26. In further 11 of the 26 analysed SRS patients we identified a total of 19 different, so far unregistered copy number alterations (CNAs). By characterising the parents of these SRS patients by microarray and microsatellite analyses parental inheritance was determined for 13 CNAs: these are probably rare familial CNVs which are regarded as likely apathogenic. For 4 of the CNAs a de-novo origin was determined. To our current knowledge it is unclear whether they are associated with the clinical features of the patients.

In summary, two out of 26 analysed SRS patients showed submicroscopic chromosomal imbalances explaining their phenotype. In four patients the pathogenic significance remains unclear. In conclusion, molecular karyotyping should be performed to exclude common microdeletion/microduplication syndromes or other submicroscopic imbalances in idiopathic SRS patients or SRS-like patients.

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## P-CytoG-196

### Craniosynostosis as a Rare Feature of 17q21.31 Microdeletion Syndrome

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Microdeletions in the region 17q21.31 are described as a definite syndrome (OMIM #610443). As of November 2010, at least 63 patients have been reported. An incidence of 1 in 16,000 has been estimated, however, this syndrome is probably underdiagnosed. Characteristic features include global developmental delay, muscular hypotonia, microcephaly, facial dysmorphism, heart defects, hearing loss, and urologic anomalies. We present the clinical and molecular findings of a nine year old girl displaying psychomotor retardation, short stature and facial dysmorphism including a bulging forehead, upslanting palpebral fissures, hypertelorism, and a bulbous nasal tip. Additionally premature closure of the sagittal suture (resulting in scaphocephaly if not operated), combined hearing loss, an atrial septal defect, and vesicoureteral reflux were diagnosed. Microcephaly was not found. CT scan of the brain showed mild widening of the ventricles but no further malformations. Her family history was normal. Conventional cytogenetic analysis revealed a normal female karyotype 46,XX. Array CGH analysis detected a 504 kb deletion in 17q21.31 containing five genes such as CRHR1, IMP5, MAPT, STH and KIAA1267. The detected microdeletion contains the 160.8 kb minimal critical region for the 17q21.31 syndrome defined in previous studies. Apart from frequent features of the syndrome, our patient presented with premature closure of the sagittal suture (operated in early childhood) as a rare feature. This condition has been found in only four of the 63 previously known patients with 17q21.31 microdeletion syndrome. A causal relationship to one of the genes affected by the deletion could not be established.

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## P-CytoG-197

### GenomeCAT- a software for the integrative analysis of DNA copy number variants

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The genomewide analysis of DNA copy number variants (CNVs) has gained increasing importance in research and diagnostics. Here we present GenomeCAT, a software dedicated to the integrative analysis of CNV data. Features for array CGH data analysis include versatile visualization tools, such as frequency plots that can be utilized for the comparative analysis of two or more CNV data sets. The search for recurrent CNVs is further assisted by comprehensive filter and query options.

In addition the software allows the simultaneous visualization of data derived from different experiment types performed on different platforms. For example, CNVs can be displayed together with data from array based gene expression analysis and ChIPSeq data on epigenetic modifications. In order to facilitate the meta-analysis of these diverse data sets, GenomeCAT can map the data to a common matrix, which can be based either on fixed genomic intervals (e.g. windows of 1kb) or genes. In this way data get feasible for statistical analysis aimed at finding out possible correlations. Much emphasis has been put on the user friendly and customizable import of external datasets and the export of own data. It is also possible to generate tables comprising all data of a selected genomic interval. GenomeCAT is a client Java application that runs on Linux and Windows. It will be distributed under GNU General Public License.

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## P-CytoG-198

### Genome stability within three-dimensional chondrocyte grafts

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Regeneration of articular cartilage defects by autologous chondrocyte implantation (ACI) is meanwhile a commonly applied clinical procedure. BioSeed®-C is a third-generation ACI tissue engineering cartilage graft that is based on autologous chondrocytes embedded in a three-dimensional bioresorbable polymer scaffold. Chondrocytes are culture-expanded in vitro and dedifferentiate. The three-dimensional assembly of the cells in polymer-based scaffolds initiates the redifferentiation. Matrix formation and tissue maturation occur in vivo after implantation of the graft. Although this technique was applied successfully to a vast number of patients during the last decade, concern was raised that the culture conditions, especially the three dimensional cultivation, may affect the genomic stability of the cells and accumulation of chromosomal aberrations in the graft may confer a tumorigenic potential. Therefore, we investigated the genomic stability within the grafts on a single cell level by karyotype analyses. In a first preliminary study we analyzed metaphases of chondrocytes in expansion phase and after 3D-cultivation from a group of post mortem donors with a mean age of 58,1 years and without inflammatory joint diseases. Within this group we found surprisingly high rates of chromosomal gains specifically affecting chromosomes 5, 7, and 8 and losses of the gonosomes. However, the aberration rates appeared not to be affected by increased passage numbers and also remained constant after three-dimensional cultivation. Analysis of single mitotic events in binucleated cells after cytochalasin B treatment also did not indicate increased nondisjunction rates caused by the in vitro cell culture. In sharp contrast to the post mortem group, the same analyses in a group of patients with cartilage damage and a mean age of 24 years revealed no increase in chromosomal gains and/or losses. Thus, we assume that the high rates of aneuploidy observed in the post mortem group represent an age and/or post mortem effect manifesting already in the uncultivated cartilage tissue and are not caused by the culture-expansion. Further investigations to confirm this hypothesis are underway.

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## P-CytoG-199

### The human genome puzzle – the role of copy number variation in somatic mosaicism

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The discovery of copy number variations (CNV) in the human genome opened new perspectives in the study of the genetic causes of inherited disorders and the etiology of common diseases. Differently patterned instances of somatic mosaicism in CNV regions have been shown to be present in monozygotic twins and throughout different tissues within an individual. A single-cell-level investigation of CNV in different human cell types led us to uncover mitotically derived genomic mosaicism, which is stable in different cell types of one individual (Mkrтчhyan et al., 2010, PLOS ONE). A unique study of immortalized B-lymphoblastoid cell lines obtained with 20 year interval from the same two subjects shows that mitotic changes in CNV regions may happen early during embryonic development and seem to occur only once, as levels of mosaicism remained stable. This finding has the potential to change our concept of dynamic human genome variation. We propose that further genomic studies should focus on the single-cell level, to understand better the etiology and physiology of aging and diseases mediated by somatic variations.

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## P-CytoG-200

### A familial case of translocation t(4;9)(p16.1;q13) and mental retardation

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A 2-year-old boy and his 4-year-old sister were taken into public welfare custody because of the difficult family situation. The mother insisted on a chromosomal analysis of the boy due to his retardation and behavioral abnormalities and it was performed against the pediatrician's opinion. During the first genetic counseling the parents seemed rather ordinary, a very talkative mother and a quite silent father. Cytogenetic analysis of the whole family revealed a translocation t(4;9)(p16.1;q13) in both father and son, while mother and daughter had a normal karyotype. Doubting the usual harmlessness of passed-on translocations in this case, a microarray-analysis was performed. The translocation was unbalanced in both son and father, bearing a 26 genes containing microdeletion of 1.7 Mb in the breakpoint region of 4p16.1. During the following genetic counseling sessions that also included the father's mother, it became clear that the father, in spite of his seemingly normal work situation, suffered a severe learning disability and was hardly able to talk at all. As a child he was given additional intensive care when his developmental problems became visible.

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## P-CytoG-201

### Overlapping microdeletions in 15q22.1-q22.2 in two patients with intellectual disability – characterization of the critical region using the DECIPHER database

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Patients with intellectual disability (ID) of unknown etiology are increasingly analyzed by molecular karyotyping using SNP arrays or array-CGH in order to identify disease-causing microaberrations and candidate genes for ID. The interpretation of the detected copy-number variations (CNVs) is often hampered

by the growing number of benign CNVs. Besides the de novo occurrence of a CNV, additional patients harboring a similar CNV and a similar clinical phenotype are important indicators suggesting causality.

Here, we report two patients with ID and microdeletions in 15q22.1-q22.2. The smallest region of overlap (SRO) of the two microdeletions contained 26 genes, 8 of which are listed in OMIM.

Patient 1, a 14 1/2 year old boy, was referred with suspected Williams-Beuren syndrome. He presented with moderate ID, slightly disproportional growth retardation, truncal obesity, strabismus and mild dysmorphisms, such as edema of the upper eyelids, a long philtrum, a thin upper lip and a prominent, everted lower lip. A SON-R test (at age 6 8/12 years) gave an IQ of 55-60. After an initial speech delay, his language skills at the age of 14 1/2 years were significantly better in comparison to his IQ. He was highly verbal with a sociable and outgoing personality.

Patient 2, a 6 1/2 year old male, was identified in the DECIPHER database. He presented with mild global developmental retardation, muscular hypotonia, uncoordinated movements, joint hypermobility, and mild dysmorphisms, including a wide nasal bridge, thin upper lip, enamel hypoplasia, short tapered fingers with broad thumbs, clinodactyly of the 5th fingers, broad 1st toes, and unilateral syndactyly of the 2nd / 3rd toes.

Molecular karyotyping detected de novo microdeletions in 15q22.1-q22.2 of 5.3 Mb and 5.5 Mb, respectively. The SRO of these deletions covered approximately 4.97 Mb and contained 8 OMIM genes. However, the critical region could be reduced tentatively by the deletion in a third patient, who was also listed in the DECIPHER database. His deletion overlaps the proximal 1.87 Mb of the SRO of patient 1 and 2, but he displayed neither ID nor obesity, muscular hypotonia or dysmorphisms, and was analyzed only because of renal failure possibly caused by nephronophthisis. Assuming that no highly penetrant haploinsufficient genes causing ID should be localized in his deletion, the critical region for ID and dysmorphisms could be narrowed down to 3.09 Mb and 11 genes, only three of which are listed in OMIM. Among those three, there are two promising candidate genes: VPS13C, which is highly expressed in the central nervous system and is involved in the trafficking of membrane proteins in the trans-Golgi network, and RORA (retinoic acid receptor), which is involved in neuronal development and may also be a candidate gene for the muscular phenotype of patient 2 since it interacts with MYOD1.

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## P-CytoG-202

### In-depth characterization of 14 patients with deletions of 5q14.3-q15: Is MEF2C the whole story?

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Microdeletions of chromosomal bands 5q14.3-q15 have been reported to cause a phenotype including severe intellectual disability / developmental delay (ID/DD), epilepsy, muscular hypotonia, and variable minor anomalies. Proximal deletions of this region including the MEF2C gene led to the identification of the transcription factor MEF2C as a frequent autosomal dominant cause of severe ID. Deletions as well as truncating and missense mutations are associated with diminished expression of MEF2C resulting in reduced transactivation of MECP2 and CDKL5.

Here, we present the clinical and genetic characterization of 14 patients with microdeletions concerning 5q14.3-q15, ten of which have not yet been published. All patients exhibited ID/DD and muscular hypotonia. Patients with proximal deletions displayed severe ID/DD and seizures. Periventricular heterotopias in three patients with 5q14.3-q15 microdeletions as published by Cardoso and colleagues in 2009 (Neurology; 72:784-92) have been excluded specifically for seven of our patients, but a wide range of mostly minor cranial MRI changes was found.

Lymphocyte RNA expression profiling of ten genes from the deleted regions demonstrated diminished expression of several genes in seven patients. Interestingly, reduced expression of MEF2C was found in one patient whose deletion did not encompass the MEF2C gene. However, MEF2C expression levels were

unchanged in two patients with more distal deletions. One of these patients exhibits a typical microdeletion 5q14.3-q15 phenotype with severe ID, epilepsy and muscular hypotonia while in the second one the ID was only mild to moderate and the muscular hypotonia was less pronounced. In order to further elucidate the role of MEF2C haploinsufficiency in different microdeletions of 5q14.3-q15, and to gain insight into which other gene(s) might contribute to the phenotype, the expression analyses are being expanded to include targeted analyses of MEF2C in twelve patients as well as genome-wide expression studies in ten patients.

## P-CANCER GENETICS

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### P-CancG-203

#### Validation of two novel unbalanced whole arm-translocations in cervical smears

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Persistent infection with a high risk human papillomavirus (HR-HPV) type is a prerequisite for the development of cervical cancer (CxCa). This process is accompanied by numerous genetic alterations including chromosomal aberrations which are already apparent in precancerous lesions (CIN). Recently, we identified two novel unbalanced translocations, der(10;14) and

der(7;21), in HPV16 immortalized keratinocyte cell lines (HPV). Both translocations, der(10;14) and der(7;21), could also be detected in 16.7% and 33.3% of CIN1, in 80% and 53.3% of CIN2/3 and in 60% and 46.7% of CxCa, respectively.

The aim of this project was to validate these chromosomal translocations in cervical smears as a basis for further diagnostic evaluation.

Cervical smears from 173 patients (women with no histological evidence for disease (n=117), CIN1 (n=29), CIN2/3 (n=21) and CxCa (n=6)) were collected and stained according to standard procedures for "Papanicolaou" (Pap)-staining. Cytological images of the areas of interest were made and the respective XY-coordinates were recorded. Coverslips were then removed using xylene and the slides were prepared for fluorescence in situ hybridization (FISH). After interphase-FISH procedure images from the identical areas of the slides (according to XY-coordinates) were taken.

Both translocations were detected in cervical smears of 10.3% of cases with CIN1, in 23.8% of cases with CIN2/3 and in 71.4% of cases with CxCa. In addition der(14;21) only was found in three cases of CIN2/3 and in one case of CxCa and der (7;21) only was found in one case of CIN2/3. The percentage of nuclei with translocations in individual lesions varied strongly but was highest among CxCa. Signals characteristic for the translocations could be detected in a maximum of 30% of the nuclei of Pap-stained dysplastic cells. None of the cytologically normal Pap-smears or normal cells in Pap-smears of women with CIN or CxCa showed one of the aberrations.

Prospective studies will have to show whether the detection of these translocations in routinely collected Pap smears will allow the identification of lesions with a high risk for progression.

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### P-CancG-204

#### Androgen receptor mutations lead to loss of transrepression and failure of antagonist treatment of prostate cancer

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Prostate cancer (PCa) is a major health problem in Western countries and most diagnosed cancer of men. The ligand-activated human androgen receptor (AR) promotes the growth of the normal prostate gland and also initially of PCa. Inhibition of human AR by androgen ablation therapy and by applying synthetic antiandrogens is therefore the primary goal in treatment of patients. A central issue in androgen ablation therapy is cancer resistance that occurs after 1 to 2 years of treatment with the current commonly used antiandrogens. The resistance often arises by reason of AR mutation. One often-detected AR point mutation in PCa is the AR T877A mutant. This change of one amino acid renders some therapeutical applied antiandrogens into AR agonists and promotes PCa progression. Interestingly, the androgen-activated

androgen receptor inhibits the expression of the catalytic subunit of the telomerase (hTERT). The expression of hTERT is associated with immortalization. hTERT is not only linked to extending telomere ends but also supports growth factor expression (non-telomere function). Analyzing tumor cells, this transrepression is lost in PCa expressing the mutant AR T877A. Also another AR mutation abrogates transrepression but not transactivation. Here, we provide the molecular basis of the loss of transrepression of AR. The wildtype AR is normally recruited to the hTERT promoter at two sites in vivo. Interestingly, however, the mutant AR T877A has lost the ability to be efficiently recruited and thus unable to mediate repression of hTERT. Our data indicate a novel role of AR mutations in the development of PCa.

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## P-CancG-205

### Unusual paediatric presentations of Gorlin syndrome

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We report three children from unrelated families with Gorlin syndrome who presented in unusual ways in childhood. The first child presented with macrocephaly, hydrocephalus, absent corpus callosum and scaphocephaly. The second child presented with macrocephaly, communicating hydrocephalus, unilateral microphthalmia, bilateral talipes, cutaneous syndactyly of the fourth and fifth toes of the right foot with duplication of the terminal phalanx of the fifth toe, pectus carinatum, an arteriovenous malformation of the right anterior chest wall, a left Sprengel shoulder and marked body asymmetry. The third child presented with macrocephaly, moderate dilatation of the lateral ventricles, bilateral cutaneous syndactyly of fingers 3-5, postaxial polydactyly of the right foot, congenital jerky nystagmus, a midline sinus on the nasal bridge and a significant leg length difference. Gorlin syndrome needs to be considered in the differential diagnosis of children who present with macrocephaly and other congenital malformations.

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## P-CancG-206

### PAX5-AUTS2 fusion resulting from t(7;9)(q11.2;p13.2) can now be classified as recurrent in B-cell acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most frequent hematological neoplasia in childhood and the incidence is highest in children 2 to 5 years of age. Many non random chromosomal aberrations leading to gene fusions have been identified e.g. t(12;21)(p13;q22) (ETV6/RUNX1), t(9;22)(q34;q11) (BCR/ABL), t(4;11)(q21;q23) (MLL/AF4) or to gene dosage changes e.g. high hyperdiploidy (51-67 chromosomes). Moreover several breakpoints have been described to be hot spots of chromosomal rearrangements e.g. 9p21 (INK4a), 14q32 (IgH) or 14q11.2 (TRAalpha). Apart from this random chromosomal aberrations will be detected occasionally in childhood ALL.

Here we present a nearly 3-year-old girl with the diagnosis of a pre-B-ALL. The patient achieved complete remission on day 33 after induction therapy (ALL-BFM 2004). Bone marrow and peripheral blood have been cultivated without stimulation for 24 hours and chromosomes were G-banded after cytogenetic preparation by using GTG banding. Routinely performed Fluorescence-in-situ-Hybridization (FISH) on bone marrow cells for t(9;22), t(12;21) and MLL-rearrangements adduced none of these specific aberrations. After cytogenetic analysis and additional FISH with specific probes for 7q31 and 9p21 the karyotype was ascertained as:

45,XX,-7,der(9)t(7;9)(q11;p13),dup(16)(p11p13)[14]/46,XX[2]

The band 9p13 is a well described breakpoint in childhood ALL and contains PAX5 which is involved in recurrent aberrations e.g. dic(9;12), dic(9;20) and fuses also to different partner genes. Hybridizations with PAX5 specific overlapping probes (BAC probes RP11-243F8 and RP11-344B23) and AUTS2 specific flanking probes (BAC probes RP4-715F13 and RP11-409J21) were performed. These FISH experiments revealed involvement of PAX5 and AUTS2 on both derivative chromosomes.

Subsequent PCR experiments and semi-nested PCR generated one product from the patients sample and sequence analysis of this PCR product showed a transcript encoding an in-frame fusion between exon 6 of PAX5 and exon 6 of AUTS2.

Kawamata et al. (2008) have reported a t(7;9)(q11.2;p13.2) in a paediatric patient with B-lineage ALL, leading to a PAX5-AUTS2 fusion transcript. Therefore the breakpoints found in our patient differed from

those observed in the previously reported case that fused the exon 6 of PAX5 with exon 4 of AUTS2. Despite these different molecular breakpoints, we can assume that the corresponding predictive fusion proteins are similar.

This second case of t(7;9)(q11.2;p13.2)/PAX5-AUTS2 we report here, shows that this translocation is recurrent in B-lineage ALL and suggests that the 7q11 region might be a hot spot of rearrangements in paediatric ALLs.

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## P-CancG-207

### SNP pattern in patients with epidermodysplasia verruciformis without typical mutation in TMC6 and TMC8

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Epidermodysplasia verruciformis (EV) is a rare skin disease, which was first described in our clinic in Basel in 1922. EV results from a genetically determined susceptibility of the skin to infection with particular types of human papilloma virus (HPV) that are considered to be innocuous for the general population. HPVs constitutes a highly diverse group of potential human pathogen DNA-viruses, which are classified depending on the homology of a specific nucleotide sequence of the virus. About 120 different types of HPVs are identified to date, which belongs to the alpha-, beta-, gamma-, my-, or ny-family. HPVs which are linked to the development of EV belongs to the beta-family, mainly HPV 5 and 8, but also HPV 9, 17, and 19 amongst others. About 20% of the unaffected population is infected by these EV-causing HPVs, but the virus is only pathologic in EV individuals. An association between HPV and the occurrence of non-melanoma skin cancer in sun exposed areas has been observed in these patients. At the UV exposed regions of the skin EV patients develop typical verrucae planae, which may develop to Bowen disease and subsequent squamous cell cancer (SCC). Common warts are only occasionally observed. A direct causal relationship between EV-HPV and skin malignancies has not been proven, but it is assumed that viral DNA is involved in the early stages of oncogenesis or acts as a cofactor in tumor formation along with UV light-induced carcinogenesis.

EV is inherited in an autosomal recessive mode. In about 75% of EV patients, two mutations in one of the two EV genes TMC6 (also known as EVER1) and TMC8 (also known as EVER2) on chromosome 17q25 have been described. The genes are orientated against each other on the complementary DNA strands and overlap about 1600 bp. Main products of both genes are 2.8 kb, but also two smaller splice variants exist. TMC6 and TMC8 belong to the transmembrane channel-like (TMC) gene family. Proteins of this family consist of 6 to 10 transmembrane domains and a conserved 120-amino-acid domain (TMC domain). Presumably TMC6 and TMC8 are localized in the endoplasmic reticulum and interact with zinc transporter 1 (ZnT-1).

We examined both TMC genes in seven patients, who present clinical EV. No typical mutation leading to a truncated protein could be found by direct sequencing. Both genes contain a lot of SNPs, and some of them result in an amino acid exchange but their influence to EV is unknown. Frequency of these SNPs in EV patients compared to the general population revealed a significant different distribution for some of them. We will discuss their distribution and a possible connection to the development of EV.

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## P-CancG-208

### Functional consequences of altered histone deacetylases inhibitors in hepatocellular carcinoma

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#### Introduction

Hepatocellular carcinoma (HCC) represents the fourth most common malignant tumour with more than 1 million persons affected worldwide per year.

Alteration of histone acetylation is a common hallmark of human tumour cells and strongly alters transcription of many genes involved in the control of proliferation, cell survival, differentiation and genetic stability. In previous studies, we have shown that histone deacetylases (HDAC1-3) are consistently upregulated in HCC. We therefore aim to understand the functional effects of altered HDAC1-3 expression during the development and progression of HCC.

#### Methods

Accordingly, we investigated the influence of histone deacetylation in extensively characterized HCC cell lines (Huh7, HepG2, HLE, HLF) and non-tumorigenic liver cell lines (THLE-2, THLE-3) by downregulation of HDAC1, HDAC2 and HDAC3 using siRNA. After downregulation of HDAC1-3 by siRNA we could measure proliferation by WST-1-Assay (Roche) as well as apoptosis by Caspase 3/7-Glow (Promega). The acetylation status was measured using Western Blot analysis or the Cyclex Cellular Histone Acetylation Kit (MBL).

#### Results

Our data indicated that treatment of HCC cell lines with siRNA against HDAC1, 2 and 3 in combination leads to increased apoptosis and acetylation whereas the proliferation decreases. Furthermore using Western Blot analysis we showed that the histone acetylation of Lys 5, 8, 12 and 16 of histone H4 is heightened.

#### Conclusion

SiRNA Knockdown of HDAC1, 2 and 3 leads to increased apoptosis and decreased proliferation in HCC cell lines caused by change in histone H4 acetylation. Since decreased acetylation is a hallmark of human cancer<sup>1</sup> our results lead us to the following conclusion: the increased acetylation induced by HDAC1, 2 and 3 inhibition and followed by a more loosely chromatin formation<sup>2</sup> ends up in an overexpression of genes involved in proliferation and apoptosis and thereby in tumor formation. To identify these epigenetic regulated tumor suppressor genes and we aim to perform microarrays of the siRNA treated cells.

#### References

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## P-CancG-209

### From gene to function: Towards a structure of the RAD51C protein

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RAD51C/RAD51L2 is one of five RAD51 paralogs (RAD51B/RAD51L1, RAD51D/RAD51L3, XRCC2 and XRCC3) which shares 20-30% amino acid homology with each other and the RAD51 recombinase, an ortholog of *E. coli* recA. All paralogs are known to be essential in a DNA repair process designated as homologous recombination (HR) for the error-free removal of DNA double-strand lesions. RAD51C functions by forming two heteromeric complexes with the other RAD51 paralogs, XRCC3-RAD51C and RAD51B-RAD51C-RAD51D-XRCC2. The importance of RAD51C protein arises in case of defects which cause several human diseases. For example monoallelic mutations in RAD51C have been reported to increase the risk of breast and ovarian cancer (Meindl et al, 2010) and biallelic mutations lead to Fanconi anemia of subtype FA-O (Vaz et al, 2010). To improve our understanding of human RAD51C function, our present studies are aimed at solving the RAD51C crystal structure. This will provide a high resolution protein structure that can be utilized to explore RAD51C on a molecular level, and thus provide important insights into its role during HR. The structure will help explaining how variants of RAD51C, like G125V, L138F are causing breast cancer and R258H, responsible for Fanconi anemia to a yet unparalleled detail. The RAD51C structure will also be used for in silico screening of suitable inhibitors. These may abrogate very specifically the removal of endogenous or therapeutically introduced DNA interstrand crosslinks as a potential application in tumor therapy. Here we describe a detailed heterologous expression analysis of RAD51C in two different expression systems - *E. coli* and insect cells. Using the *E. coli* system we analyzed different expression constructs with N- or C-terminal His-Tags, in several *E. coli* expression hosts under varying experimental conditions (temperature and time). The best expression could be achieved using a codon optimized RAD51C gene in a pETM11 vector and *E. coli* strain BL21 (DE3) CodonPlus-RIL as expression host. Subsequent biochemical characterization of the resulting protein revealed, however, that RAD51C could be only in higher oligomeric aggregates. In thermal unfolding experiments we could show that the purified RAD51C was not properly folded indicating *E. coli* might not serve as an appropriate expression host. As an alternative we will be pursuing insect cell expression in order to provide a eukaryotic expression host that might yield properly folded protein.

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## P-CancG-210

### **Molecular karyotyping and DNA methylation pattern analyses in primary fibroblasts of patients with childhood cancer**

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Malignancies in children are unlikely to be due to environmental hazards or unhealthy lifestyle. Instead, genetic factors may play a predominant role for most malignancies in children. Therefore it is plausible to assume that genetic and epigenetic changes are the primary cause for juvenile tumor development. In particular, modulation or dysregulation of DNA repair- and cell cycle-associated genes may contribute to cancer predisposition. With the help of the German Childhood Cancer Registry, we recruited in 2005-2006 a cohort of (1) 20 individuals who survived a childhood malignancy and then developed a second cancer and (2) 20 carefully matched controls who survived a childhood cancer without developing a second malignancy. Aiming to identify new candidate loci for cancer susceptibility factors we performed an Affymetrix Genome-Wide Human SNP Array 6.0 analysis and an Infinium Methylation 27k Bead Chip analysis to compare the genome-wide SNP DNA profiles and DNA methylation patterns of primary fibroblasts from two-cancer patients, one-cancer patients, and a pool of healthy controls. We developed a stepwise general and detailed analysis method for analyzing the numerous SNP data. After eliminating known CNVs without apparent impact in neoplasma development, we identified microdeletions and microduplications spanning several genes, variants within known tumor genes, variants within candidate genes or loci, and variants within genes or loci with yet unknown assignment. The results are now classified according to the tumor type and matched patient pairs and compared to the genome-wide methylation profiles. In addition, genomic Q-PCR, RT-PCR and bisulphite pyrosequencing experiments for a subset of conspicuous gene loci will be carried out to validate our data. Our results may reveal novel genes particularly involved in initial steps of tumorigenesis.

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## P-CancG-211

### **Genomewide analysis of paraffin embedded HER2-positive breast tumors by SNP-array**

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Amplification and overexpression of the ERBB2 (HER2) oncogene on chromosome 17q12 is found in approximately 15-25% of invasive breast cancer. HER2-positive tumours define a clinically important breast cancer subgroup accessible to therapeutically target the HER2 protein by monoclonal antibodies or tyrosine kinase inhibitors. Here we studied the feasibility of expanded genomewide screening by SNP-array on paraffin-embedded breast tumor tissue to identify additional recurrent aberrations potentially acting in HER2-positive tumors.

We investigated cases with positive HER2 IHC-score (2+ or 3+), known HER2/TOP2A-FISH status and an estimate of at least 70% tumor cells. SNP-array was performed on Illumina HumanCytoSNP-12 DNA Analysis BeadChips and analyzed using the Illumina iScan System with BeadStudio/Karyostudio Software.

Tumor tissues were considered as somatic mosaics with normal cells and the results were expected to reflect this. Hence, changes in LogR ratios (copy number alterations) should be accompanied by B-allele-frequency alterations (BAFA) with intermediate percentage. Vice versa, a BAFA without visible change in LogR ratio was interpreted as genomic imbalance, either reflecting a copy number change in only a fraction of the tumor cells or a copy-number neutral LOH. This combinatorial analysis of LogR ratio and BAFA largely circumvents the problem of reduced call rates of DNA from paraffin-embedded material. Thus, cases with call rates as low as 80% could be reliably analyzed. Regarding the HER2 gene locus, it could be roughly discriminated between tumors showing a distinct HER2 amplicon peak and tumors in which numerical or structural aberrations were approved. These findings corresponded well with the results of HER2/TOP2A-FISH. HER2-amplified cases frequently showed further aberrations of chromosome 17, especially additional amplifications in 17q proximal and/or distal to the HER2 amplicon and 17p-aberrations. Genomewide, a cumulative higher complexity of aberrations, accompanied by additional amplifications in a number of cases, was recognized along with an increasing number of involved chromosomes. Besides, 8p12, a region prone to amplification, seems to include a putative recurrent breakpoint.

SNP-array analysis can be successfully performed on paraffin-embedded tissue as shown here for HER2-positive breast tumor samples with only some restrictions. This allows the collection of additional data on upcoming aberrations in the course of tumor development in expanded patient collectives and might lead to the identification of changes affecting the therapeutic success.

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**P-CancG-212****Loss of the Y chromosome in MDS as clonal abnormality or age-related phenomenon in individual patients**

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It is still subject of debate whether loss of the Y chromosome in patients with myelodysplastic syndromes (MDS) is an age-related phenomenon or a clonal abnormality. Y loss is observed in 5% of MDS patients. Analyzing our multicentre, international DACH-, ICWG- and IMRAW-database we previously could show that the frequency of loss of the Y chromosome in MDS karyotypes increases with the age of the patients, supporting the hypothesis that -Y is age-related. A biological background of this phenomenon is supported by the fact that those patients with -Y as a single abnormality are significantly older at first diagnosis as MDS patients with other abnormalities. Although higher age is an adverse prognostic marker in MDS, the overall survival of MDS patients with -Y as single abnormality is significantly better than that of MDS patients with normal karyotype. The aim of this study was to evaluate if Y loss is an age- and/or MDS-associated phenomenon in a single MDS patient. Studying sequential karyotypes we observed -Y occurring during karyotype evolution. We also identified a patient with -Y at first diagnosis in a mosaic karyotype with normal cells that later developed additional aberrations in the cells with -Y during the course of the disease. These data strongly suggest that -Y is MDS-associated in these patients. To demonstrate that -Y is occurring in the clonal CD34+ cellular compartment as a somatically acquired event we enriched clonal CD34+ cells and CD3+ T-cells not belonging to the MDS clone from peripheral blood of twelve patients by immunomagnetic cell sorting. This allowed us to study loss of the Y chromosome in CD34+ and CD3+ cells separately using Fluorescence in situ hybridization (FISH) analysis. The percentage of cells with -Y was significantly increased in CD34+ cells compared to CD3+ cells (p<0.0001). It is not clear whether the low proportion of -Y in CD3+ cells that exceed our laboratory threshold in 7/12 cases only slightly is due to age related -Y in T-cells or to contamination of the CD3+ cells with clonal cells. However, we could confirm the clonal origin of -Y in patients where we found additional abnormalities in cells with -Y. The data of our study suggest a clonal nature of this karyotype anomaly at least in a subset of patients with MDS. In other patients this finding is clearly age-related. Identifying patients with non-age associated loss of the Y chromosome at early stages of the disease is of value in determining clonality and in predicting prognosis and treatment outcome.

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**P-CancG-213****SNP Array analysis on CD34+ progenitor cells of peripheral blood is feasible to detect additional abnormalities in MDS**

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Cytogenetic aberrations are important prognostic markers in myelodysplastic syndromes (MDS). Using conventional chromosomal banding analysis, aberrations are detectable in about 50% of patients. In addition to these aberrations, microdeletions/microamplifications and loss of heterozygosity (LOH), e.g. as a consequence of uniparental disomy (UPD), could have prognostic significance. Especially aberrations that are acquired during the course of the disease (karyotype evolution) could be associated with progression into higher stages of MDS. We aim to detect these newly acquired aberrations in sequential analyses "on time" using high resolution SNP arrays. To avoid repeated bone marrow biopsies, but to allow frequent monitoring, we started to perform SNP array analysis on circulating CD34+ cells of peripheral blood. Recently, we could show that the detection of chromosomal anomalies on these blood cells is possible by FISH analysis (Bräulke et al., 2010). In a pilot study we were able to perform SNP array analyses using pre-amplified DNA isolated from CD34+ cells from the peripheral blood of six patients and pre-amplified DNA isolated from CD34+ cells from the bone marrow of two patients. Three patients were analysed using the Affymetrix Cytogenetics Whole-Genome 2.7M array, two using the Affymetrix Genome-Wide Human SNP 6.0 array, and three using both arrays in parallel. We compared results from SNP array analyses with banding analysis and FISH and could demonstrate that for clone sizes >40% the method is feasible to detect the same chromosomal aberrations on CD34+ cells as observed on bone marrow cells by conventional banding analyses. We could also detect additional microdeletions not detected by chromosomal banding analysis: One patient showed a microdeletion (1.5 MB) in 15q15.1, in the genomic region containing RAD51 as a candidate gene. This microdeletion was detected by both arrays in parallel. Seedhouse et al. (2004) have previously shown that a polymorphism in the RAD51 gene promoter (135G-/C, 5' -UTR, rs1801320) is

implicated in t-AML risk. The risk to develop t-AML was significantly increased when a variant RAD51-135C allele was present (irrespective of other genotypes). Sequence analysis in the promoter region of our patient also showed the rare variant RAD51-135C allele. To determine whether the deletion in 15q15.1 is inherited or acquired, we have enriched CD3+ cells and perform SNP array analysis on this additional specimen. Sequencing of RAD51 in CD34+ and CD3+ cells are in progress. Our results show that SNP array analyses of circulating CD34+ cells is a feasible method to gain more information about the cytogenetic course of the disease, karyotype evolution, rare abnormalities, their clinical and prognostic impact and biologic significance of common microdeletions/microamplifications.

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#### **P-CancG-214**

### **Pyrosequencing based methylation analysis of the CHD2, GNG7, IRF4 and SYK genes in classical Hodgkin lymphoma cell lines**

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By analyzing classical Hodgkin lymphoma (cHL) cell lines with combined high resolution array-CGH and expression profiling we recently identified CHD2 and GNG7 as potential tumor suppressor gene candidates in cHL. As promoter hypermethylation is besides mutation and deletion a common alteration leading to inactivation of tumor suppressor genes we performed pyrosequencing based methylation analysis of the promoter regions of these candidates. Moreover, two other genes were included in the study: the SYK gene that was recently reported to be silenced in cHL due to the loss of the B-cell identity of Hodgkin and Reed-Sternberg (HRS) cells and the IRF4, a B-cell gene, known to be expressed in cHL.

Bisulfite treated DNA from seven cHL cell lines (L428, HDLM2, KMH2, L1236, SUPHD1, UHO1, L540) served as template for PCR amplification of CpG islands that overlapped or were located in close vicinity of the transcription starting sites of the genes. Methylation levels in the following CpG islands have been analyzed: CpG island at chr15:91227156-91227755 bp and CpG island at chr15:91248561-91248827 bp for CHD2; CpG island at chr19:2653533-2653987 bp for GNG7; CpG island at chr6:336189-338790 bp for IRF4 and CpG island at chr9:92603597-92604367 bp for SYK. Bisulfite treated commercial totally methylated DNA and pooled DNA from peripheral blood samples served as controls.

No hypermethylation was detected for the CHD2 and IRF4 genes indicating that the genes are not affected by promoter hypermethylation. In contrast, recurrent hypermethylation was identified in the SYK promoter region in all 7/7 analyzed cHL cell lines. In case of GNG7, hypermethylation of the promoter region was identified in the 3/7 cell lines namely the L428, KMH2 and L540. Together with its homozygous deletion in HDLM2 we detected alterations of the GNG7 gene in 4/7 cHL cell lines.

Our results confirm the previously published findings of recurrent hypermethylation of SYK in cHL demonstrating that epigenetic silencing contributes to the observed loss of B-cell identity of HRS cells. We detected also no hypermethylation for the IRF4 gene with retained expression in cHL. Interestingly, we observed a recurrent hypermethylation of the GNG7 gene. GNG7 encodes the guanine nucleotide binding protein 7, a G protein with GTPase activity whose role in carcinogenesis is poorly understood. It has been speculated that G proteins may be involved in cell contact induced growth arrest and function as tumor suppressor genes. In line with this hypothesis, downregulation of GNG7 has been associated with bad prognosis and shorter survival in pancreatic and gastrointestinal tumors.

In summary, our results confirm that the SYK gene is recurrently hypermethylated in cHL and indicate that the recurrent transcriptional inactivation of GNG7 detected in cHL and other human neoplasms is to some extent of epigenetic origin.

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#### **P-CancG-215**

### **Is there a risk for BRCA1 mutation carriers by the frequent use of mammography?**

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Women carrying a BRCA1 mutation have an increased breast cancer risk. Therefore, an intensive surveillance program is recommended including annual mammography by the age of 30 years. Due to a

reduced repair capacity for DNA double strand breaks mutation carriers can accumulate chromosomal damages after radiation in therapeutic dosages (~25x2 Gy). Our question was, whether this increased radiosensitivity is detectable after mammography (comparable to 0.005 Gy), too. To evaluate the effect of therapeutic and diagnostic radiation we analysed the percentage of aberrant metaphases in lymphocyte cultures of 19 BRCA1 mutation carriers after exposure to 2, 4 and 6 Gy, and after mammography. Aberration rates were estimated by means of chromosome banding analysis (n=10) and fluorescence in situ hybridization (FISH) using whole chromosome painting probes (WCP) for chromosomes 1, 2 and 4 (n=19). The results in BRCA1 mutation carriers were compared to identically irradiated age-matched controls (seven healthy women without family history of breast/ovarian cancer) and to unirradiated lymphocytes of each proband.

After radiation with 0 Gy, 2 Gy, 4 Gy and 6 Gy, and after mammography the mean percentage of aberrant metaphases detected by chromosome banding analysis was 11%, 53%, 79%, 87%, and 16%, respectively, in mutation carriers but only 6%, 43%, 62%, 82%, and 8%, respectively, in controls. By means of WCP-FISH the mean percentage of aberrant metaphases after 0 Gy and 4 Gy was 6% and 44% in mutation carriers but only 2% and 29% in controls.

Thus lymphocytes of BRCA1 mutation carriers showed significantly more aberrant metaphases compared to non carriers after radiation with different therapeutic and diagnostic dosages. Moreover, these results suggest that the increased radiosensitivity of BRCA1 mutation carriers is measurable after mammography by means of chromosome banding analysis as well as WCP FISH despite the low dosage.

Supported by the German Cancer Aid

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## **P-CancG-216**

### **DNA methylation mapping of the DLK1 locus in 14q32**

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Human chromosome 14q32.2 carries cluster of protein-coding paternally expressed genes (PEGs), such as DLK1 and non-coding maternally expressed genes (MEGs), for example MEG3 (also known as GTL2). Patients with Temple Syndrome due to constitutional deletion in 14q32 pat or UPD14 mat show hypomethylation in the differentially methylated region (DMR) of MEG3. In addition to constitutional changes, somatic deletion del(14)(q24q32) is also a recurrent event e.g. in B-cell lymphomas. Moreover, Khoury et al. (Blood, 2010) recently described that an insulator located 18kb upstream of DLK1 plays an important role in regulating DLK1 imprinting and, in addition, showed loss of imprinting in acute myeloid leukemia (AML). To characterize constitutional and somatic changes at the imprinted region in 14q32 we here investigated 101 CpGs at this site in controls (n=20), patients with Temple Syndrome (n=3), lymphomas with del(14)(q24q32) (n=31) and lymphoma cell lines (n=13). To this end, we established 15 bisulfite pyrosequencing assays targeting the MEG3-DMR locus, the CpG-island upstream of DLK1 and a CpG island located within the gene DLK1, respectively. The range of "normal" DNA methylation for each individual CpG was determined in DNA isolated from each 10 peripheral blood samples from healthy males and females. Pooled peripheral blood DNA from 20 healthy donors (10 males and 10 females) and enzymatically methylated DNA acted as controls in bisulfite pyrosequencing. Whereas patients with Temple Syndrome showed hypomethylation only in the MEG3 DMR, the tumor samples displayed a complex and heterogenous pattern of DNA methylation changes. Similar to the results reported by Khoury et al., we observed recurrent gain of methylation at the CpG-island upstream of DLK1 in the lymphoma samples and lymphoma cell lines. Nevertheless, at the MEG3 DMR we observed both gain and loss of DNA-methylation in lymphomas with del(14). This suggests that the parental methylation status is maintained in these lymphomas what contrasts the uniform gain of methylation in AML described previously. Our results suggest constitutional and somatic DNA methylation aberrations to differ at the 14q32 imprinted region.

This study is supported by the Imprinting Network, BMBF

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## P-CancG-217

### **Genomic instability in colorectal adenomas is associated with multiple oncogene amplifications and predicts adenoma recurrence and synchronous carcinoma**

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Individual colorectal adenomas have different propensities to progress to invasive disease. Here, we explored if these differences could be explained by gene copy number alterations. We evaluated 18 adenomas of patients without synchronous or subsequent carcinoma (6.5 years follow up), 23 adenomas of carcinoma patients and six related carcinomas. All samples were measured for their DNA ploidy status. Centromere probes for chromosome 17 and 18 as well as gene specific probes for SMAD7, EGFR, NCOA3, TP53, MYC, and RAB20 were assessed by multi-colour fluorescence in-situ hybridization. An increased genomic instability index of CEP17, SMAD7 and EGFR as well as TP53 deletions and MYC amplifications defined adenomas of patients with synchronous carcinoma ( $p < 0.05$ ). Diploid NCOA3 signal counts were associated with longer adenoma-recurrence free surveillance ( $p = 0.042$ ). In addition, NCOA3-, MYC-, EGFR- and RAB20- amplifications as well as TP53 deletions correlated with increased DNA stem line values and/or aneuploidy in adenomas ( $p < 0.05$ ). Aberrations of NCOA3, MYC, and RAB20 were furthermore associated with histopathologically defined high-risk adenomas ( $p < 0.05$ ). RAB20 amplifications were also correlated with high-grade dysplastic adenomas ( $p = 0.002$ ). We conclude that genomic instability in colorectal adenomas is reflected by EGFR-, MYC-, NCOA3-, and RAB20-amplifications that do correlate with histomorphological features and are indicative for adenoma recurrence and the presence of synchronous carcinomas.

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## P-CancG-218

### **Screening of childhood cancer and familial breast cancer patients for constitutional epimutations in tumor suppressor genes reveals a constitutional BRCA1 epimutation in a discordant monozygotic twin with childhood cancer**

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In Western societies the most common disease related cause of death in children is cancer. Leukemias and cancers of the brain and central nervous system account for more than 50% of childhood cancer, whereas breast, lung and colon cancers predominate in adults. There is growing evidence that in addition to classic DNA sequence mutations constitutional epigenetic alterations in tumor suppressor genes can predispose to cancer. Promoter methylation can lead to transcriptional silencing of an otherwise intact allele (first hit) in all or a subset of somatic cells. Constitutional epimutations originate either in the germline or during early embryonic development. By bisulfite pyrosequencing we screened primary fibroblasts of 22 childhood cancer patients for constitutional epimutations in several tumor suppressor genes (BRCA1, BRCA2, RAD51C, PTEN, TP53, MLH1 and ATM). One female patient with acute lymphatic leukemia in childhood and then thyroid cancer displayed a significantly increased BRCA1 methylation (12%). Subsequent bisulfite plasmid sequencing demonstrated that 13% (of 50 analyzed) BRCA1 alleles were hypermethylated (at the majority of analyzed CpGs), resulting in gene silencing. Interestingly, this patient had a healthy monozygotic twin sister who did not show aberrant methylation in BRCA1 or any other analyzed gene. Evidently, the BRCA1 epimutation in the cancer twin arose postzygotically, most likely predisposing to childhood cancer. In order to define the role of constitutional epimutations in hereditary breast cancer, we analyzed the promoter methylation of BRCA1, BRCA2, RAD51C and other tumor suppressor genes in blood cells of 99 patients with familial breast cancer and no detectable DNA sequence mutation in BRCA1/BRCA2. Moreover, we studied 22 females from breast cancer families with known mutations and intrafamilial variation in age of disease onset. All analyzed breast cancer patients showed normal methylation values (1-6%) at BRCA1, BRCA2, and other tumor suppressor genes. Our results suggest that constitutional epimutations in tumor suppressor genes do exist but may account for only a small subset of childhood and/or hereditary cancer patients.

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**P-CancG-219****Functional analyses of unclassified variants in the human MMR protein hMLH1**

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The HNPCC-Syndrom (Hereditary Nonpolyposis Colorectal Cancer) is associated with germline mutations in the mismatch repair genes hMLH1, hMSH2, hMLH3, hMSH6, hPMS2 and hPMS1. Beside clear pathogenic mutations, caused by stop or frameshift as well as splice mutations, many missense mutations are found, whose function usually remains unclear. The ability to distinguish between a loss-of-function-mutation and a silent polymorphism is important for inherited diseases like HNPCC, where the opportunity exists for early diagnosis and preventive interventions. In the database of the German HNPCC consortium all mutations, found in hMSH2, hMLH1, and hMSH6 in German patients, are registered. Many of the mutations are missense variants of undefined pathogenicity (unclassified variants, UV).

In previous studies we analysed 23 missense variants in the hMLH1 gene registered in the German database of HNPCC patients, to determine the functional significance of amino acid replacements. Seven of these UVs mapped to the ATPase-domain and 16 to the protein interaction domain of the hMLH1-protein. In addition we used 2 non functional controls described by Kondo et al. 2003. These were studied in the yeast *S. cerevisiae* using two different functional assays. First we investigated the ability of hMLH1 to interact with its interaction partner hPMS2. Furthermore we analysed the potential of hMLH1 to interfere with the yeast mismatch repair system. After these studies 13 of the missense variants were suggested to be pathogenic, four were classified as normal and six missense variants remain unclear in its characterisation.

To obtain further information about the pathogenicity of these and untested variants we constructed RFP(red fluorescence protein)-hMLH1 fusion proteins in a eukaryotic expression vector, which can be expressed in human cells. These missense variants were first tested for their stability in human cells (HEK 293T). In the next step we analysed the subcellular localisation of the hMLH1 wild type protein and the variants. Furthermore the colocalisation of the hMLH1-variants with a hPMS2-GFP fusion protein in HEK 293T cells is an indication of their normal protein-protein interaction. Therefore the cotransfection of hMLH1 variants labelled in red and hPMS2 labelled with a GFP tag and immunofluorescence analysis indicates whether a functional interaction took place. We are currently studying the previously in yeast analysed 23 missense variants as well as new variants not studied before in any assay. These studies will provide additional information on the functionality of these missense variants.

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**P-CancG-220****High- resolution genomic profiling and classical cytogenetics in patients with benign or atypical meningiomas**

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According to the World Health Organization meningiomas are classified as benign, atypical and anaplastic meningiomas. While the majority of meningiomas are sporadic solitary benign tumors with a good chance for a total surgical treatment and relatively optimistic outcome, prognosis for patients with atypical and anaplastic meningiomas remains poor. High- resolution genomic profiling revealed more information about meningiomas. Therefore, we analyzed genomic aberrations of benign and atypical meningiomas using SNP-A technique, combined with GTG-banding, spectral karyotyping, and locus-specific FISH. We could confirm frequently detected chromosomal aberrations, and identified novel genetic events. Applying SNP-A analyses we could identify de novo loss/gains within chromosome 22 in normal control DNA and tumor cells in three cases. Copy neutral LOH regions 4p16.1, 7q31.2, 8p23.2, and 9p22.1 were found de novo in primary meningioma cells. Using GTG-banding and spectral karyotyping, we detected a novel balanced reciprocal translocation t(4;10)(q12;q26) in a benign meningioma. The previously described novel paracentric inversion within 1p36 was detectable as a recurrent chromosomal aberration in benign and atypical meningiomas. Advances in acquiring SNP array data on an increasing number of tumors and matched normal samples will help further elucidate the role of UPD in neoplasms.

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## P-CancG-221

### Copy number variation analysis in 134 unrelated patients with mutation negative adenomatous polyposis.

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Background: Adenomatous polyposis syndromes are characterised by multiple colorectal adenomas and a high lifetime risk of colorectal cancer. Germline mutations in two genes are involved in different forms: the autosomal dominant Familial Adenomatous Polyposis (FAP) is caused by mutations in the tumor suppressor gene APC on chromosome 5q22 and the autosomal recessive MUTYH-associated Polyposis (MAP) by mutations in the base excision repair gene MUTYH on chromosome 1p34.1. However, in up to 50% of families no germline mutation could be identified although a genetic cause is likely. Copy number variants (CNVs) have recently been recognised as important forms of structural variation which also predispose to human disease. In particular, heterozygous microdeletions contribute significantly to the mutation spectrum of hereditary tumor syndromes and thus it could be hypothesised that those heterozygous deletion CNVs might also be the underlying cause in yet unidentified genes responsible for adenomatous polyposis syndromes. Methods: Genomic DNA from 134 unrelated mutation negative patients with clinically verified adenomatous polyposis was used for genome-wide SNP genotyping with the HumanOmni1-Quad BeadArray (Illumina). Putative CNVs were identified by the QuantiSNP v2.2 algorithm. To search for rare, non-polymorphic deletions, the CNVs were filtered according to various criteria by use of the Cartagenia Bench™ software, by in-silico analysis, and by comparison with 531 healthy controls. Results: In the 134 patients we identified 54 unique heterozygous deletions between 10 kb and 1 Mb which were not present in the healthy controls and which contain 79 protein coding genes. The vast majority of CNVs was found in one patient only, no CNV was presented in more than 3 patients (2%). So far, 25 out of 34 examined CNVs could be verified by quantitative PCR (qPCR). Interestingly, the CNVs include four predicted tumor suppressor genes (TSG) and three more genes which play important roles in cancer development. Conclusion: By applying stringent filter criteria we identified a group of rare deletion CNVs which might contain predisposing genes or susceptibility loci for adenoma formation including TSGs. Further work will include complete CNV validation by qPCR, screening for duplications and homozygous deletions and subsequent gene prioritisation according to gene function and pathway involvement. The most promising candidate genes will be sequenced in all patients to search for pathogenic germline point mutations. The study was supported by the German Cancer Aid (Deutsche Krebshilfe).

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## P-CancG-222

### The proportion of clonal plasma cells in bone marrow versus single chromosomal abnormalities – predicting progression from smouldering to symptomatic multiple myeloma

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Smouldering MM (sMM) is a plasma cell disorder defined by the presence of  $\geq 10\%$  plasma cells in bone marrow and/or a monoclonal protein level of  $\geq 3$  g/dl in serum without organ damage. The aim of this retrospective study was to analyze whether genomic abnormalities confer prognostic information in patients with sMM who are at high risk of progression into symptomatic MM. We analyzed the prognostic value of 12 chromosomal abnormalities using fluorescence in situ hybridization (FISH) in a series of 200 sMM patients. Hyper-/non-hyperdiploidy (HD/NHD) was defined by absence/presence of gains of chromosomes 5, 9, and 15. In addition, the percentage of clonal plasma cells (cPC) in the bone marrow is based upon the largest percentage of existing chromosomal aberrations.

Interphase-FISH-analysis on CD138-enriched plasma cells detected gains of chromosomes 1q21 (31%), 5p15/5q35 (35%), 9q34 (45%), 11q23 (41%), 15q22 (40%), and 19q13 (41%), as well as deletions of chromosomes 8p21 (9%), 13q14 (37%), and 17p13 (7%). Furthermore, the IgH translocations t(11;14), t(4;14), t(14;16), and IgH translocations with unknown partner were observed in a frequency of 24%, 10%, 5%, and 22%, respectively. The median percentage of cPC was 85.5 (range: 62 – 95).

For the entire group, the median follow-up time was 27.2 months (range: 18.2 - 33.5). We analyzed the prognostic impact of each chromosomal aberration on time to progression (TTP). Of all chromosomal

abnormalities analyzed, only deletion 8p21 and the percentage of cPC showed a significant impact on TTP. The TTP at 3 years for patients with deletion 8p21 was 53% (versus 73% for those without;  $p=0.01$ ). A 10% increase of cPC in the bone marrow was associated with an elevated risk to develop a symptomatic MM of 33% (unadjusted  $p<0.001$ ). After adjustment of  $p$ -values for multiple testing, only the percentage of cPC showed a statistically significant impact on TTP ( $p=0.02$ ).

Our results show that FISH-analysis on CD138-enriched plasma cells is a useful technique in the study of sMM, because it allows myelomatous plasma cells to be discriminated from their normal counterparts. In addition, our findings suggest that the proportion of cPC (analyzed by FISH) rather than single chromosomal abnormalities predict progression from sMM to symptomatic MM. FISH-based information can be obtained easily at the time of diagnosis, which would help to establish an individually adapted follow-up strategy.

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## P-CancG-223

### Analysis of frequency ERCC2 gene polymorphism (rs3916891) in Polish patients with differentiated thyroid cancer

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Thyroid carcinomas are the most often carcinomas of endocrine system with growing up frequency. The most often occurs papillary and follicular thyroid cancer, which belong to group of tumors with well prognosis, 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 polymorphism c.\*171G>C (rs3916891) in ERCC2 gene. Groups of 280 patients with differentiated thyroid cancer and 320 individuals from population group were examined. Sequence variants were determined by pyrosequencing.

There were no observed differences in allele and genotype frequencies in patient with differentiated thyroid cancer and population group. Allele C was present with frequency 0,04 and allele G with frequency 0,96.

The differences were observed when considerate men and women separately. Allele C in males with DTC was observed with smaller frequency comparing with females population control. In patient group we did not regard frequencies in these groups, because of much higher frequency of occurring differentiated thyroid cancer in woman.

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## P-CancG-224

### VHL and PTEN act cooperatively in tumour suppression

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Germline mutations in the von Hippel-Lindau (VHL) tumour suppressor predispose to retinal and CNS haemangioblastomas (HBs), pheochromocytomas (PCCs) and clear cell renal carcinomas (ccRCC). The established physiological function of VHL is that of a ubiquitin E3 ligase adaptor for HIF $\alpha$  subunits, but a role in many other cellular processes, like microtubule formation, and cilia assembly, was described. How VHL acts as a tumour suppressor remains unknown. To date, knockout models of VHL disease failed to show relevant endogenous tumour formation in vivo. Thus, loss of Vhl function alone is not sufficient to initiate tumorigenesis in mice. Intriguingly, premalignant cystic lesions of VHL patients exhibit a strong activation of the phosphoinositide-3 kinase (PI3K) pathway and downstream mammalian target of rapamycin (mTOR) signalling. PTEN, a negative regulator of the PI3K pathway, is one of the most frequently inactivated tumour suppressors in sporadic human malignancies. As even a subtle reduction of Pten dosage in mice initiates tumour formation in a tissue-specific manner, and PCCs are part of the Pten-associated tumour spectrum, we hypothesized that Vhl and Pten may interact functionally in tumour suppression.

Here, we present Vhl knockin mouse models with endogenous tumour formation recapitulating part of human VHL disease. VHL syndrome subtype-specific mutations associated with PCC (VHL type II disease) were introduced into the Vhl locus of mice, and the natural cause of Vhl type IIB (V2B) and Type IIC (V2C) germline mutations was studied over several generations. Furthermore, Vhl knockin mutations were also combined with hemizygous inactivation of Pten (Pten+/-), and the influence on tumour spectrum, incidence, and tumour progression was analysed in over 200 mice of various genotypes at the ages of 3 to 12 months. As previously described, Pten +/- mice developed various tumours at 9 and 12 months of age, like lymphomas and ovarian carcinomas, independent of the Vhl genotype. However, there was a clear Vhl genotype-dependent effect on the development of kidney hypertrophy and cystic lesions in V2B compound animals. Furthermore, both incidence and tumour mass of PCCs was significantly increased in V2B, and V2C, compound heterozygous mice, in accordance with tissue-specific action of these germline mutations observed in the human. We have thus established relevant model systems of VHL disease, and provide direct genetic evidence for a cooperative action of both tumour suppressors in the pathogenesis of PCCs. Detailed analyses of different tumour stages will help to unravel the underlying molecular mechanism(s) of this interaction.

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## P-CancG-225

### A patient with MDS and two clones with different deletions in 5q: a case report

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal bone marrow disorders which lead to an ineffective haematopoiesis with peripheral cytopenias and a risk for progression to an acute leukemia. Chromosomal aberrations occur in 50 – 80% of patients with MDS and play a major part in pathogenesis, prognosis and therapy. One of the most frequent chromosomal aberrations in MDS is a deletion of the long arm of chromosome 5, also known as 5q- syndrome. This clinical entity is characterized by an interstitial deletion within chromosome 5 as sole karyotype abnormality and is associated with refractory anemia, abnormalities in the megakaryocytic lineage and low risk for leukemic transformation. In MDS clonal genetic instability and regional instability in the long arm of chromosome 5 concerning the regions q22 and q31 are well known features.

Here we present a patient with MDS and two clones with different deletions in 5q detected by GTG-banding and Fluorescence In Situ Hybridisation (FISH) analysis. Based on the chromosome analysis we assumed two different cell clones. A reliable distinction was not possible because the aberrations were hardly visible. With the following FISH two different deletions were detected: del(5)(q31q33) and del(5)(q33q?33). To our knowledge four cases with two 5q- clones with different deletions are found in literature. This case is a further example for genetic instability of chromosome in MDS.

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## P-CancG-226

### Differentiation of Westphal variant of Huntington's disease and juvenile Parkinson's disease

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**INTRODUCTION:** The Westphal variant as the juvenile type of Huntington's disease, onset before 20 years, is not characterized by choreatic movements. Contrary to Huntington's chorea, stiffening and loss of movements, rigidity, akinesia and mutism are the main symptoms. These symptoms recall the Parkinson's disease which is characterized also by bradykinesia, rigidity, tremor, dystonia, and gait disturbances. However, cognitive impairment and development of dementia are constant signs in the Huntington's disease.

**CASE REPORT:** We report about a 44-years-old female patient with gait disturbances (atactic-athetotic-choreatic) and a progressive movement disorder beginning in the late teens. Neuropsychological testing reveals a mild organic brain syndrome and ongoing dementia. Application of L-Dopa shows advanced movements. Chronic neurogenic changes were seen in neurophysiologic investigations. MRI imaging of the brain was normal. In the family history dementia of her father (died at an age of 65 years), father's sister (died in her 8th decade) and father's cousin were noticed.

**MOLECULAR GENETICS:** DNA analysis of the HTT gene for Huntington's disease revealed repeated numbers within the normal range (15 and 16 CAG). However, in the PARK2 gene two mutations have been identified: 1) the frameshift mutation c.622insA (p.F208fsX211) and 2) a gross genomic deletion affecting exon 3. Testing relatives revealed the frameshift mutation for the mother and the exon 3-deletion for both

sisters proving the compound heterozygous nature of the two mutations for the patient. These results exclude Huntington's disease and confirm the diagnosis of Parkinson's disease 2, the autosomal recessive juvenile form (OMIM 600116).

**CONCLUSIONS:** There are overlapping symptoms in both, juvenile Westphal variant of Huntington's disease and juvenile Parkinson's disease. The differences may be the non- or late and slow development of dementia and response to L-Dopa in juvenile Parkinson's disease. However, gene analysis should be done to exclude one and confirm the other form of juvenile bradykinesia.

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## **P-CancG-227**

### **Non-random transmission of mutant alleles to female offspring of BRCA1 and BRCA2-carriers?**

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**Background:** Mechanisms such as meiotic drive, in utero selection against deleterious mutations or maternal-fetal incompatibility have been implicated in observations of transmission distortion of Mendelian loci and traits (e.g. Zöllner et al, Am J Hum Genet 74, 2004; Imboden et al, NEJM 355, 2006). Since there a number of (controversial) reports suggesting a skewed ratio of maternal transmission of mutant alleles to female offspring of BRCA1 mutation carriers, we analyzed the transmission of mutant alleles in a small series of BRCA1 and BRCA2 maternal carriers.

**Patients and methods:**

Maternal and paternal carriers were ascertained from families with a history of breast and/or ovarian cancer who were referred for genetic counseling. Offspring of carrier mothers and fathers were tested for mutation status. We assumed that 50% of the unknown (persons who were not tested) are carriers.

**Results:**

BRCA1: among a total of 62 daughters, there were 36 carriers, 13 non-carriers and 13 unknown (carrier = 42,5; non-carrier = 19,5;  $\text{Chi}^2 = 7,8065$ ;  $p = 0,0052$ ).

BRCA2: among a total of 76 daughters, there were 48 carriers, 6 non-carriers and 22 unknown (carrier = 88; non-carrier = 22;  $\text{Chi}^2 = 38,409$ ;  $p = 0,0000$ ).

**Conclusion:** Given the low frequency of BRCA mutant alleles in the general population, there are no prospective population-based studies of allele transmission which would avoid various types of bias which are the likely explanation for a putative transmission distortion such as reported here for a small cohort of BRCA carriers.

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## **P-CancG-228**

### **Analyzing driving forces for BWS and Wilms tumors**

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Beckwith–Wiedemann syndrome (BWS) is a complex overgrowth syndrome with variable phenotypic appearance and increased risk to develop embryonic/early-childhood tumors like Wilms tumor (WT). While only a small proportion of BWS patients show germ line mutations in the CDKN1C gene or translocations within the KCNQ1 gene, all other aberrations are epigenetic mutations. Yet despite their difference all defects seem to be able to cause a full spectrum of phenotypic characteristics of BWS, with the exception that to date no BWS with WT and CDKN1C mutation has been reported. If the character of individual (epi-) mutations in BWS patients designate some BWS patients do develop tumors, it is intriguing to speculate that the relevant (epi-)genetic alterations result in transcriptionally "translated" driving forces for tumorigenesis. The main candidate for such a genetic driving force is the fetal growth factor IGF2 that displays its main effects during embryonic development and is found with an increased dosage in the majority of sporadic BWS cases. Tumor cells differ from their original cells by having acquired alterations in their cell cycle, which is influenced by different kinase signaling pathways like Akt or Erk1/2. Increased Akt signaling has a variety of effects including inhibition of death promoting effects of wild type p53 thereby rescuing cells from apoptosis. Thus a critical IGF2 threshold could trigger tumorigenesis by critically altering the relevant pathway. Since WTs originate from persisting embryonic cells, we utilized a human embryonic kidney cell line (HEK293) to test this idea. We assessed the integrity of the relevant Akt-mTOR pathway in HEK293 cells, in fibroblasts from BWS individuals and in isolated WTs. These cells most likely have the cellular signaling equipment to test the effects of putative pathogenic driving forces. In addition we genetically

engineered the HEK293 cells to overexpress the IGF2 isoforms that are hyperactivated by epimutations and analyzed for IGF2 dosage and activation of Akt/mTOR and Erk1/2.

The results of these experiments indicate that IGF2 stimulates Akt activation in nephroblasts and therefore may be the pre-disposing factor for tumorigenic growth and also may explain why BWS patients with LOI of IGF2 nevertheless have normal IGF2 serum levels.

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## P-CancG-229

### IRF4 polymorphisms and B-Cell Lymphoma risk

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IRF4 (interferon regulatory factor 4) gene on 6p25 is a transcription factor important for hematopoietic development and immune processes, being a key regulator of lymphocyte development and proliferation. Recent genome wide association studies in chronic lymphocytic leukemia (CLL) have shown that single nucleotide polymorphisms (SNPs) in IRF4 influence CLL risk.

In order to validate the association between the IRF4 locus and CLL, we genotyped two SNPs located in the 3'UTR region of IRF4 (rs872071 and rs4959853) in a series of 601 CLL cases and 633 controls from Spain. In this population we confirmed the previously reported association for the SNP rs872071 (OR=1.26, 95%CI:1.08-1.48; adjusted P-value=0.02). Similarly, the analysis of these SNPs in an independent cohort of 97 CLL patients from a single center in the United Kingdom (as compared to the Spanish controls) revealed a significant association of both SNPs with CLL (rs872071, OR=1.54, 95%CI:1.12-2.11, adjusted P-value<0.001; rs4959853, OR=2.05, 95%CI:1.25-3.37, adjusted P-value=0.01).

To explore the relationship between IRF4 and B-cell lymphomas/leukemias others than CLL, we analyzed the frequencies of these SNPs in 390 samples of several subtypes of cytogenetically characterized Non-Hodgkin Lymphomas. Remarkably, Follicular Lymphoma in children was also associated with a high frequency of the rs872071 risk allele (G=0.61), whereas a depletion of the risk allele was observed in lymphomas with a somatic translocation affecting the IRF4 locus (G=0.34). However, these results were not yet significant due to the limited size of these groups.

Our data validate the relationship between common polymorphisms in the IRF4 locus and risk for CLL and, moreover, suggest that inherited variation in B-cell developmental genes might vary between different genetic subtypes of B-cell lymphoma.

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## P-CancG-230

### Multiple recurring gene rearrangements in osteosarcoma cell lines as revealed by molecular karyotyping

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Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. About 70% of the tumors show a multitude of chromosomal aberrations and highly complex karyotypes. Several recurring gains and losses of chromosomal regions involving well known oncogenes and tumor suppressor genes, respectively, have been identified. But, so far, no recurring chromosomal translocations or gene rearrangements have been detected. In a previous study of OS cell lines we combined chromosome banding analysis with multicolor karyotyping to increase the resolution of the metaphase chromosome analysis, but failed to detect recurring chromosomal changes apart from genomic gains and losses. Therefore, we included comparative genomic hybridization with DNA microarrays (aCGH, 44k, Agilent) to determine the breakpoints of the genomic imbalances more precisely in 19 OS cell lines. A total number of 4266 breakage events were counted. 22% of the breaks were located in or close to centromeric regions. A non-random distribution of the breakpoints was observed with the chromosome arms 6p, 8q, 9p, 11p, 11q, 12p, 13q, 14q, 17p, 17q, 18p, 19p, 19q, 20q and 21q being affected more frequently than expected. 1628 (38%) of the breaks were observed in two or more cell lines and, therefore, considered as recurrent breakpoints. These recurrent breakpoints led to rearrangements of a total of 397 different genes as revealed by a shift of the fluorescence ratio within the respective genes. Among these, 57 genes were identified, which play a role in tumor genesis and progression, function as a tumor suppressor, or are involved in cell growth and proliferation, bone development or organization of the mitosis apparatus. Thus, oncogenic fusion

genes, oncogene activation or tumor suppressor gene inactivation may result from these recurring rearrangements. Detailed analyses of the breakpoints and chromosomal imbalances using high resolution arrays and array painting with flow sorted or microdissected chromosomes, next-generation-sequencing of target chromosomes, in combination with gene expression analyses may help to clarify the genetic mechanisms underlying the recurring gene rearrangements in OS.

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## **P-CancG-231**

### **Establishment of a stable cell line with constitutionally down-regulated HMGA2 expression**

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The High Mobility Group A2 (HMGA2) protein is an oncofoetal protein that is expressed during embryogenesis and shows increased expression on the mRNA and protein levels in several types of cancer such as leukaemias, in a variety of malignant tumours as well as in benign mesenchymal tumours as e.g. pulmonary chondroid hamartomas, uterine leiomyomas, and lipomas. HMGA2 usually cannot be detected in fully differentiated tissues unless with highly sensitive methods like qRT-PCR. This points to a pivotal role in the development of tumours and an involvement of HMGA2 in neoplastic transformation that also seems to be true in other mammals like the dog (*Canis lupus familiaris*). Dogs also develop spontaneous tumours such as prostate carcinomas that show a high grade of HMGA2 expression. Hence they represent a good model organism for the development of cancer and the contribution of HMGA proteins to tumourigenesis, especially as dogs often live under the same conditions as their human owners. Here we describe the transfection of a spontaneously immortalised cell line derived from a canine prostate carcinoma (CT1258) with a vector coding for a microRNA that is directed against HMGA2. This miRNA is co-cistronically expressed with GFP thus allowing the tracking of the miRNA expression with a fluorescence microscope. Additionally a gene promoting the resistance against Blastidicin allowed for the positive selection of transfected clones and the creation of stable cell lines with a constitutional reduction (52%-56%) of the HMGA2 expression level compared to the wild-type cell line. These cell lines can thus provide valuable insights into the role of HMGA2 in the development and growth of tumours.

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## **P-CancG-232**

### **Absence of TP53 mutations in patients with BRCA1/2-negative early-onset breast cancer or a significant family history**

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#### Introduction

Germline TP53 mutations cause a rare and highly penetrant cancer predisposition syndrome known as Li-Fraumeni-Syndrome (LFS), characterized by a variety of early onset tumours. Breast cancer is part of the Li-Fraumeni tumour spectrum. We aimed to clarify the role of TP53 germline mutations as a potential cause of early onset hereditary breast cancer.

#### Material and Methods

DNA samples from blood leukocytes of 62 BRCA1/2-negative women from families fulfilling the criteria for hereditary breast and ovarian cancer were investigated. Among them, 22 women had early onset breast cancer, diagnosed at or before the age of 34 years (range 22 to 34 years). 31 women fulfilled Chompret (8) Li-Fraumeni criteria or Eeles/Birch (23) Li-Fraumeni-like criteria. Furthermore, seven cases of very early onset tumours, among them tongue cancer (age 17), ovarian cancer (age 33), cervical cancer (in both twins at age 31), and colon cancer (age 25, FAP and HNPCC excluded), were analysed. The entire coding region and surrounding intronic regions of the TP53 gene were investigated via direct sequencing. Screening for large genomic rearrangements in the TP53 gene was performed using MLPA (SALSA MLPA kit P056).

#### Results

Neither TP53 mutations nor TP53 large genomic rearrangements could be detected in our collective. However, two women have been identified as CHEK2 mutation carriers (c.1100delC, deletion of Exon 9), applying the TP53 MLPA kit. Only one of the two families fulfilled the Li-Fraumeni-like criteria according to Eeles.

#### Conclusions

According to our data, germline mutations in the TP53 gene do not seem to play a causative role in the aetiology of early onset breast cancer within the German population.

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**P-CancG-233****MUTYH gene mutation in Polish polyposis patients**

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Colorectal adenomatous polyposis, autosomal recessive MIM #608456 also called MUTYH-associated polyposis (MAP), is an autosomal recessive disorder predisposing to colorectal cancer. MAP was described in 2002 by Al-Tassan. The number of polyps observed in MAP patients not exceeds 1000. The colonic phenotype of disease is corresponded to attenuated form of FAP. There were extra colonic features, described in some MUTYH biallelic mutations carriers but their prevalence was not established. Penetrance of CRC in MAP patients is approximately 100% by age 65 years. The colorectal cancer risk in MAP patients is 93 time higher than in general population. The MAP is caused by mutations of MUTYH gene in homozygotic or compound heterozygotic state. The MUTYH gene encoding protein with glycosylase activity, involved in repair of oxidative DNA damages. The MUTYH activity avoids the chances of pairs G:C to A:T accruing as results of oxidative DNA damages. In MUTYH gene the increased frequency Y165C and G382D was observed. Those two mutations in homozygotic or biallelic state are observed in most cases of MAP with diagnosed mutation. In our study we checked prevalence of Y165C and G382D in 338 polyposis probands with out APC gene mutation. In group with one of above mentioned mutations detected, the entire coding sequence of MUTYH gene was studied by mutations screening and direct PCR product sequencing method. In our studies we detect the MUTYH mutations in 19 probands. One probant was homozygote of Y165C, five probands were compound heterozygote and remaining 13 were heterozygotes.

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**P-CancG-234****STK11 mutation status in Polish Peutz-Jeghers syndrome patients**

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Peutz-Jeghers syndrome (PJS; MIM 175200) an autosomal dominant disorder associated with increased risk of malignancies. The frequency of PJS is estimated from 1/29,000 to 1/120,000 people. During second or third decade of life in most patient the hamartomatous polyps are observed. Polyps can be located throughout digestive tract. Risk of colorectal cancer is lower than in FAP or HNPCC, but PJS can be reason of many gastrointestinal discomforts like bowel obstruction, intussusception or bleeding. In Peutz-Jeghers syndrome high risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. Second manifestations are mucocutaneous hypermelanocytic lesions. The pigmentations are usually brown, dark or blue spots developing on lips, hands and feet, in the mucosa of the nose, conjunctiva or rectum appear in about 90% cases. Mutations in STK11 gene are detected in 70 % patients with familial form of PJS and in 30 to 70% sporadic cases of disease. STK11 gene is located on chromosome 19p13.3. Protein coding by STK11 gene is serine/threonine kinase. Loss of STK11 function causes many defects, since STK11 participates in very important cell signaling pathways. Here we present the study considering 30 Polish families with PJS. The STK11 point mutations were detected 9 families and large STK11 gene rearrangements were detected in 6 families.

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**P-CancG-235****Do RMRP mutations affect telomerase function?**

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Cartilage-hair hypoplasia (CHH) is a rare autosomal recessive disorder characterized by short stature, metaphyseal dysplasia, and variable extraskelatal features, including sparse hair, immunodeficiency, and a

predisposition to malignancy, e.g., EBV-associated lymphomas. CHH is caused by mutations in the RMRP gene which encodes the RNA component of the RNase MRP complex. This ribonucleo-protein complex is involved in the replication of mitochondrial DNA, processing of the 5.8S ribosomal RNA, and control of cell cycle progression. Recently, the RMRP RNA was found to form a complex with TERT, the protein component of the telomerase complex. Unrelated to the elongation of telomeres, the RMRP-TERT complex was shown to use RMRP RNA as a template for Dicer-dependent production of miRNA.

We thus hypothesized that mutations in the RMRP RNA may also have an effect on telomerase function. As a pilot experiment, we determined the mean telomere length in DNA extracted from blood and primary fibroblasts of CHH patients in comparison to healthy controls of the same age and sex. We performed telomere length assays based on southern analysis of terminal restriction fragments (TRFs). These analyses, however, did not reveal significant differences. As immortalization with Epstein Barr virus greatly increases telomerase activity and telomere length in blood cells, we assumed that transformation may provoke differences in telomere maintenance. TRF analysis of DNA extracted from EBV-transformed lymphoblastoid cell lines did indeed demonstrate clear differences in telomere length. In spite of no apparent differences in immortalization and proliferation, however, the telomeres of lymphoblasts from the CHH patient cohort were significantly shortened in comparison to age-matched controls. Ongoing experiments correlate telomere length to telomerase function and associated cellular functions, like the DNA damage response. Our results indicate that RMRP mutations do have a negative effect on telomerase function and that pathogenesis of CHH may be, at least in part, be due to telomerase dysfunction. This effect of RMRP mutations may be the molecular basis for an increased incidence of EBV-associated lymphomas and the increased general tumor risk observed in CHH patients.

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## P-CancG-236

### Gain of the Centromeric Region of Chromosome 8, a new Germline Alteration in Juvenile Myelomonocytic Leukemia

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Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive childhood myeloproliferative disorder characterized by clonal hyperproliferation of myelomonocytic cells. The pathogenesis of JMML involves disruption of signal transduction through the RAS pathway, which may be an early event during leukemogenesis. Additional genetic lesions may be necessary for full malignant transformation.

Material and Methods: We applied microarray BAC/PAC and high resolution 244k oligo array comparative genomic hybridisation (aCGH) to bone marrow samples from 21 JMML patients in order to identify subtle genomic alterations.

Results: In 8 of 13 JMML patients with normal karyotype and 2 of 8 JMML patients with monosomy 7, additional copy number alterations were identified. A recurrent deletion of around 1.4 Mb at 17q11.2 targeting the NF1 gene was identified in 2 patients with clinical diagnosis of neurofibromatosis. In addition to the monosomy 7, one patient (D600) with a somatic mutation in PTPN11 showed an additional marker chromosome. The origin of this marker chromosome was resolved by aCGH. The BAC/PAC array indicated a gain of the centromeric region: arr 8p12q11.21(37,204,000-49,686,000)x3. Breakpoints were refined to arr 8p12q11.21(36,673,794-50,142,678)x3 by high resolution oligo aCGH. Interestingly, in patient D703 without monosomy 7, but with a heterozygous CBL germline mutation and homozygosity in hematopoietic cells, we detected an almost identical gain arr 8p12q11.21(40,076,917-49,622,319)x3. The gain was confirmed by means of FISH using a centromere-specific probe for chromosome 8 showing three signals. This patient was also found to have a small additional marker chromosome. In order to rule out that the marker chromosome was inherited, we performed chromosome analyses on the parents of patient D703. Both had a normal karyotype and did not carry an extra copy of the region 8p12-q11.21. However, FISH on buccal epithelial mucosa cells of patient D703 revealed a mosaic constellation with 34% cells containing 3 CEP8 signals. Likewise, in the second patient, the dup 8p12-q11.21 (D600) was constitutional as 38% of fibroblasts showed 3 CEP8 FISH signals.

Discussion: There has been a longstanding discussion as to whether trisomy 8, one of the most frequent aberrations in hematological malignancies, may - at least in some cases - represent a constitutional mosaic leading to a propensity to malignancies, especially to hematological malignancies. Our findings provide evidence that even a partial trisomy 8 can be present as a constitutional mosaic in patients developing

JMML. Further work is needed to determine how constitutional partial trisomy 8 mosaic contributes to leukemogenesis in different mutational subtypes of JMML.

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## P-CancG-237

### Correlation of SHOX2 Gene Amplification and DNA Methylation in Lung Cancer

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#### Background

Recently, DNA methylation in the SHOX2 locus was used to reliably detect lung cancer patients at a high specificity in a group of critical controls based on the analysis of bronchial lavage samples, even in cytologically negative samples with no visible tumor cell content. This study aimed to investigate, if the methylation correlates with SHOX2 gene expression and/or copy number alterations. An amplification of the SHOX2 gene locus together with the observed tumor-specific hypermethylation might explain the good performance of this marker in bronchial lavage samples.

#### Methods

SHOX2 expression, gene copy number and DNA methylation was determined in lung tumor tissues and matched morphologically normal adjacent tissues (NAT) from 55

lung cancer patients. Quantitative HeavyMethyl (HM) real-time PCR was used to detect SHOX2 DNA methylation levels. SHOX2 expression was assayed with quantitative real-time PCR, and copy numbers alterations were measured with conventional real-time PCR and array CGH.

#### Results

A hypermethylation of the SHOX2 locus in tumor tissue compared to NAT was detected in 96% of tumors from a group of 55 lung cancer patients. This correlated highly significantly with the frequent occurrence of copy number amplification ( $p < 0.0001$ ), while the expression of the SHOX2 gene showed no difference.

#### Conclusions

Frequent gene amplification correlated with hypermethylation of the SHOX2 gene locus. Thus SHOX2 DNA methylation is eligible as a biomarker for lung cancer diagnosis, especially when sensitive detection is needed, i.e. in bronchial lavage specimens.

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## P-CancG-238

### Characterization of genetic changes in oligodendroglial tumors including glioblastomas with oligodendroglial component

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In contrast to astrocytic tumors including glioblastomas (GBM), oligodendrogliomas (O) show a better prognosis and increased responsiveness to chemotherapy. Interestingly, a small subgroup of glioblastomas contains areas with histological features of oligodendroglial differentiation. In the current WHO classification (2007) these tumors are now included as a variant of glioblastomas and are called glioblastomas with oligodendroglial component (GBMO). However, definitive diagnostic criteria still do not exist.

In this study we used a genome wide approach (chromosomal comparative genomic hybridization (CGH) and molecular karyotyping) in combination with interphase fluorescence in situ hybridization (FISH) (regions 1p, 1q, 7q, 10q, 17p, 19q, cen18, 21q) to genetically characterize GBMO and "classical" O. We analyzed the oligodendroglial and the astrocytic glioblastoma parts of 13 GBMO separately by chromosomal CGH of microdissected paraffin embedded tumor tissue and interphase FISH on paraffin sections. Furthermore, freshly frozen material of eight oligodendrogliomas (3 WHO grade II, 5 WHO-grade III) and two oligoastrocytomas (OA) WHO grade II was examined genome wide by using the standard Agilent 400K chip.

We identified four distinct genetic subtypes in GBMO: an "astrocytic" subtype (9/13) characterized by +7/-10; an "oligodendroglial" subtype with -1p/-19q (1/13); an "intermediate" subtype showing +7/-1p (1/13), and an "other" subtype having none of the former aberrations typical for gliomas (2/13). In contrast, most "classical" O (7/8) and one of two OA showed combined 1p/19q deletion, corresponding genetically to the

“oligodendroglial” subtype. Five of the O with 1p/19q codeletion showed additional aberrations (e. g. -4, -14, -9, -18). The different histological tumor parts of GBMO revealed common genetic changes in all tumors and showed additional aberrations specific for their oligodendroglial and astrocytic parts. These findings demonstrate the monoclonal origin of the tumor followed by the formation of the astrocytic and oligodendroglial components. We are currently evaluating the array CGH results of the oligodendrogliomas, which will also be presented.

The diagnostic determination of these genetic signatures may allow for a better prognostication of the patients. Our results underline the importance of molecular cytogenetic analyses to supplement the histological diagnosis of gliomas.

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## **P-CancG-239**

### **Inhibition of PDGFR $\beta$ in colorectal cancer cells leads to decreased proliferation and G2 cell cycle arrest**

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the western world and growth factors and their receptors play a significant role in the regulation of CRC growth, angiogenesis, and metastasis. The platelet-derived growth factor receptor (PDGFR) has attracted increasing attention as potential target of anti-tumor therapy in CRC. In the present study the influence of the  $\alpha$ - and  $\beta$ -subunit of the PDGFR (PDGFR $\alpha$ , - $\beta$ ) was investigated in different CRC cell lines. PDGFR $\alpha$  expression was not observed in all cell lines tested, however, varying expression levels of PDGFR $\beta$  could be detected both at the RNA and the protein level in the cell lines CaCo-2, DLD-1, SW480 and SW837. To study the function of PDGFR $\beta$  in CRC cell lines, SW480 cells showing the highest PDGFR $\beta$  expression were used for receptor blockade. The inhibition was performed by two different strategies: firstly, PDGFR $\beta$  expression was down-regulated by three different PDGFR $\beta$ -specific siRNAs. Secondly, the tyrosine kinase activity of the PDGFR $\beta$  was inhibited by the pharmacological inhibitor Ki11502. Both approaches resulted in a significant decrease of SW480 cell proliferation, whereas an increased rate of apoptosis could not be detected. Both treatments reduced the activity of the PI3K signaling cascade, while the MAPK signaling cascade was not affected. Further experiments showed that the treatment with the PDGFR $\beta$  inhibitor led to a G2 arrest in SW480 cell cycle progression. Taken together, these results support the assumption that PDGFR $\beta$  could act as a therapeutical target in CRC treatment.

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## **P-CancG-240**

### **Aberrant splicing of APC in patients with mutation negative adenomatous polyposis**

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Background: Familial adenomatous polyposis (FAP; MIM 175100) is an autosomal-dominant cancer predisposition syndrome characterised by the appearance of numerous colorectal adenomas, which, if not detected early and removed, inevitably result in colorectal cancer. In up to 50% of patients with colorectal adenomatous polyposis no germline mutation in the currently known genes – APC and MUTYH – can be identified using routine diagnostic methods such as sequencing of the coding exons and deletion/duplication analysis by MLPA. However, pathogenic variants localised in promoter regions and deep within introns remain undetected with this approach. To uncover aberrant transcripts which might be caused by intronic mutations we performed a systematic transcript analysis of the APC gene in 66 mutation negative patients with clinically verified adenomatous polyposis. Methods: Total RNA was isolated from leukocytes and transcribed into cDNA. The APC cDNA was amplified in five overlapping fragments using specific primers. PCR products were analysed on agarose gels in comparison to controls. Subsequently, PCR-products of patients with deviant pattern were sequenced. Results: In 4 out of 66 patients (6%) the gels showed additional larger bands indicating aberrant splicing. In one patient, sequencing of the whole PCR product revealed an insertion of 83 basepairs from intron 10 between exon 10 and 11. Sequencing of genomic DNA with primers flanking the corresponding region of intron 10 showed a point mutation at the 3' end of the inserted region (c.1408+731C>T) generating a new splice donor site with predicted high splice efficiency (99%). A cryptic splice acceptor site at the 5' end of the region (splice efficiency of 97%) was predicted by in-silico analysis. The insertion results in a frameshift leading to a premature stop codon (p.Gly471SerfsX55). The genomic variants underlying the other three aberrant transcripts remain to be identified yet. Conclusion: According to the preliminary result of our cDNA analysis, intronic APC variants of potential pathogenicity are

relevant in FAP patients with no germline mutation in the coding regions and adjacent intronic parts of the gene. Since those variants are missed in routine mutation screening, transcript analysis should be considered as an additional diagnostic option in mutation negative patients. The study was supported by the German Cancer Aid (Deutsche Krebshilfe).

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#### **P-CancG-241**

### **Congenital Hypertrophy of the Retinal Pigment Epithelium and intrafamilial phenotypic variation: examination of a FAP family**

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Familial adenomatous polyposis (FAP) is a well-described autosomal dominant inherited disease caused by germline mutations in the adenomatous polyposis coli (APC) gene. The syndrome is characterized by the development of hundreds to thousands of adenomatous polyps in the colorectum. Without early diagnosis and treatment almost all patients will develop colorectal cancer. FAP is associated with further extracolonic manifestations such as duodenal cancer, desmoid tumors, epidermoid cysts, lipomas, fibromas, osteomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE). Colonic phenotype and some extracolonic manifestations are predicted to correlate with the location of the mutation in the APC gene.

In the present study we describe 28 FAP patients from one family. Although all patients carry the same mutation at codon 1981 in APC they differ in phenotype. In this study we focus on the occurrence of CHRPE, a pigmented fundus lesion associated with FAP. CHRPE is known to be a very common extracolonic manifestation and is accepted to be of interest in the early diagnosis of FAP patients. As these retinal lesions are variable in size, localization, and pigmentation, they can be classified into 4 groups referring to Berk's classification: oval, pigmented, and surrounded by a halonaevus (type A); round, small, and pigmented (type B); round, large, and pigmented (type C) and round, large, and depigmented (type D). The most characteristic lesions in FAP patients occur as multiple, usually bilateral lesions and often with a depigmented halonaevus (according to Berk type A), but most frequently lesions of type B are found.

Aim of this study was to analyze FAP-associated CHRPEs in individuals with the same APC mutation and to compare the phenotypes with recent findings. A remarkable result was the high presence of CHRPE associated eye manifestations in our patient cohort but also the intrafamilial variation. The majority of patients showed distinct eye pigmentations and more than one third of them were defined as FAP associated according to Berks' classification. Recent findings set a mutation area between codons 311 and 1444 in APC for the occurrence of CHRPE in FAP. Our result is therefore noteworthy as it doesn't correspond to this range. To conclude, our study shows that the occurrence of CHRPEs could reveal a high intrafamilial variability at least in patients with mutation in codon 1981.

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#### **P-CancG-242**

### **Colorectal, endometrial and metachronous colorectal-endometrial cancer risks in Lynch syndrome**

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**Introduction.** Lynch syndrome patients have a high lifetime risk for colorectal (CRC) and endometrial cancer (EC). These patients are also at a considerable risk for developing metachronous tumours. In the present analysis we were interested in estimating the risk for CRC and EC, as well as the risk to develop EC after CRC and vice versa, depending on the affected gene (MLH1, MSH2 or MSH6).

**Materials and Methods.** For estimation of colorectal cancer risk we analysed 639 mutation carriers (235 MLH1, 320 MSH2, 84 MSH6), who were not index patients. For estimation of EC risk we analysed 735 female mutation carriers (293 MLH1, 361 MSH2, 81 MSH6), including index patients. A total of 328 and 91

female mutation carriers were analyzed for estimating the risk of developing EC after CRC and vice versa. Kaplan-Meier analysis was used to estimate age-dependent cumulative risks and the log-rank test was used for group comparisons.

Results. CRC risk at 60 years was 60%, 55%, and 22% for MLH1, MSH2 and MSH6 mutation carriers ( $p < 0.001$ ). Male carriers had a significantly higher risk for CRC ( $HR = 1.8$ ,  $p < 0.001$ ). EC risk at 60 years was 24%, 16%, and 29% for MLH1, MSH2 and MSH6 mutation carriers ( $p = 0.92$ ). The cumulative risk for developing EC 20 years after CRC was 14% (95% CI 6%-23%). Vice versa, the cumulative risk for developing CRC 20 years after EC was 76% (95% CI 63%-89%). No significant differences by affected MMR gene could be shown.

Discussion. Risk for CRC in Lynch syndrome patients differs by gender and affected MMR gene. MSH6 mutation carriers have a significantly lower CRC risk especially in younger years; a less dense surveillance programme might therefore be appropriate. The risk for developing metachronous CRC and EC is high, but more data is needed to show risk differences between the different MMR genes.

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## P-CancG-243

### **A BACH2-BCL2L1 fusion gene resulting from a t(6;20)(q15;q11.2) chromosomal translocation in the lymphoma cell line BLUE-1**

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Abnormalities of the long arm of chromosome 6 are a common feature in various B-cell malignancies. In most cases the genes involved have not yet been clearly identified. We have molecularly characterized the recently established Burkitt lymphoma cell line BLUE-1 that carries a t(6;20)(q15;q11.2) rearrangement in addition to the typical t(8;14) with MYC-IgH fusion. To identify the gene loci involved on both chromosomes we applied a sequential BAC clone mapping strategy. Using RT-PCR we were finally able to detect a fusion mRNA transcript showing a fusion of the first (non-coding) exon of BACH2 (basic leucine zipper transcription factor 2) on 6q15 to the second exon of BCL2L1 (BCL-X) on 20q11. Various fusion transcripts were detected for different BCL2L1 (BCL-XL) isoforms. The fusion ultimately results in strong expression of the Bcl2l1 (Bcl-xL) anti-apoptosis protein, as demonstrated by immunoblotting. This is the first report that shows involvement of both BCL2L1 and the transcription factor BACH2 in a chromosomal rearrangement. It points to BACH2 as a possibly important target in lymphomas with 6q aberrations, although other genes on 6q are probably also involved in these cases. Moreover, it suggests that other members of the BCL2 anti-apoptosis gene family other than BCL2 itself might also be involved in lymphoma.

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## P-CancG-244

### **Phenotypic heterogeneity in GPR56-associated polymicrogyria overlapping with cobblestone lissencephaly complex**

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Polymicrogyrias (PMG) are a heterogeneous group of cerebral malformations characterized by increased number of small gyri and sulci. PMG can be subdivided into different forms depending on the anatomical pattern. There are bilateral frontal, frontoparietal, perisylvian, parasagittal parieto-occipital and generalized forms. Due to the excessively increased number of cortical gyri with consecutive partial fusion the polymicrogyric cortex can present on cT/cMRI as pachygyria and needs special attention. In 2004, mutations of the GPR56 gene (G-protein coupled receptor) could be associated with a distinct form of bilateral frontoparietal polymicrogyria (BFPP) and later assigned as BFPP1 to distinguish from cases without GPR56 mutations, which were classified as BFPP2. As characteristic features patchy lesion of the white matter in combination with hypoplasia of the cerebellar vermis and the pons have been observed. Here, we

present the clinical and genetic findings of 8 patients from 5 families with GPR56-associated PMG. Particularly, we observe that patchy white matter lesions previously reported as characteristic for GPR56-associated PMG may be only transiently present, and are hence not an obligate feature of BFPP1. In addition, we demonstrate that GPR56-associated PMG may extend into the occipital region, resembling generalized PMG in axial images. Additionally, there is a considerable intrafamilial variability pointing to further genetic and/or exogenous modifying factors. The appearance of an encephalocele in one patient and structural eye abnormalities in two additional patients provides further evidence for the phenotypic overlap of GPR56-associated PMG with cobblestone lissencephalies, like Walker-Warburg syndrome (WWS) or muscle-eye-brain disease (MEB). This clinical overlap is further underlined by the GPR56 animal model indicating pathologic similarities like the overmigration of neurons into the leptomeninges due to clefts of the glia limitans. Taken these characteristics together, our data further provide evidence that the phenotypic spectrum of GPR56-associated migrations disorders should be considered at an early point for the genetic testing strategy.

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## **P-CancG-245**

### **Investigation of non-CpG DNA-methylation in human neoplasia using targeted DNA-microarrays**

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DNA cytosine methylation is an epigenetic modification and is found throughout the vertebrate-genome almost exclusively in a CpG dinucleotide context. Recent analyses of the human methylome of human embryonic stem cells has found about 17% of cytosine-methylation in a CHG- and 7% in a CHH-context (H = A, C or T; Lister 2009).

We aimed to investigate the presence of CHG/CHH-methylation in tumor-DNA which might be of relevance for further studying its function in tumorigenesis. In addition detection of CHG/CHH-methylation as well as distinct methylation patterns could serve as biomarker for tumor diagnostics.

We have analysed DNA methylation of MCF-7 breast cancer cell line (8 replicates) and childhood ALL (acute lymphatic leukemia, n=8) samples, as well as DNA from blood of healthy persons (n=8) using the methylation sensitive restriction enzyme PspGI and the methylation-insensitive isoschizomer BstNI specific for "CCWGG", allowing detection of methylation patterns in a non-CpG context. Upon MSRE digestion DNA was amplified in multiplex-PCRs and amplicons read-out on microarrays. By this approach we were targeting 360 CpG islands of genes known methylated in cancerous disease. Thereof 250 analysed 5'-gene regions harbored PspGI sites and provide a read-out for elucidation of DNA-methylation in a CHG-context. Class comparison of chip-data derived from BstNI and PspGI digests elucidated several significant genes corresponding to non-CpG methylation in DNA from the breast cancer cell line as well as ALL samples. However, qPCR analyses of these marker-candidates did not confirm findings. Thus data from our analyses show that non-CpG methylation is, when even present, a very rare event in samples analysed, and thus would not be a useful target for cancer biomarker development.

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## **P-CancG-246**

### **Differential DNA repair gene regulation in untreated and irradiated primary fibroblasts of individuals with childhood malignancy and second cancer**

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The etiology of most cases of childhood cancer is still unknown. Because children are usually not exposed to environmental hazards or an unhealthy lifestyle, it seems reasonable to assume that genetic differences play a critical role in tumor development. With the help of the German Childhood Cancer Registry we recruited 20 individuals who survived childhood malignancy and then developed a second cancer as well as 20 matched individuals with childhood malignancy but without a second cancer that served as controls. We screened primary fibroblasts of these patients for modulation / dysregulation of genes involved in DNA repair, cell cycle regulation and other related regulatory pathways under untreated and different treatment conditions. Using customized cDNA microarrays we first compared the DNA repair transcriptomes of untreated primary fibroblasts from one-cancer and two-cancer patients with those of a pool of healthy controls and identified 55 genes that were differentially regulated in at least two cancer patients. By real-time qPCR we verified the microarray results for 17 of the candidate genes in all patient cell lines. and identified

three genes, VIM, RAD9A and RFC2, to be differentially regulated in the two-cancer patient group compared to the one-cancer patient group.

Then, we irradiated the fibroblasts of the cancer patients with a dose of 1 Gray and again used real-time qPCR to analyze expression levels of the candidate genes at different time points (1 h, 4 h and 24 h) after DNA damage. Significant expression differences between two-cancer and one-cancer patients were detected for the cell cycle control gene CDKN1A and the DNA repair gene RAD9A. CDKN1A showed a moderate upregulation in the untreated cells and 1 h after treatment for the two-cancer patient group compared to the one-cancer patient group.

Marked differences were found for RAD9A with significantly lower expression levels in the two-cancer patient group in untreated cells, as well as 1 h and 4 h after damage. Further, Western blot and antibody microarray experiments of untreated cells were performed that confirmed the downregulation of RAD9A in the two-cancer patient group also at the protein level. In addition, the antibody microarray experiments revealed differential protein expression levels between the two-cancer and the one-cancer patient group for the tumor suppressors BRCA1 and BRCA2 as well as the DNA repair protein RAD51. Since RAD9A is an important regulator involved in cell cycle arrest and DNA damage repair, the observed RAD9A downregulation may result in a higher susceptibility to tumor formation in two-cancer patients.

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## P-CancG-247

### **Improved MLPA analysis identifies the first pathogenic PMS2 allele that results from intra-chromosomal non-homologous recombination between PMS2 and PMS2CL**

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The role of the mismatch-repair (MMR) gene PMS2 for Lynch syndrome (LS) may have been underestimated in the past. This is largely explained by the attenuated LS phenotype of PMS2-mutation carriers, but also by the difficulties of PMS2 mutation analysis arising from sequence exchange between the duplicated regions of PMS2 and its pseudogene PMS2CL. Using a reliable and sensitive RNA-based assay developed in our lab for PMS2 mutation analysis and research purposes, we showed that due to the sequence exchange one third of all functional PMS2-alleles, termed "hybrid"-alleles, carry pseudogene-derived sequence variants (PSVs) in exons 13 to 15. While "hybrid"-alleles with PSVs only in exon 15 may result from a number of different recombination and/or gene conversion events, we found that "hybrid"-alleles with pseudogene-derived sequences in the more 5' exons 13 and 14 can be traced back to a single ancient intrachromosomal non-homologous recombination event. Now we report the identification of the first pathogenic PMS2 allele that has most likely arisen from a similar mechanism. Since this pathogenic allele harbors PSVs in exons 11 to 15, it escapes the detection by PMS2-specific exon sequencing from gDNA. Using improved MLPA analysis containing gene, pseudogene and unspecific probes run with pre-selected control-DNA samples harboring two non-hybrid PMS2 and PMS2CL alleles we uncovered this allele which was further confirmed by direct cDNA sequencing. Characterizing the recombination breakpoint in intron 10 enabled the design of a simple PCR assay specific for this mutation. Clinically of note, this mutation was identified in a patient who was originally suspected to suffer from attenuated familial adenomatous polyposis since he presented with two synchronous colorectal cancers and multiple (20) colonic adenomas. However, the patient tested negative for an APC or a MUTYH mutation. Subsequent immunohistochemical analysis showed isolated PMS2 expression loss in the microsatellite instable tumors which prompted PMS2 mutation analysis. Currently we are testing 25 patients with so far unexplained isolated loss of PMS2 expression in the tumor for the presence of this mutation as well as intragenic deletions and duplications by improved MLPA analysis together with our allele-specific PCR assay. These studies will hopefully clarify the relevance of this allele and intragenic copy number changes for PMS2-associated LS. Furthermore, they may also determine whether the development of multiple colonic polyps is a unique feature in one patient or is more frequently observed in carriers of this particular mutation or PMS2 mutations in general.

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**P-CancG-248****Underlying molecular mechanisms of the histone deacetylase inhibitor valproic acid in the therapy of prostate cancer**

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The histone deacetylase inhibitor valproic acid is a well known remedy for epilepsy and was also shown to have several anti-cancer effects in a variety of human cancers. In the field of prostate cancer it is so far hardly studied. We discovered an antiproliferative effect of valproic acid on primary mouse prostate cancer cells. These cells were generated from a well established mouse model called the TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse model. We could also show that these cells are less invasive under the treatment with valproic acid. A cDNA microarray analysis revealed several differentially expressed genes after the treatment of prostate cancer cells with 1mM valproic acid for 24 hours. These differentially expressed genes could be confirmed by both quantitative realtime RT-PCR and Western blot analysis. Additionally, we could show that these genes are differentially expressed in a dose and time dependent manner. One of these genes is the Cyclin D2 gene which encodes for a key regulator of cell cycle progression and we could demonstrate that Cyclin D2 was strongly upregulated after the treatment with valproic acid. We downregulated Cyclin D2 expression in prostate cancer cells via siRNA and checked the impact on valproic acid-related effects. Furthermore, valproic acid is known to induce apoptosis in several cancers and using flow cytometry, we received first results that this also occurs in our cellular model. Moreover, we could show that the pro-apoptotic protein Bim is upregulated in prostate cancer cells under the treatment with valproic acid. In summary, valproic acid could be shown to have promising therapeutical effects on prostate cancer cells and hence it could become a potent drug for the therapy of prostate cancer in the future.

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**P-CancG-249****Analysis of promoter methylation of Fanconi anemia genes in familial breast cancer**

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Familial breast cancer accounts for 5-10% of breast tumors. Half of these are caused by mutations in BRCA1 or BRCA2 (FANCD1). In rare familial cases mutations of the Fanconi anemia (FA) genes FANCI (BRIP1) and FANCD2 (PALB2) have been detected. Recently, hypermethylation of the BRCA1 promoter has been observed in some familial breast cancers. We hypothesized that aberrant promoter methylation might affect FA gene function in familial breast cancer. Therefore we analyzed 24 paraffin-embedded breast cancer samples (5 of BRCA1-positive, 3 of BRCA2-positive and 16 of BRCA1/2-negative female patients with family history of breast cancer), 22 blood samples of these patients and 9 breast cancer cell lines by bisulfite-pyrosequencing of FA gene promoter regions. Tissue specific methylation was defined by bisulfite-pyrosequencing of eight normal breast tissues and blood samples of 10 healthy controls. Hypo-/hypermethylation were defined as the mean methylation level of tissue/blood controls plus 3 standard deviations below/above.

FANCA, FANCD2, FANCL, FANCI and FANCD1 showed no difference in DNA methylation in tumors and cell lines compared to tissue controls. In contrast, compared to tissue and blood controls, respectively, FANCA showed hypomethylation in 10 of 24 (42%) tumors and in 5 of 9 (56%) cell lines. In contrast, cell line HCC 1937 and 6 blood samples of the breast cancer patients showed hypermethylation. Four of these patients showed hypermethylation in blood and hypomethylation in the tumor. FANCB was hypomethylated in 9 of 18 (50%) tumors and in 7 of 9 (78%) cell lines. In contrast, FANCB was hypermethylated in 8 of 18 (44%) tumors.

We conclude that hypo- as well as hypermethylation of FANCA and FANCB is recurrent in familial breast cancer and might therefore be involved in carcinogenesis. The methylation pattern of FANCA can differ between blood and tumor in the same patient. The functional relevance of these observations remains to be resolved.

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## P-CancG-250

### **Ring chromosome 22 and neurofibromatosis type II: proof of two hit model for the loss of the NF2 gene in the development of meningioma.**

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Carriers of a ring chromosome 22 are mentally retarded and show variable facial dysmorphism. They may also present with features of neurofibromatosis type II (NF2) such as vestibular schwannomas and multiple meningiomas. In these cases, tumourigenesis has been suspected to be caused by the loss of both alleles of the NF2 gene, a tumour suppressor localized in 22q12.2.

We describe a 18-year-old patient with constitutional ring chromosome 22 and mental retardation who developed rapid onset spastic paraparesis at the age of 15 years. The causative spinal meningioma at the level of T3, which compressed the spinal cord, was surgically removed, and the patient regained ambulation. Array CGH and MLPA analyses in blood revealed a terminal deletion in 22q13.32, not comprising the NF2 gene. In tumour tissue, loss of the whole ring chromosome 22 including one NF2 gene due to mitotic instability constituted the likely first hit, while a point mutation in the other allele of the NF2 gene (c.784C>T, p.R262X) was demonstrated as second hit.

Our analyses prove that the two hit model with biallelic loss of the NF2 gene is the pathogenetic mechanism leading to the development of meningioma in patients with ring chromosome 22. We review all cases from the literature and suggest clinical guidelines for surveillance of patients with ring chromosome 22.

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## P-TECHNOLOGY AND BIOINFORMATICS

### P-Techno-251

#### **Targeted resequencing of the CFTR gene using multiplexing, sample barcoding and the SOLiD™ Next-Generation Sequencing System: a pilot study**

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Cystic fibrosis (CF) is one of the most common diseases with an autosomal recessive inheritance worldwide, and more than 1800 different mutations are reported in the disease associated CFTR gene. Sanger sequencing of the CFTR gene is the diagnostic gold standard, but is rarely used due to the high workload and costs of the method. Although using a Next-Generation Sequencing System and sample barcoding should significantly reduce costs, it still would provide results with the highest mutation detection rate possible. Utilising a high-throughput next generation sequencing platform for such a clinical diagnostic setting requires highly specific resequencing of the coding and flanking intron regions of the CFTR gene. While PCR amplification has proven to be a reliable method for amplification of gene specific target regions, it has suffered from limited multiplex capability. Here, we describe a pilot study with 20 patient samples with different CFTR gene mutations, using a Multiplicon™ multiplexing assay for the CFTR gene, sample barcoding and subsequent Next-Generation Sequencing with the SOLiD™ platform. The results of SOLiD™ sequencing are compared with Sanger sequencing data of all 20 samples.

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### P-Techno-252

#### **MS-SNuPE as a diagnostic tool for the detection of aberrant methylation patterns in growth retarded patients**

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In the last decade the crucial role of imprinted genes in human growth and development has become evident. A number of diseases have been found to be directly linked to aberrant methylation of specific genes including Silver-Russell syndrome (SRS), Beckwith-Wiedemann syndrome (BWS) and Transient Neonatal Diabetes Mellitus (TNDM). To identify these epigenetic changes a number of methods have been described. MS-MLPA (methylation specific multiplex ligation dependent probe amplification) and MS-PCR as well as MS-pyrosequencing and bisulfite sequencing are meanwhile well established methods. Whereas

quantification of the methylation status is difficult by MS-PCR, MS-MLPA, bisulfite sequencing and MS-pyrosequencing allow a direct quantification. However, they are either time-consuming and/or labor intensive or complex to establish.

As recent publications show a considerable fraction of SRS, BWS and TNDM patients exhibit loss of methylation at other than the disease specific loci (multilocus hypomethylation MLH). It is therefore necessary to have simply adjustable assays to analyze different loci with the possibility of direct quantification in single-tube reaction formats available.

Methylation-specific single nucleotide primer extension (MS-SnuPE) is based on the AB Prism® SNaPhot™ technology: after initial bisulfite conversion of DNA multiplex PCRs for the different loci can be performed followed by a primer extension step. A unique colored and base specific dye is added to the extension primer allowing the detection of single base changes directly adjacent to the primer annealing site. The extension primers were designed to anneal directly beside the C of a differentially methylated CpG. For length discrimination the primers were tagged. With this technique, multiplexing is possible and the MS-SNuPE products can be quantified.

We established MS-SNuPE to analyze the methylation status of several imprinted loci and to elucidate the applicability of MS-SNuPE in research and routine diagnostic. We chose the loci of GRB10, MEST, IGF2R, H19, LIT1, DLK1/GTL2 (chromosomes 6, 7, 11, 14) because methylation of these differentially methylated regions is frequently disturbed in MLH. The method was validated by screening a cohort of probands with (epi)genetic disturbances at the different imprinted loci.

In all cases, the aberrant methylation identified in previous analyses by other techniques could be confirmed by the newly developed MS-SnuPE approaches. In comparison to the other MS techniques, multi-locus MS-SnuPE allows the parallel quantitative analysis of numerous CpG islands and can therefore be regarded as a suitable method for the detection of aberrant methylation.

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## P-Techno-253

### **Targeted Re-Sequencing in ALS / FTD patients and patients with dementia – a new tool, successfully solved cases and an unexpected discovery**

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**Background:** Several genes have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and other types of dementia. As each gene accounts for only a small fraction of cases, it is crucial to develop an efficient tool for investigating all genes simultaneously. Here we present a very fast and cost-efficient targeted re-sequencing approach to screen for all known ALS / FTD and dementia associated genes and potential candidate genes simultaneously. This will avoid long lasting diagnostic procedures in the hope to identify the underlying cause of the disease. If the causing gene is identified, predictions about disease progression, examination of relatives and personalised treatment options according to the underlying genetic basis become possible. We expect to identify major genetic risk factors in a significantly larger proportion of cases as compared to the cases identified with the conventional sequencing method. In addition, careful collection of all clinically relevant information will allow us to investigate how the genotype determines the phenotype in the different subsets of neurodegenerative diseases.

**Methods:** Genomic DNA is enriched using a custom made Agilent SureSelect in solution kit. Sequencing of 50 genes (360kb in total) is performed using barcoded libraries on one oct (1/8 slide) on the SOLiD 4 platform generating approximately 5 Million mappable 50 basepair reads per patient.

The basic data analysis is performed with Bioscope v1.2. Additionally, we developed a diagnostic pipeline to (i) identify regions that are underrepresented with reads (coverage <20 per base), (ii) identify potentially pathogenic SNVs, (iii) identify small insertions and deletions and (iv) identify larger indels including whole exon deletions and duplications. All variants and putative deletions are then re-sequenced by the gold standard Sanger sequencing or quantitative PCR, respectively. The gene list and the Agilent SureSelect Design are updated, extended and balanced with each round of ordering of the Agilent SureSelect in solution kit.

**Results:** We analysed 16 patients with clinical diagnoses of ALS, FTD or neurodegeneration with dementia. Overall 99% of all 1052 targeted exons were represented by reads with an average coverage of >200. A mean of 40 SNVs and 2 indels were identified in all regions of interest per individual. This number can be reduced to less than five if synonymous SNPs and frequent polymorphisms are excluded. The most likely pathogenic variants are validated by the gold standard Sanger. We will present the solved cases and in particular a family with neurodegeneration and dementia in which the underlying genetic cause was unknown

for many years . The finding was unexpected and highlights the potential of high throughput screening in diagnostic sequencing.

Conclusion: Taken together, we introduce a fast and highly efficient screening tool for variants in ALS and dementia associated genes using next generation sequencing. We present solved cases and troubleshooting of unsolved cases. In ongoing experiments we validate novel rare variants in known genes as well as in candidate genes with potential pathogenicity by segregation analysis.

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## **P-Techno-254**

### **Whole Exome and Targeted Re-Sequencing in Parkinson patients**

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Background: 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. The aim of our study is to identify novel high penetrance PD genes in familial cases. In order to exclude all known PD genes first, we enrich a set of 50 genes (candidates included) in index patients and screen for variants. If no potential or known pathogenic variant is found, we apply whole exome sequencing as the next most suitable approach as it has recently become possible to sequence all coding exons in one single experiment.

Methods: Genomic DNA is enriched using the Agilent Whole Exome SureSelect in solution kit. Sequencing is performed on one quad (¼ slide) on the SOLiD 4 platform generating approximately 80 Million 50 basepair reads per exome. Targeted re-sequencing of 50 genes (300kb in total) is performed using barcoded libraries on one oct (1/8 slide).

Results: Overall 98,4% of targeted exons were represented by reads with an average coverage of 70. In total, up to 98% of all mappable reads are on target (± 200 bp) using the Agilent enrichment and SOLiD sequencing protocol. We compared different SNP calling algorithms and third party softwares and identified on average around 400 previously unknown non-synonymous variants per individual per exome. After applying several prediction tools the number of potentially pathogenic variants can be reduced to around 50. These variants are then verified by conventional Sanger sequencing, tested for segregation and genotyped in large control cohorts.

Taken together, we introduce a fast and highly efficient screening tool for variants in PD genes using next generation sequencing. In addition, we use whole exome sequencing to identify novel high penetrance PD genes in familial cases. In ongoing experiments we validate novel rare variants with potential pathogenicity by Sanger sequencing and segregation analysis.

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## **P-Techno-255**

### **Disease gene identification by exome sequencing**

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Exome resequencing is a potentially powerful technique for identifying the genetic cause underlying monogenic Mendelian disorders. We have successfully applied this method to unravel the cause of a dominant disorder by analyzing exomes of 4 affected individuals with Schinzel-Giedion syndrome (Hoischen et al.; Nat Genet. 2010); identifying heterozygous de novo mutations in SETBP1. All missense mutations clustered to a ultra-high conserved 11bp exonic region, which suggests a dominant-negative or gain-of-function effect. In line with this, CNVs overlapping SETBP1 cause different phenotypes, which explains why conventional approaches did not reveal candidate loci for this syndrome. Exome sequencing is particularly useful for identifying gain-of-function mutations for which no other genomewide approach is applicable. More recently we showed that for a recessive condition sequencing the exome of even a single individual can be sufficient to identify the causative gene (Gilissen et al.; Am J Hum Genet 2010). In addition to mutation detection, we further evaluated the use of exome sequencing data for direct genotyping, e.g. homozygosity mapping, and CNV calling from exome coverage data.

Within 8 months we have sequenced 150 human exomes using a combination of Agilent's SureSelect(TM) exome enrichment and LifeTechnologies' SOLiD(TM) 4 sequencing, demonstrating that this approach is robust and high-throughput. Currently we are optimizing this approach to reach: 1.) higher throughput by barcoding and automation 2.) higher quality by using a 50Mb exome kit, paired-end sequencing, and optimization of CNV detection from exome data. Driven by the initial success we have expanded our operations to 3 SOLiD(TM) 4 sequencers and will soon upgrade these to the 5500XL systems.

With this, we are convinced that high-throughput exome sequencing will become the standard tool for disease gene identification in the coming years.

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## P-Techno-256

### Development of a Novel Screening Method for the Cystic Fibrosis Transmembrane Conductance Regulator Gene based on a Macroarray DNA-Chip Platform

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#### Background:

Cystic Fibrosis (CF) is a disease caused by mutations within the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The autosomal recessive disorder affects approximately one in 2500 births; hence CF is one of the most common congenital metabolic diseases in the Caucasian population. CF affects primarily the epithelial cells in the intestine, the respiratory system, the pancreas, the gall bladder and the sweat glands. Poor mucociliary clearance, coupled with excessive mucus production, causes obstructive lung disease and chronic bacterial infections leading to respiratory failure, the main cause of mortality in CF patients. The 230 kb long CFTR gene is located on chromosome 7q31.2 and encodes for a 1480 amino acid protein, which is a member of the ABC (ATP-binding cassette) superfamily of transporters. Over 1300 mutations have currently been identified in the CFTR gene associated with CF disease. For the early detection of CF, screening of potential mutation carriers is essential, but most methods, like e.g. DNA sequencing are expensive and time consuming, due to the multiplicity of mutations.

We here present a novel screening method for 47 markers within the CFTR gene, based on a cost effective DNA macroarray chip platform and a timesaving automated analysis software. The reaction chip carrying immobilised detection spots is integrated into a common 1.5 ml reaction tube. This feature enables technicians to handle the chip with equipment found in common laboratories. Moreover, instead of using a fluorescence based method, the detection scheme is based on a robust precipitation reaction, that enormously reduces the costs for the reading device.

#### Methods:

The PCR amplification is performed using 5 different PCR master mix tubes, amplifying the targets for all 47 markers. Probe design was performed according to the Santa Lucia TM calculation algorithm. The probes were designed in a way that the respective variant is located in the centre of the probe. One perfect match and one mismatch probe was designed in order to cover both possible alleles. Allele discrimination was obtained by the difference in the melting behaviour of the two probes. The final visualisation of the targets bound to the probes is performed using an enzyme coupled detection scheme, resulting in a genotype specific precipitation pattern.

#### Results:

Genotyping with respect to allele discrimination was successfully established for 47 alleles of the CFTR gene. The automated analysis software classifies genotyping results regarding the ethnic ancestry.

#### Discussion and Conclusion:

With the described method it is possible to detect 47 genetic markers of the CFTR gene in a simple and fast way. The novel developed method can help to improve the essential screening for CFTR mutations by increasing cost-effectiveness, since it delivers a high amount of information in short time and at considerably lower cost expenses if compared to other currently available assays.

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## P-Techno-257

### Validating massive parallel sequencing as a diagnostic tool for seizure disorders

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Introduction: The epilepsies are common neurological disorders with a strong genetic impact. Consequently, understanding the genetic basis of seizure disorders will provide novel insights into the underlying pathophysiology and result in novel diagnostic and therapeutic avenues. With our approach we aim to reveal the genetic basis of epileptic disorders in so far unresolved cases. The detection of causative mutations in known epilepsy genes might expand our understanding of their respective phenotypes. Furthermore, the screening of several putative candidate genes might enable to delineate known and

unknown disorders and even might contribute to a better understanding of their aetiology and pathophysiology.

**Methods:** Genomic DNA is enriched for a panel of 485 genes using a custom designed Agilent SureSelect in solution kit. 265 of the 485 genes are known causative genes for seizure disorders comprising a broad range of phenotypes, such as Idiopathic Generalized Epilepsies, Epileptic Encephalopathies, Epilepsy in X-linked Mental Retardation Syndromes, Congenital Disorders of Glycosylation, Ceroidlipofuscinosis, Coenzyme Q Deficiency, Joubert Syndrome and Related Disorders, Selected Mitochondrial and Metabolic Disorders, Neuronal Migration Disorders, Syndromic Microcephaly, Mucopolysaccharidosis, Neuro-cardio-facio-cutaneous Syndromes and others. The remaining genes on the panel represent putative candidate genes for epileptic disorders. Sequencing is performed using barcoded libraries on one Oct on the SOLiD 4 platform. The basic data analysis is undertaken with Bioscope v1.2. Additionally, we developed a diagnostic pipeline to (i) identify regions that are underrepresented with reads (coverage <20 per base), (ii) identify potentially pathogenic SNVs, (iii) identify small insertions and deletions and (iv) identify larger indels including whole exon deletions and duplications. All variants and putative deletions are then re-sequenced by the gold standard Sanger sequencing or verified by quantitative qPCR, respectively.

**Results:** We sequenced 20 so far genetically undiagnosed cases with a broad spectrum of epilepsy phenotypes. We present an overview of the number of detected sequence alterations comprising mutations, possibly damaging variants as well as benign SNPs in both well known epilepsy genes and putative candidates.

**Conclusion:** We have successfully established a fast and cost efficient genetic screening method to detect sequence variations in genes that are most likely involved in disease pathogenesis based on the patient's clinical presentation. If this restricted targeted analysis fails to reveal the genetic cause of the patient's seizure disorder the data analysis can be extended to all 485 panel genes including several potential candidate genes. By applying this approach we hope to uncover both known and unknown sequence variants and give new insights in genetic factors involved in epileptogenesis.

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## **P-Techno-258**

### **Next generation genetic testing and candidate gene analysis in retinal degeneration.**

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Retinal degenerations and dysfunctions are characterized by a tremendous genetic heterogeneity and clinical variability of symptoms. More than 20 different clinical diagnoses involve mutations in more than 170 genes. Some of them are accompanied by extraocular clinical manifestations (syndromic forms), including for example deafness, mental retardation or renal abnormalities. During the last 3 years, therapeutic intervention has become a reality for these so far untreatable diseases, although it is not yet clinical routine. Genetic heterogeneity and clinical variability require efficient and reliable diagnostic tests in order to apply specific treatment. So far, high throughput genetic testing is not available for patients with these diseases.

We make use of next generation sequencing and established an efficient diagnostic strategy to screen for mutations in 168 known retinal disease genes. In addition, a few thousand candidate genes have been selected and are also included in the screening. Two genomic DNAs with 31 known sequence alterations in ABCA4, PRPH2, and ROM1 have been used as positive controls to evaluate the reliability of the technique. We confirmed more than 90% of the previously detected variants including missense and nonsense mutations as well as a single base pair insertion. Coverage of the corresponding genomic positions showed high variability. In addition, 14 patient samples were analyzed for causative mutations and several potentially pathogenic sequence alterations were detected.

This strategy will lead to the identification of disease-causing mutations in known genes but also in novel genes, which will unravel new biological processes underlying these diseases. Moreover, this approach will enable us to detect possible disease-modifying sequence variations.

**HAR4 and human brain development - new insights from comparative gene expression, 2D-3D structures and interactomes**

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The most prominent features that distinguish our species from other primates are the enlarged brain size and cognitive abilities in humans. The specific brain structure and function has its basis in the human genome and transcriptome. We have investigated non-coding DNA elements, called human accelerated regions (HARs) that were rapidly evolving since the human/chimpanzee divergence 6-8 million years ago and are generally assumed to play an important role in gene regulation. To support a role for HARs in human brain evolution, we examined the expression patterns, RNA secondary and tertiary structure and interaction partners of genes in close proximity to these regions. HAR4 is relatively long and thus its folding pattern may be particularly interesting. 2D and 3D models were estimated by bioinformatic means for human, chimpanzee, gorilla, orang-utan, macaque, lemur and spider monkey HAR4 sequences. Gene expression data were generated from frontal cortex tissue of humans, chimpanzees, baboons and marmosets using Illumina human bead arrays. To largely exclude artefacts due to sequence mismatches between humans and other primates, we aligned the 50mer oligonucleotide probes spotted on the array to the respective sequence homologues of the analyzed primates. Only interspecific hybridization to probes with no or at most one nucleotide sequence difference was considered as reliable. ATBF1, a transcriptional repressor that is known to be upregulated in schizophrenia, showed a 1.9, 2.9 and 6.4 fold upregulation in chimpanzee, baboon and marmoset brain, respectively, compared to humans. Interacting genes that have been associated with similar functions and/or diseases (brain development, autism, schizophrenia) showed the same expression differences between humans and primates. In the secondary and tertiary structure of the RNA, significant differences in the general shape and folding patterns between human and primate sequences were observed. Taken together, we show by RNA folding and by interaction data combined with gene expression analysis a number of features discriminating between primates and man around the HAR4 RNA and its interactions.

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**P-Techno-260**

**Predicting the disease potential of gene mutations with MutationTaster**

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The evaluation of the disease potential of DNA alterations by wet lab means is time and cost intensive. Especially when it comes to Next Generation Sequencing projects and thousands of variations have to be tested for their possible effect, in silico approaches are inevitable.

Here we present MutationTaster, an automatic solution to disease potential prediction. MutationTaster performs various tests both on protein and DNA level which are then scored by a Bayes classifier. In contrast to similar tools, MutationTaster is not limited to single amino acid substitutions. Besides, it has a higher performance and is much faster. To streamline deep sequencing projects, MutationTaster includes an analysis pipeline compatible with any of the three major NGS platforms.

Since its official release in 2009, the protein and genetic data used by MutationTaster was updated several times and a better splicing model was developed. Further harmless polymorphisms and disease mutations were employed to train and optimise the software.

MutationTaster has already become a widely used prediction tool with far more than 1,000,000 queries by external groups each day.

In this presentation, we will illustrate the biological tests included in MutationTaster. We will give examples to demonstrate the intuitive use with single alterations detected by Sanger sequencing. Additionally, we will show how NGS data can be quickly analysed with our pipeline and MutationTaster's batch query system. Finally, we will highlight why MutationTaster performs better than other tools and which limitations exist.

## **P-Techno-261**

### **Finding disease genes in consanguineous families with HomozygosityMapper**

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Linkage analysis in consanguineous families poses high demands on IT infrastructure and cannot make use of all genotypes provided by modern genotyping arrays. Besides, linkage software is difficult to handle. The analysis is therefore usually not performed by the researchers or physicians themselves but by dedicated bioinformaticians. The whole process is hence extremely time consuming and requires fast and efficient communication between clinicians and bioinformaticians – a delicate matter.

Here we present a novel version of HomozygosityMapper, our web-based application for homozygosity mapping. It is extremely easy to use: researchers can upload their genotype files directly from the genotyping device, define patients and unaffected relatives and analyse their data. They do not have to care for complex pedigree files, correct genetic maps, allele frequencies or genome builds. Within a few minutes, all possible disease regions are detected, scored and displayed in a graphical interface. Users can also zoom into the underlying genotypes and inspect them visually. To eventually identify the disease gene, HomozygosityMapper provides a direct interface to our candidate gene search engine GeneDistiller.

Since its official release, more than 3.2 billion genotypes from more than 1,500 different projects have been analysed with and permanently stored in HomozygosityMapper. Many user requests have been integrated since then; it is now possible to either exclude genetic heterogeneity or to allow a far greater degree of heterogeneity than before. Furthermore, HomozygosityMapper is no longer restricted to humans and was already successfully applied to identify the gene responsible for generalised progressive retinal atrophy in dogs.

In this presentation, we will demonstrate the use of HomozygosityMapper and its integration with GeneDistiller on the example of lipodystrophy. Additionally, we will describe other settings which are ideally suited for our software such as the search for recessive founder mutations and loss of heterozygosity studies. We will also show how data can be shared with collaborators and eventually published on the HomozygosityMapper website.

HomozygosityMapper is freely available at <http://www.homozygositymapper.org>.

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## **P-Techno-262**

### **Hunting disease genes with GeneDistiller**

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Linkage analysis is still the first choice in family-based studies. However, linkage intervals usually comprise hundreds of genes, making the determination of candidate genes a cumbersome task.

Here we present a new release of GeneDistiller, our search engine for candidate genes. GeneDistiller integrates information from various databases in a web application with an easy-to-use interface. In contrast to purely automatic solutions, GeneDistiller is not limited to one approach, i.e. literature, co-expression or protein interaction network analysis. It hence allows the researchers to use their full background knowledge of the diseases to define a suitable candidate gene.

Since its introduction, GeneDistiller was continuously improved according to user requests. Sequencing primers for all known exons of a gene, including all transcripts, can be designed with one click. GeneDistiller now features more data, such as the Human Phenotype Ontology and the STRING protein interaction database, both of which can be used to search for similarities with known disease genes. It also offers output as tabular plain text files thus facilitating queries by computer programs. Additionally, it produces Microsoft Excel spreadsheets allowing researchers to easily highlight and comment relevant parts of the results. Furthermore, the integration with HomozygosityMapper has been improved so that homozygosity around the potential disease gene can be used as filter and prioritisation criteria.

In a recent study, we successfully applied GeneDistiller to identify the gene causing lipodystrophy. On this occasion, we will re-enact our strategy in this case and give an overview about the functional scope of GeneDistiller. We will also briefly demonstrate the use of GeneDistiller on other diseases where the background knowledge of the disease led to completely different strategies. Finally, we will discuss the pros and cons of GeneDistiller compared to purely automatic approaches.

GeneDistiller is freely available at <http://www.genedistiller.org>

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

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Hereditary retinal dystrophies (RDs) and optic nerve degenerations (ONDs) are amongst the genetically most complex and heterogenous conditions. Up to now more than 180 genes have been described to be implicated in RDs and ONDs. However it has been estimated that mutations in the known genes account for only ~ 50% of all affected subjects. Up to now genetic testing on a diagnostic basis was only offered for a selection of genes and certain subgroups of clinical diagnoses, or already known mutations (i.e. Asper chips). For many genes, no prevalence data are available. In addition, current genetic diagnostics based on mutation scanning techniques (e.g. HRM, dHPLC, SSCP) and Sanger sequencing on capillary sequencing machines frequently takes several month or even years and, due to the need for the investigation of many large genes, is laborious and cost-intensive. Such considerations, as well as the demand to clarify the remaining 50% of unsolved cases, have resulted in an initiative to transfer hereditary ophthalmic disease diagnostics to the high-throughput sequencing (HTS) arena.

Methods: Genomic DNA is enriched for the coding and flanking intronic/UTR sequences of a panel of 190 genes known to be associated with RD and OND diseases using a custom designed Agilent SureSelect in solution kit. Sequencing is performed using barcoded libraries on a single Oct on the SOLiD 4 platform. The basic data analysis is performed with Bioscope v1.2. Additionally, we developed a diagnostic pipeline to (i) identify regions that are underrepresented with reads (coverage <20 per base), (ii) identify potentially pathogenic SNVs, (iii) identify small insertions and deletions and (iv) identify larger indels including whole exon deletions and duplications. All variants and putative deletions are then re-sequenced by the gold standard Sanger sequencing or quantitative PCR, respectively. The gene list and the Agilent SureSelect Design are updated, extended and balanced with each round of ordering of the Agilent SureSelect in solution kit.

Results: We present the results of the high throughput analysis in 50 patients. First, we analyzed the number of targets captured per individual with coverage sufficient for SNP calling and the overall percentage of reads on target. Then, we performed SNV and indel calling. We will demonstrate how so far genetically undiagnosed cases could be solved by our newly developed method. Most interestingly, we are able to detect different types of mutations including SNVs, as well as copy number variations ranging from small, large up to whole exon deletion.

Conclusion: We have successfully established a HTS platform for known RD and OND genes as a tool to perform in-depth massively parallel mutation screening with the aim to define the unbiased prevalence of these genes, to elucidate di- or polygenic mechanisms of the disease and to support refined phenotype/genotype correlation.

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## **P-NORMAL VARIATION / POPULATION GENETICS / GENETIC EPIDEMIOLOGY / EVOLUTIONARY GENETICS**

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### **P-NormV-264**

#### **Association of mitochondrial haplogroups and Control Region polymorphisms with Age-Related Macular Degeneration: a case control study**

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Age-related macular degeneration (AMD) is a major cause of irreversible vision loss among the elderly in the developed world. Studies have implicated genetic and exogenous factors, including oxidative stress in the development of AMD.

Mitochondria play a central role in cell survival, as they supply almost all of the energy of a cell. Thus organs requiring large amounts of energy, such as brain, heart, muscle and retina, are dramatically affected by mitochondrial dysfunction. Furthermore, the mitochondrial electron transport chain is the main producer of reactive oxygen species (ROS) and the retina is highly susceptible to oxidative stress. Therefore, variations

in the mitochondrial genome could contribute to the development of AMD by modification of intracellular ROS production.

A study in Australia reported an association of haplogroup (HG) H with a lower prevalence of AMD (Jones et al., 2007), and a study in the US reported higher frequencies of the mitochondrial Control Region (CR) polymorphisms C16069T and T16126C in patients with AMD compared to controls (Udar et al., 2009).

The aim of the present study was to analyse the frequency of mitochondrial HGs and polymorphisms of the CR of the mitochondrial DNA in patients with AMD of Middle European descent in Austria.

This study included patients with AMD (n=202) and a control group 1 (n=468), both enrolled at the Department of Ophthalmology, Paracelsus Medical University, Austria, and a second control group (n=1598) (control group 2), enrolled at the Department of Internal Medicine.

The nine major European HGs and the CR polymorphisms were identified by means of primer extension analysis and sequencing, respectively.

After applying logistic regression involving the confounding variables age and sex, mitochondrial HG H was found with a significantly reduced prevalence in AMD (AMD vs. control group 1: 36.1% vs. 44.9%,  $p=0.019$ ; AMD vs. control group 2: 36.1% vs. 44.0%,  $p=0.035$ ). In addition, the following polymorphisms of the mitochondrial CR were significantly associated with AMD: C16069T (AMD vs. control group 1: 15.8% vs. 9.5%,  $p=0.042$ ), T16126C (AMD vs. control group 1: 28.7% vs. 19.0%,  $p=0.017$ ) and T152C (AMD vs. control group 1: 27.7% vs. 18.0%,  $p=0.009$ ; AMD vs. control group 2: 27.7% vs. 22.7%,  $p=0.026$ ). Furthermore, a trend of a higher frequency of HG J in AMD was observed (AMD: 14.9%; control group 1: 9.8%; control group 2: 11.1%), which is consistent with the fact that C16069T and T16126C polymorphisms are associated with mitochondrial HG J.

In conclusion, our data confirm an association of AMD with mitochondrial DNA variability, as previously observed in Caucasians, recruited in Australia and the US.

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## P-NormV-265

### A potentially misleading marker SNP for CAD risk evaluation: When ancestry matters

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Several large scale studies have recently described the variant allele of dbSNP rs3798220 in LPA to be associated with an increased risk for coronary artery disease (CAD; odds ratio app. 1.7). The LPA locus controls the quantitative apolipoprotein(a)/Lipoprotein(a) (apo(a)/Lp(a)) trait and encodes apo(a). apo(a) and a LDL-like particle form Lp(a), which shows significant inter-population differences in its plasma concentration but is highly heritable. In all populations, a large fraction of the variation in Lp(a) levels is explained in a causal way by a transcribed copy number variation (CNV) in LPA, the KIV-2 CNV, and the resulting apo(a) isoforms of variable size. There is an inverse correlation of apo(a) size with Lp(a) plasma concentrations. In Europeans, high Lp(a) plasma concentrations together with short apo(a) proteins have been established as a risk factor for atherothrombotic disease. With the advance of cheap high throughput genotyping assays, SNPs become feasible for diagnosis of risk alleles in LPA, often replacing the laborious KIV-2 CNV size assessment. In individuals of European descent, where the variant allele of rs3798220 is associated with high Lp(a) and short KIV-2 CNVs alleles, this SNP has been confirmed to serve this purpose. Here, according to published studies, its population minor allele frequencies range from app. 1 to 2 %. In South Eastern (Thai, n = 155) and Eastern Asian (Japanese n = 200, Chinese n = 200) populations we have analysed by TaqMan® Assay and re-sequencing, the variant allele reaches much higher frequencies (7.1 to 11.5 % resp.). In both North and South Indian populations (n = 122, resp. N = 347) it is very rare (MAF ~ 0.15%), this also applies for CAD cases from these populations. In autochthonous African populations the variant allele has not yet been observed at all, as our analysis in samples from Gabonese Bantu (n = 119), Khoi San (n = 331) and Egyptians (n = 109) has confirmed.

The variant allele of rs3798220 is not associated with elevated Lp(a) or short KIV-2 CNV alleles in Asian populations. This argues against it having any causal effect on Lp(a) levels. Instead, the strong LD of the variant allele with short KIV-2 CNV alleles (app. 73 kb upstream) can explain its association with CAD and elevated Lp(a) concentrations in Europeans. The marked differences between Europeans and Asians in respect of rs3798220 and its association with the apo(a)/Lp(a) trait make this SNP unfeasible to assess the risk for CAD in individuals of Asian ancestry or in admixed populations.

**Analyses and Comparison of Imputation Runs Based on Genotyped Datasets of Different Size**

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**Introduction**

Genotype imputation is a common used method for genome wide association analysis. This technique allows geneticists to accurately evaluate the evidence for association at genetic markers that are not directly genotyped. Genotype imputation is particularly useful for combining results across studies that rely on different genotyping platforms. Genotype imputation methods take a varying number of genotyped SNPs per person and a reference sample as input. The latter serve as a reference, from which untyped sites are derived. Genetic effects may then be calculated not only at the genotyped SNPs, but also at the imputed sites.

**Materials and Methods**

Based on imputed SNPs, strong genetic effects could be shown for the genetically complex age related macular degeneration (AMD), a major cause of blindness in industrialized countries. In a genome wide association analysis the AMD case group consisting of 335 unrelated individuals is compared to 1636 unrelated, population based controls. Both groups were genotyped on the Affymetrix GeneChip® Human Mapping 250k Styl array.

Imputation was performed, using Beagle 3.3.0 in combination with HAPMAP Release 22 data files as reference panel, consisting of 2.5 million SNPs on 60 individuals with Northern and Western European ancestry. Different regions were chosen, representing different chromosomal regions of varying linkage disequilibrium (LD) and marker density. Throughout these regions, comparisons of imputation quality and the influence of minor allele frequencies (MAF) were performed.

**Results**

Here, we have addressed the question of how much information might get lost by performing a genome wide analysis, only having data of the 250k Styl array as compared to a 500k dataset as input files for imputation. The 500k dataset consists of the Affymetrix GeneChip® Human Mapping 250k Styl array and the Affymetrix GeneChip® Human Mapping 250k Nspl array.

Analyses showed that imputation quality can be improved across all SNPs by approximately 15%, whereas the specific influence on each SNP may vary. More than 80% could generally improve their imputation quality while more than 40% showed an improvement of more than 10%. Approximately 20% increased imputation quality by 30% or more, whereas the highest increase of more than 50% could be observed at approximately 10% of all SNPs.

These results are exceeded by the well established AMD locus CFH (complement factor H), which showed an increase in imputation quality in general, in regions of small, as well as in regions of high increase of imputation quality.

**Conclusion**

After comparison one can see some increase in imputation quality of imputed SNPs in the 500k imputation run. However, this has to be weighted against higher costs of genotyping when including two instead of one array. Together, this makes genome wide association analyses with one 250k chip reasonable, particularly since the minor overall decrease in imputation quality needs to be compared to 50% cost reduction.

**CARD15/NOD2gene 3020insC mutation among Polish patients with Crohn's disease**

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**Introduction:** Crohn's disease (CD), next to ulcerative colitis (UC), belongs to Inflammatory Bowel Diseases (IBD). The etiology of the disease still remains unidentified, but it is known, that it is characterized

by polygenic background. So far, the best known gene laying in the background of CD is CARD15/NOD2 gene with insertion of cytosine mutation in the 11th exon.

Aim: the aim of the study was the analysis of 3020insC (rs5743293, p.Leu1007fsinsC) mutation among Polish patients with CD.

Materials and methods: 185 Polish patients with the diagnosis of CD were qualified for research. Insertion analysis was performed with pyrosequencing. The results were compared with an population group (213 individuals), consisting of both men and women.

Results: The CARD15/NOD2 gene 3020insC mutation was observed significantly more frequent among CD patients than in population group: 78,4% didn't have insertion in any of alleles, 16,8 were heterozygous (C/-), and 4,9% were carriers of two affected alleles (C/C). Although in Polish healthy population C/- genotype was observed (8,5%), none individuals presented genotype C/C. Further studies will focus on correlating the presence of 3020insC mutation with phenotype and localization of symptoms of the disease, especially with predisposition to malignancies of the gastrointestinal track.

Conclusions: Leu1007fsinsC mutation in CARD15/NOD2 gene is related with CD in Polish population. It remains in agreement with other studies in other populations where this mutation was related to IBD.

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## P-NormV-268

### **A novel concept for walk-away automated sample preparation for direct-to-consumer genetics and pharmacogenomic studies**

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The analysis of genetic information continues to gain importance in all aspects of life ranging from individual ancestry research to pharmacogenomic studies in drug development. Here we introduce a novel method of sample collection specifically designed for high DNA yields from saliva samples. The novel saliva stabilization buffer supersedes cooling of samples and stabilizes genomic DNA for months at room temperature. The non-invasive saliva collection method reduces puncturing-associated infection risks and is designed to integrate with automated nucleic acid extraction procedures.

In this study the saliva samples are purified using a new magnetic particle processor for variable sample volumes from 50 to 5000 µl. By a unique magnetic separation tool combined with reliable pipetting technology and a newly developed incubator the instrument automates diagnostic extraction protocols for nucleic acids with full in-process control. Furthermore, high and reproducible yields are obtained in extraction protocols of DNA from saliva and blood. As much as 90 % of contained nucleic acid are recovered in the automated process.

The yield and purity of the extracted DNA is perfectly convenient for the performance of genotyping procedures that meet highest quality standards of molecular genetic diagnostic applications. Genotyping with DNA templates extracted with this method revealed a reproducible high rate of analyzable results respectively a low rate of genotyping failures.

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## P-NormV-269

### **Severely reduced human genetic diversity on the island of Nias/Indonesia - epidemiological data from a large population based screening**

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The origin of the native inhabitants of Nias is unknown. A variety of attempts have been made by collecting oral literature up to 40 – 60 (!) generations (J.M.H.) and by comparative language studies and archeological excavations in caves going back about 12,000 years BP. Recently we came across an unexpected island effect (van Oven et al. 2010). An extreme bottleneck was detected by severely reduced Y-chromosomal but also to a lesser extend mtDNA diversity. This is in contrast to most other populations in Island South-East Asia. Consequently epidemiological studies should show endemic diseases not found outside of Nias and monogenic disorders which might be very rare on Nias but very common on neighboring islands and vice versa. Among more than 5,000 people we found (1) two endemic syndromes one with familial disproportionate short stature and bone dysplasia in two consanguineous sibships suggestive of autosomal recessive inheritance and one with doubled thumbs and dysmorphic feet affecting the father and his three children demonstrating an autosomal dominant mode of inheritance. (2) Two otherwise rare monogenetic disorders are very common on the island predominantly albinism and a form of X-recessive gout. The prevalence rate of albinism might even exceed clusters e.g. known in Timor or Nigeria. (3) Among

multifactorial disorders diabetes and hypertension are very common also in aggregate. These results will lay the foundation for segregation studies and whole genome scans aimed to identifying causal genes.

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#### **P-NormV-270**

### **Comparative methylation analysis of human-specific FOXP2 target genes in adult cortices of humans and chimpanzees**

*Seifert D.<sup>1</sup>, Schneider E.<sup>2</sup>, El Hajj N.<sup>2</sup>, Kondova I.<sup>3</sup>, Bartsch O.<sup>1</sup>, Haaf T.<sup>2</sup>, Zechner U.<sup>1</sup>*

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The molecular mechanisms governing language acquisition in the human brain are largely unknown. Up to now, mutations in the gene encoding the transcription factor FOXP2 (forkhead box P2) are the only reported monogenic cause of language and speech disorders in humans (Lai et al., 2001). A recent study analyzing differential gene expression in human neuronal cells overexpressing human or chimpanzee FOXP2 as well as in human and chimpanzee brains provided evidence for human-specific transcriptional regulation of FOXP2 target genes to be implicated in the development and evolution of language circuitry in humans (Konopka et al., 2009). We set out to investigate if the observed differential expression of FOXP2 target genes may be due to differential methylation of their promoter regions in human and chimpanzee brains. Overall, we quantified the methylation levels of 19 differentially expressed target genes in adult frontal cortices of 13 humans and 6 chimpanzees using bisulphite pyrosequencing. For three genes, the observed mean methylation levels were significantly different in human and chimpanzee samples ( $p < 0.05$ ): The TAGLN gene showed significantly lower mean methylation levels in the human samples (30.2% methylation in humans vs. 38.7 % methylation in chimpanzees) whereas the mean methylation levels of the NPTX2 (7.6% methylation in humans vs. 4.7 % methylation in chimpanzees) and GJA12 (37.3% methylation in humans vs. 27.6 % methylation in chimpanzees) genes were significantly higher in the human samples. The remaining 16 genes displayed very similar mean methylation levels that did not differ significantly in human and chimpanzee samples. Our findings suggest that differential methylation of regulatory regions is involved in regulating differential expression of at least a subset of FOXP2 target genes in human and chimpanzee brains. In addition, our study provides additional support for the use of comparative methylation analyses as an important methodology to detect evolutionary changes in gene regulation that have operated in human-specific brain development and function.

Lai et al., 2001, Nature 413: 519–523

Konopka et al., 2009, Nature 462:213-217

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#### **P-NormV-271**

### **Evidence for an increase in the sex ratio of normal newborn and children with trisomy 21 due to the Chernobyl reactor accident**

*Sperling K.<sup>1</sup>, Koerblein A.<sup>2</sup>, Neitzel H.<sup>1</sup>*

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The sex ratio at birth is a prevalence measure. Firstly it reflects the relative number and the fertilising capacity of sperm with either an X or Y chromosome (primary sex ratio). Secondly, it reflects the relative survival of male and female conceptuses (secondary sex ratio). The secondary sex ratio among newborn of caucasian ancestry is about 106 males to 100 females. Changes occur over time, however, they are very small and have been explained by improvement in nutritional standards, general health and safer family planning. After the Chernobyl accident the population of large parts of Europe was exposed to additional ionizing radiation. Here, we have analysed whether this event had an effect on the sex ratio among normal newborn and those with Down syndrome (trisomy 21). The study is based on the annual live birth data per gender from 1950 to 2009 in Germany (and separately in Bavaria) and the relevant monthly data in all 96 Bavarian counties (Landkreise) from 1980 to 1992. In addition, the sex ratio was calculated in newborn with Down syndrome, diagnosed in West-Berlin from 1980 to 1991 and fetuses with trisomy 21 diagnosed prenatally 1986 in Germany. We found a significant increase in the sex ratio in 1987 in Germany (and Bavaria) beginning in January 1987 and accompanied by a shift in the sex ratio towards male Down syndrome probands in Germany. The data point to a causal relationship between radioactive exposure and a shift in the sex ratio in man. This is compatible with an epigenetic effect of low doses of ionizing radiation around conception leading to a loss of female embryos. Moreover, there is clear evidence that chromosome disjunction during human oogenesis is also an error-prone process and, consequently, low dose irradiation around fertilization, when the first and second oogonal meiotic divisions take place, leads to an increase in

trisomy 21. The distorted sex ratio in the trisomy 21 cases is, therefore, considered to be the combination of faulty epigenetic processes and meiotic non-disjunction induced by low-dose irradiation around fertilization. Thus, these data throw a new light on the well known but poorly understood phenomenon of male excess in DS.

## **P-PRENATAL DIAGNOSIS / REPRODUCTIVE MEDICINE**

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### **P-Prenat-272**

#### **The importance of the M2/ANXA5 haplotype for prothrombotic recurrent pregnancy loss and obstetric complications**

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Recurrent pregnancy loss (RPL) of prothrombotic etiology accounts for 40 – 70% of RPL cases. The haplotype M2/ANXA5 is a confirmed risk factor for RPL in populations of Central Europe, Germans and Italians. With a carriership incidence of 15 to 19% it is an important hereditary risk factor to consider as RPL predisposition. The population risk measured is 2.5 - 3 and grows to 4 when compared with uneventful pregnancies. Follow up studies to our original work show in addition higher risk for various obstetric complications related to prothrombotic placental pathology: preeclampsia (PE), gestational hypertension (GH), fetal growth restriction (FGR) and pregnancy-related venous thromboembolism (VTE). Genetic analyses of other populations confirm similar RPL risk for M2/ANXA5 carriers. Thus, we could argue that M2/ANXA5 is generally conferring elevated risk for prothrombotic placental pathology and in contrast to the 'classical' thrombophilic factors, FVL and PTm, this is more pronounced in early pregnancies, < 15 wks.

In our original study (2007) we demonstrated that M2 reduces the promoter activity of the ANXA5 gene in vitro. Our last expression analyses indicate that ANXA5 mRNA is allele-specifically reduced in placentas of M2 carriers. Italian data previously showed reduction of the ANXA5 mRNA in placentas of PE and FGR patients. Thus, the offending event in these prothrombotic pregnancy complications seems to be related to the expression of placental anticoagulant protein, annexin A5 in the relevant organ. Indirect evidence from anticoagulant treatment in antiphospholipid syndrome (APS) patients suggests that it could be successful in M2/ANXA5 carriers, because reduced placental presentation of ANXA5 is the underlying pathology in APS-related RPL.

Since M2/ANXA5 is far more abundant than FVL and PTm in Western populations and the associated RPL risk for carriers is similar, molecular diagnostics for this haplotype could be proposed to risk populations, such as women with previous unexplained pregnancy losses, women over 35 and couples seeking assisted reproduction. Diagnostic solutions can be very direct, employing proprietary haplotypization technology.

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### **P-Prenat-273**

#### **Detection of trisomy 22 in chorionic villus cells: a challenge in prenatal diagnostics**

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Trisomy of chromosome 22 is the second most common autosomal trisomy found among spontaneous abortions, accounting for 3-5% of all cases. Miscarriage usually occurs within the 8- to 14-week gestation range.

Survival of cases with nonmosaic fetal trisomy 22 to term is very rare. Of all nonmosaic trisomies, with the exception of trisomies 13, 18 and 21, it is only trisomy 22 which may be associated with limited postnatal survival.

Nonmosaic trisomy 22 is characterized by intrauterine growth restriction, microcephaly, hypertelorism, epicanthal folds, hypoplastic or low set ears, midface hypoplasia, hypoplastic distal phalanges and genitalia anomalies in males. Other frequent features include cleft palate, cardiac and/or renal anomalies and anal atresia/stenosis.

However, the detection of trisomy 22 in mosaic or nonmosaic state in chorionic villus cells is a diagnostic challenge as the degree of concomitant fetal involvement may vary considerably. Careful follow-up analyses in close cooperation of gynecologist and human geneticist are therefore mandatory to clarify a specific prenatal situation.

We present cytogenetic and clinical data of a case of trisomy 22 diagnosed in chorionic villus cells. Chorionic villus sampling (CVS) was performed due to an increased level of the free  $\beta$ -chain of the human choriongonadotropin (free  $\beta$ -HCG, 14.4 MoM) in maternal serum in the 13th week of gestation. Chromosome analysis revealed a trisomy 22 in all 15 cells analyzed. Cordocentesis was performed consecutively and none of 200 fetal blood cells analyzed presented a trisomy 22.

As the inconspicuous cytogenetic result of the fetal blood sample does not exclude a fetal mosaic trisomy 22 and ultrasonographic examinations revealed a hypoplastic left heart syndrome, parents decided to terminate the pregnancy. Post mortem, dysmorphic features were noted. Details of our findings are discussed in the context of the literature.

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#### **P-Prenat-274**

### **Cryptic unbalanced translocation in a fetus with holoprosencephaly and pulmonary atresia detected by prenatal array CGH**

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We report on a first spontaneous pregnancy of a 39-year-old woman after 15 years of infertility. In the 21st week of pregnancy the following sonographic abnormalities were present in the fetus: microcephaly, protruding eyes, alobar holoprosencephaly, hypoplastic right heart with pulmonary atresia and dysplastic tricuspid valve. Chromosome analysis of amniotic fluid cultures showed a normal male karyotype. Parental chromosomes were investigated because of the long unexplained infertility and were normal with standard karyotyping. In order to find the cause of the multiple unusual malformations an array CGH analysis was performed revealing an 18 Mb deletion of 7q34-qter and an 11 Mb duplication of 3q27.2-qter.

An unbalanced translocation was suspected and verified by FISH analysis with subtelomeric probes of chromosome 7 and 3 on cultured amnion cells. FISH analysis on parental chromosomes detected the father as a carrier of the balanced translocation t(3;7)(q27.2;q34). The couple chose to terminate the pregnancy. Examination of the fetus showed facial dysmorphism (hypotelorism, flat nose, low set ears, protruding eyes) and confirmed the alobar holoprosencephaly and the complex heart defect with atresia of the pulmonary artery, dysplastic tricuspid valve with intact ventricular septum. The holoprosencephaly can be explained by loss of one copy of the SHH gene on 7q36. There is no gene located for hypoplastic right heart with pulmonary atresia without VSD, however it is thought to occur as a single mendelian trait in a subset of patients (OMIM 265150).

The parents were counselled that early prenatal diagnosis is possible in a next pregnancy by chorionic villi analysis including FISH. PGD could help in their case in achieving a pregnancy without an unbalanced translocation.

This report highlights the importance of including array CGH analysis in the diagnostic work up of fetuses with multiple malformations. Prenatal array CGH should be added to conventional cytogenetics if more than one organ system is involved.

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#### **P-Prenat-275**

### **Detailed sonographic, cytogenetic and molecular findings in five new prenatal cases with tetrasomy 9p**

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An isochromosome is a mirror-image chromosome with two identical arms on either side of the centromere. When present as a supernumerary chromosome, it imposes a tetrasomic state for the chromosomal arm concerned. The mode of isochromosome formation is a misdivision at the centromere. Subsequent mitotic loss in one cell lineage can lead to a mosaic condition (Gardner and Sutherland 2004). Additionally isodicentric isochromosome of the short arm of chromosome 9 is a rare chromosomal abnormality and there is described a wide tissue-to-tissue variation in mosaicism.

Here we report about five prenatal cases with supernumerary dicentric isochromosome 9p [idic(9p)] and elaborately description of ultrasound findings, cytogenetic and molecular analysis. 4/5 cases showed several ultrasound observations. Only in one case the ultrasound was normal and amniocentesis was done due to advanced maternal age. 3/4 fetuses had cleft-lip-palate, 2/4 had congenital heart defects and 3/4 brain malformation including Dandy-Walker malformation, dilation of cisterna magna and/or corpus callosum agenesis. Additionally sonographic results were kidney anomalies in two cases, IUGR in two and limb anomalies in three cases. In all cases the patients decided to terminate the pregnancy after comprehensive genetic counseling.

Furthermore, we performed detailed cytogenetic/molecular studies on different fetal tissues. For all isochromosomes the breakpoints were determined to the long arm q11/q12. In two cases we found in 100% of the cells of amniotic culture the supernumerary idic(9p), whereas three cases had varying degrees of mosaic idic(9p). Moreover in three cases we investigated chorion villi, muscle, skin, cartilage and Achilles`tendon by cytogenetics, FISH and array-CGH. In one case chromosome analysis of cultured fibroblasts of the skin detected no idic(9p), but the array-CGH on DNA of the skin surprisingly revealed the existence of this aberration in mosaic. In one case we could determine maternal origin of the supernumerary idic(9p).

Phenotypes of isochromosome 9p described previously in the literature are very variable, but there are some common ultrasonography features like Dandy-Walker malformation, cleft lip/palate, IUGR and limb malformations we observed in our four prenatal cases.

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## **P-Prenat-276**

### **Overexpression of mouse Pxt1 results in male infertility.**

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Mouse peroxisomal testis specific 1 (Pxt1) gene encodes a male germ cell-specific expressed protein which contains functional peroxisomal targeting signal (PTS). The expression of Pxt1 starts at the primary spermatocyte stage of spermatogenesis. In order to analyze the function of PXT1 in the testis, we have generated transgenic lines overexpressing the c-MYC-PXT1 fusion protein under the control of hPGK2 promoter. Because transgenic male founders were infertile, female founders were generated to establish transgenic lines. The expression of c-MYC-PXT1 fusion protein was detected first in primary spermatocytes. Histopathological analysis revealed that increased apoptosis in male germ cells is the main reason for infertility. We have observed the degeneration of germ cell epithelium and we found that spermatocytes are the most affected cells. In older animals, Sertoli cell only-like phenotype was observed. These results pointed us to analyse the pro-apoptotic character of PXT1. At the N-terminus of PXT1 we could identify a pro-apoptotic BH3-like domain. Also overexpression of the EGFP-PXT1 fusion protein in different cell lines resulted in apoptosis, while after deletion of the BH3-like domain apoptosis was strongly reduced. Our results indicate that PXT1 acts as a BH3-only pro-apoptotic protein. To elucidate which molecular apoptotic pathways are activated by overexpression of PXT1, a qPCR array experiment was performed. Here we present the candidate genes for the PXT1-induced apoptotic pathway. We found, that these genes are differential expressed in transgenic testis as compared to the testes of wild type animals. Taken together, we provide the evidence that peroxisomes might be directly involved in apoptosis regulation during spermatogenesis.

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## **P-Prenat-277**

### **Successful polar body-based preimplantation genetic diagnosis for aniridia**

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Aniridia is a severe congenital ocular malformation caused by mutations in the paired box gene-6 (PAX6; 607108) on chromosome 11p13. Here we report on a woman with aniridia who suffers from high-grade limited vision, cataract and nystagmus. A de-novo autosomal-dominant splice site mutation IVS10+2T>C in one allele of the PAX6-gene was previously diagnosed. For children the risk of inheriting the disease from their mother is 50%. The patient and her husband asked for polar body diagnosis as prenatal diagnosis was not an acceptable option for them.

We developed an individual test system to detect the mutation with two flanking single nucleotide polymorphisms (SNPs) simultaneously. Detection was performed using an initial multiplex-PCR amplification of the DNA followed by melting curve analysis after real-time PCR with specific FRET probes (fluorescence resonance energy transfer probes, TIB Molbiol). Testing of both polar bodies was possible in less than 20 hours after intracytoplasmic sperm injection (ICSI) according to the German Embryo Protection Law.

The linkage between the two SNPs and the mutation was determined by the analysis of single chromosomes 11 isolated by microdissection from GTG banded metaphases of our patient. Later on the results were confirmed in blood cells of her unaffected son who was born in the meantime. Validation was done by analysing 75 single blood cells of our patient and revealed an allele dropout rate of less than 12% for the mutation screening and the SNP test systems respectively. Incorrect results in all three test systems were seen in two cells indicating an error rate of 2-3%.

In the first in vitro fertilisation (IVF) cycle 27 oocytes were retrieved after hormonal ovarian stimulation. ICSI was performed with 16 mature oocytes. One hour later the first polar bodies were isolated from 15 oocytes. Nine hours after ICSI the second polar bodies could be removed from 12 oocytes. Four oocytes were diagnosed preconceptionally to contain the wildtype allele and were recommended for transfer. One of these embryos showed developmental arrest, two embryos were transferred on day 5 and one embryo was vitrified. Transfer of the first two embryos was followed by an initial elevation of human chorionic gonadotropin (hCG) but pregnancy did not continue. No pregnancy was achieved with the vitrified embryo that was transferred two months later. In a second IVF cycle, again 27 oocytes were obtained and 16 of them did undergo ICSI. This time analysis of 15 first polar bodies and 12 second polar bodies resulted in six oocytes carrying the wildtype allele. Only three of them differentiated normally and all three were transferred on day 5 but again pregnancy was not ongoing although hCG was initially increased.

In conclusion, maternal aniridia mutation was detected successfully and reliably by polar body diagnosis.

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## P-Prenat-278

### **Mutation analyses of the TSPYL1 gene in infertile patients with azoospermia, cryptozoospermia or oligozoospermia**

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Background: Male infertility is responsible for approximately 50% of all couple infertilities, and it is thought that genetic abnormalities account for 15%-30% of causes of male infertility. Recently, mutations in the TSPYL1 gene have been reported to be associated with an autosomal recessive sudden infant death with dysgenesis of the testes syndrome (SIDDT) and with 46,XY disorder of sex development (DSD) and male idiopathic infertility, respectively. Currently, five autosomal intronless TSPY-like genes (TSPYL1, TSPYL3, TSPYL4, TSPYL5, TSPYL6) have been identified in the human genome encoding proteins that share a highly conserved SET/NAP domain, characteristic among TSPY/SET/NAP1 family members. Members of this protein family execute diverse functions in cell cycle regulation, DNA replication, nucleosome assembly, transcription and translation by direct protein interaction via their SET/NAP domains. Notably, a specific frameshift mutation (c.457dupG) in the TSPYL1 gene, which leads to a truncation of the SET/NAP domain, has been demonstrated to be causative for the SIDDT syndrome in an Amish family. Although it was further shown, that TSPYL1 is able to bind in vitro to the zinc finger protein 106 (ZFP 106) at its SET/NAP domain, the exact function of TSPYL1 in testicular development and spermatogenesis is uncertain.

Methods: Mutation analysis was performed for TSPYL1 in a cohort of 106 infertile men with idiopathic non-obstructive azoospermia, cryptozoospermia and oligozoospermia, and in a control group of up to 105 men with proven paternity.

Results: 8 known SNPs were detected in the TSPYL1 gene of which none was significantly associated with male infertility. One azoospermic man was heterozygous for the yet undescribed missense mutation c.419C>G (p.Ser140Cys). This sequence variation was not observed in 102 fertile men with proven paternity. Additionally, one out of 101 fertile men was shown to be heterozygous for the missense change c.487G>A (p.Val163Ile). The p.Val163Ile variant was not identified in 106 infertile men. We analyzed both missense mutations with the online-software "Mutation Taster" ([www.mutationtaster.org](http://www.mutationtaster.org)), "PolyPhen2" (<http://genetics.bwh.harvard.edu/pph2/>) and "PMut" (<http://mmb.pcb.ub.es/PMut/>), and none of these programs predicted a disease causing effect for c.419C>G (p.Ser140Cys) and c.487G>A (p.Val163Ile), respectively. These assessments await confirmation by functional studies.

Conclusions: Mutations in the TSPYL1 gene do not seem to play a major role in the pathogenesis of idiopathic male infertility. Mutation screening of the TSPYL1 gene can currently not be recommended in diagnostics of idiopathic male infertility.

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## P-Prenat-279

### **Preimplantation genetic diagnosis: Array CGH after trophoctoderm biopsy**

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Preimplantation genetic diagnosis (PID, PGD) of pluripotent trophoctoderm cells has been legalized by the German Supreme Court in July 2010 for families at high risk for severe genetic disorders. So far, investigations to detect maternal derived mutations or chromosomal translocations were carried out on polar

bodies using PCR based techniques or Fluorescent-in-situ hybridisation of selected chromosome probes. Thus, only the maternal component of the fertilised oocyte could be tested. The court decision from Juli 2010 has changed this setting. Through the permission to test pluripotent trophoblast cells after trophectoderm biopsy (TEB) at day 5 after fertilisation both the maternal and the paternal genetic component can be investigated. In collaboration with the Kinderwunschzentrum Regensburg and the Zentrum für Humangenetik Regensburg, we performed array CGH analysis (24sure and 24sure+, BlueGnome) on trophectoderm cells from families at risk for chromosomal translocations and monogenic diseases (Muscular Dystrophy, Duchenne Type, DMD). On day 5, blastocysts were subjected to mechanical trophectoderm biopsy. Cell samples contained 1-10 cells derived from the pluripotent trophoblast cells.

We report here our results of the array CGH analysis of trophectoderm cell samples from seven IVF cycles. The objective of the analysis was the investigation of mosaicism which occurs within one single biopsy (sample separated in two parts), the detection of aneusomies of entire chromosomes or chromosome segments derived from an unbalanced translocation and the detection of a small deletion within the DMD gene. The preliminary data show that the cellular integrity of the cell sample is critical for the success rate of whole genome DNA amplification (WGA) and subsequent genetic testing. Amplification failed for partially lysed cells or samples derived from embryos arrested in development, suggesting biopsy induced problems and/or degradation effects. Using Array CGH, normal euploid samples were detected as reliable as chromosomal aneuploidies (monosomies and trisomies). Further, the application of the 24sure and 24sure+ protocols clearly demonstrates the power of the Array CGH to detect even a 300 kb deletion in the DMD gene. In addition, Array CGH analysis revealed the appearance of chromosomal mosaicism in two separated samples from a single biopsy.

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## P-Prenat-280

### The effects of cryopreservation on mouse sperm: epigenetic analysis of sperm and offspring

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Cryopreservation of sperm is a very cost-attractive alternative to the commonly used embryo cryopreservation for backup and maintenance of mouse breeding colonies in animal facilities. It further plays an important role for the application of assisted reproductive technologies (ARTs) in humans. Several studies already demonstrated a negative impact of ARTs like superovulation and embryo culture on the epigenome and, in particular, imprinted gene methylation after fertilization. However, the possible effects of cryopreservation on epigenetic modifications have not been thoroughly analyzed up to now. Thus, we used limiting dilution bisulfite pyrosequencing combined with SNP analysis to analyze parent-specific imprinted gene methylation patterns at single allele resolution in two cell-stage (C57BL6/J x Cast/Ei)F1 mouse embryos generated by in vitro fertilization (IVF) with frozen, respectively, fresh sperm from the same male. In parallel, the same frozen and fresh sperm samples were directly investigated for imprinted gene abnormalities by normal bisulfite pyrosequencing. Our preliminary data show aberrant genomic imprinting of the paternally methylated H19 gene (19 embryos from frozen and 28 embryos from fresh sperm derived from 8 different males) as well as the maternally methylated Snrpn (19 embryos from frozen and 32 embryos from fresh sperm derived from 8 different males) and Igf2r (8 embryos from frozen and 22 embryos from fresh sperm derived from 8 different males) genes in both embryos from frozen and fresh sperm. We found abnormalities in H19 (18.6%, respectively 28.4% abnormally methylated CpG sites in frozen, respectively fresh sperm) and Snrpn (14.9%, respectively 22.4% abnormally methylated CpG sites in frozen, respectively fresh sperm) methylation that tended to be more pronounced in embryos from fresh sperm. For the Igf2r gene, however, the observed aberrant paternal and maternal methylation patterns more markedly affected embryos from frozen sperm (40.6%, respectively 17.6% abnormally methylated CpG sites in frozen, respectively fresh sperm). Interestingly, the H19, Snrpn and Igf2r methylation patterns of the analyzed sperm samples were normal and not different between the two groups. These findings are currently complemented using a highly multiplexed gene-expression profiling system that allows us to simultaneously investigate the expression levels of 29 different pluripotency markers and candidate reprogramming genes in single embryos from frozen and fresh sperm. Our preliminary results may suggest a negative impact of sperm freezing specifically on imprinting of the Igf2r gene in two cell-embryos. The findings for H19 and Snrpn, however, rather indicate that the majority of observed methylation abnormalities is due to the IVF procedure that was used for the generation of embryos from both experimental groups. These aspects should be taken into consideration when performing ARTs.

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## P-Prenat-281

### Chromosomal abnormalities and permanent embryo arrest

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#### OBJECTIVE

In vitro fertilization makes it possible to examine the earliest stages of embryonic development. Despite strongly improved culture conditions approximately 10 – 20 % of IVF embryos show a permanent cell cycle arrest state. The underlying reasons are the subject of various research activities and probably consist of intrinsic and extrinsic factors and/or a combination of both. Among others errors of the embryonic genome activation, spindle- as well as interphase nuclear- anomalies are related to embryonic growth arrest. Of special interest for this study is an increased rate of chromosomal abnormalities found in embryos with early developmental arrest.

#### MATERIALS/METHODS

Arrested embryos used in this study were donated by patients undergoing IVF treatment for infertility, and written patient consent was obtained in each case. The study was approved by the local ethical review board and can be accomplished in accordance with the strict Austrian legal regulations, since only fertilization products without development potential are used for analysis. Embryos were considered to be arrested when no cleavage had occurred during 48 h (< 5 cells on day 3 post fertilization, < 8 cells on day 4, < 12 cells on day 5).

Blastomeres of embryos with developmental arrest were analysed using Fluorescence in situ hybridisation (FISH). Arrested embryos were classified into different categories: normal diploid (euploid), homogeneously abnormal (aneuploid), abnormal mosaics, chaotically (complex), polyploid and haploid.

#### RESULTS

To date the most frequent finding in the study was the chaotic pattern with various numerical chromosome abnormalities, which can be different from cell to cell. Polyploidy comes a close second and represent a special case, since here probably not the polyploidy is the reason for embryonic arrest, but the arrest is the cause for the polyploidy. It seems that although cellular division stops in arrested embryos DNA synthesis continues in some cases.

#### CONCLUSION

Almost 70% of the analysed arrested embryos displayed chromosomal abnormalities, suggesting that the elevated levels of chromosomal abnormalities represent a not negligible factor for developmental arrest.

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## P-Prenat-282

### A prenatally diagnosed de novo duplication of chromosome 6q (dup(6)(q15q25)): signs, symptoms and clinical course

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Pure de novo duplications of chromosome 6q are very rare. To our knowledge, there are currently only 12 cases reported in the literature. The majority of cases with duplication 6q result from malsegregation of a reciprocal translocation, leading to terminal 6q duplication and partial monosomy of another chromosome.

Although involving several different breakpoints, affected patients share phenotypic characteristics, including severe mental retardation, short stature, feeding difficulties, microcephaly, prominent forehead, downslanting palpebral fissures, flat nasal bridge, tented upper lip, micrognathia, short webbed neck, and joint contractures. Based on these findings, a „6q duplication syndrome“ has been established as a separate clinical entity.

We describe a case of a de novo 6q duplication diagnosed prenatally. Ultrasound investigations showed generalised hydrops and cardiac anomalies. Post mortem examination following intrauterine fetal death during 35th week of pregnancy revealed facial dysmorphisms (including tented upper lip), hand and feet malformations, vitium cordis, anal atresia, hydrops fetalis, and signs of the oligohydramnion-sequence. Radiographs showed signs of a hypokinesia sequence.

Chromosome analysis of fetal blood cells demonstrated a derivative chromosome 6 with a prolonged q-arm. Chromosome painting showed that the entire derivative chromosome was derived from chromosome 6. The rearranged chromosome was identified as a dup(6)(q15q25). Karyotypes of both parents were normal, indicating that the derivative chromosome resulted from a de novo rearrangement. The clinically severe phenotype seen in our case can be explained by the position of breakpoints and extent of the duplicated

region of 6q. A comparison and discussion of cytogenetic and clinical data of our case and the literature is given.

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#### **P-Prenat-283**

### **Neonatal manifestation of mucopolysaccharidosis type VII (Sly syndrome) through hydrops fetalis**

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Hydrops fetalis, characterized by abnormal accumulation of fluid in nonvascular compartments or body cavities of the fetus, is an end-stage symptom of a variety of disorders. We diagnosed a case of Mucopolysaccharidosis type VII (MPS VII, Sly syndrome) prenatally through enzyme diagnostics in amniotic fluid. Sly syndrome is a rare form of a lysosomal storage disease. This autosomal-recessive disorder is caused by the deficiency of the enzyme beta-glucuronidase, which is responsible for degrading glucuronic-acid containing glycosaminoglycans.

In ultrasound, the fetus of a non-consanguineous couple was first conspicuous by means of an increased nuchal transparency and showed progressive hydrops fetalis later in pregnancy. More common causes for nonimmune hydrops fetalis like fetal heart diseases or chromosomal aberrations were excluded. There was no detectable activity of beta-glucuronidase in amniotic fluid and sequencing analysis of the GUSB gene on fetal DNA confirmed the diagnosis of MPS VII. One of the two mutations that we found has not been described in the literature. We recommend enzyme diagnostics in amniotic fluid for cases of nonimmune hydrops fetalis, where more common causes have been excluded, to prenatally detect known autosomal-recessive metabolic disorders like MPS VII. Also, we outline the differential diagnoses of nonimmune hydrops fetalis.

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#### **P-Prenat-284**

### **Array CGH analysis in men with spermatogenic failure**

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Infertility, which affects 10-15% of all couples, is attributed to a male (co )factor in around 50%. Male infertility is mostly caused by spermatogenic failure, clinically noted as oligo- or azoospermia. However, the reasons for the decreased sperm production remain largely unclear. We hypothesised that the number or specific patterns of CNVs may disturb chromosome synapsis during meiosis and cause meiotic incompetence responsible for reduced sperm output. On the other hand, specific CNVs might demask mutations in individual genes important for spermatogenesis.

Here we describe the analysis of 89 patients with idiopathic oligozoospermia and 37 patients with Sertoli-cell-only syndrome (SCOS) by array-CGH using the 244A and the 400k Array Set (Agilent Technologies) using DNA extracted from peripheral blood cells. We compared the array-CGH results of these 126 patients with the results of 100 normozoospermic men. All patients and controls were selected retrospectively.

The mean number of CNVs and the amount of DNA gain/loss were comparable between all groups (patients and controls). Ten recurring CNVs were only found in patients with severe oligozoospermia, three only in SCOS and one CNV in both groups with spermatogenic failure but not in normozoospermic men. Sex-chromosomal, mostly private CNVs were significantly overrepresented in patients with SCOS. CNVs found several times in all groups were analysed in a case-control design and four additional candidate genes and two regions without known genes were associated with SCOS (P<0.001).

In conclusion, by studying CNVs in spermatogenic failure for the first time, we provide a number of candidate genes possibly causing or being risk factors for the men's spermatogenic failure. The recurring, patient-specific and private, sex-chromosomal CNVs as well as those associated with SCOS are candidates for further, larger case-control and re-sequencing studies.

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## P-Prenat-285

### **Detecting preeclampsia 4 to 5 weeks before its onset – a new method in prenatal diagnosis**

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Preeclampsia is a severe placental complication in late pregnancy leading to preterm deliveries and to life threatening diseases like eclampsia or HELLP syndrome.

So far only a specialized Doppler ultrasound of the uterine arteries was of diagnostic value in order to find pre-symptomatic signs of a beginning preeclampsia. Recently a simple biochemical examination of the two angiogenic factors sFlt-1 (soluble fms like tyrosine kinase 1) and PlGF (placental growth factor) was established which may diagnose about 90% of the early and severe forms of preeclampsia at a false-positive rate of 3%.

We give a description of the new methodology and report preliminary experiences and results collected at our laboratory.

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## P-Prenat-286

### **Prenatal Array CGH for characterization of a small supernummary marker chromosome**

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Small supernummary marker chromosomes (sSMC) are common, the majority of these sSMC is proven to be de novo. Nevertheless, it often remains difficult to correlate the presence of an sSMC with a clinical outcome, especially in prenatal cases. To predict the risk of an adverse clinical outcome in cases of de novo sSMCs a precise marker characterization is essential.

We report a case of a 39-year-old pregnant woman. Chorionic villi sampling was performed because of advanced maternal age. Cytogenetic analysis showed mosaicism for a sSMC in both the direct preparation and the culture. Both parents had normal karyotypes. For precise characterization of the sSMC Array CGH analysis was done showing a gain of signal intensity extending over 15.5 Mb corresponding to the cytogenetic region p11.2 to q11.2 of chromosome 16 (arr 16p11.2q11.2(29,614,874x2,29,734,338-45,256,643)x2~3,45,270,602x2).

FISH analysis was performed for confirmation and in order to enable rapid diagnosis for the presence of the marker in amniotic fluid. Probes for the centromere 16 (alpha satellite D16Z2) and probes corresponding to positions 30.9-31.0Mb and 32.2-32.3Mb confirmed the results from the array.

FISH analysis of amniotic fluid cells using the alpha satellite probe revealed the presence of the sSMC in 26% of the investigated cells.

Although chromosome 16 contains a highly variable block of heterochromatin in the pericentromeric region, the duplicated chromosomal material consists of 2 Mb euchromatin comprising numerous genes. In the literature no directly comparable case could be found, however, there are patients with slightly larger and smaller duplications, respectively, showing various dysmorphic features, behavioural problems, seizures or mental retardation. In addition the marker chromosome overlaps with the known 16p11.2 microdeletion/microduplication syndrome which typically spans positions 29,528,190 to 30,107,184 in 16q11.2. According to this data an increased risk for psychomotor and mental retardation cannot be excluded, despite the absence of pathological sonographic findings in the 16th week of the pregnancy.

This report underlines the importance of Array CGH analysis in the diagnostic work up of prenatally detected sSMC.

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## P-Prenat-287

### **Molecular karyotyping in prenatal diagnosis: “new method” and “old problems”**

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Applying molecular karyotyping (array CGH = aCGH) in prenatal diagnosis offers some advantages, like faster turnaround times and increased resolution. Therefore, aCGH was implemented in several prenatal diagnostic centers and several reports have suggested that aCGH is ready for mainstream use in prenatal diagnosis. In contrast to these reports, in November 2009 the American College of Obstetrics and

Gynaecology released a Committee Opinion, regarding molecular karyotyping with targeted arrays only as an appropriate adjunct to routine karyotyping in pregnancy, until more study data are available. To get own experience with aCGH in the prenatal setting, we started in 2009 a prospective study, offering aCGH with a low density targeted BAC-array to patients undergoing chorionic villous sampling (CVS) and conventional karyotyping, because of risk parameters in first trimester screening. 38 DNA samples, either isolated from native chorionic villi or CVS-long term cultures were tested. In our experimental setting, the "new method", targeted aCGH using DNA from CVS samples allowed a fast (within 48 hours) and reliable diagnosis of all common chromosomal aneuploidies, with low copy number variation rates (CNV)-rates and without CNV interpretation problems. aCGH increased the diagnostic power, in cases with unbalanced structural aberrations. Nevertheless, a low interstitial backbone resolution can miss small structural aberrations and detection of mosaicism is problematical. Additionally, an "old problem", confined placental mosaicism (CPM) is also critical in CVS diagnostics by aCGH. Therefore, basic cytogenetic knowledge and experience in prenatal diagnostics is an important part for the correct understanding and interpretation of aCGH results. Furthermore, an international standardized array design and common analysis parameters would be very helpful for the implementation of aCGH in routine prenatal diagnosis.

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## **P-Prenat-288**

### **DNA methylation alterations in trisomy 21**

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DNA methylation changes as well as complex karyotypes are well known aberrations in cancer cells. We hypothesized that genetic aberrations as known from syndromal disease could also cause epigenetic differences in order to compensate gene-dosage effects as known from X-inactivation.

We aimed to investigate the presence of methylation changes in trisomic DNA which might be of relevance for further studying its function in gene-regulation in syndromal disease. In addition, detection of aberrant methylation patterns could serve as biomarker for diagnostics.

We have analysed DNA methylation in methanol-acetic acid fixed cell suspensions from cytogenetic analyses of patients with trisomy 21 (n=11) and from healthy persons (n=10) using an assay targeting 360 different sites for testing DNA methylation changes. In principle the assay is based on methylation sensitive restriction enzyme (MSRE) digestion followed by amplification of undigested methylated DNA which is then hybridized onto a microarray. Bioinformatic analyses elucidated several genes not located on chr21 significantly differentially methylated in patients and controls. Microarray results of these samples and additional DNA samples isolated from whole blood were then validated by qPCR.

These findings strengthen our hypothesis, that chromosomal aneuploidy affects DNA methylation patterns and thus epigenetic regulation. Therefore we would hypothesize that similar effects could be expected in other syndromal disease, and might even occur when only small structural changes are the disease causing reason.

Although until today there is no confirmation that these methylation changes found in peripheral blood mononuclear cells (PBMNCs) of patients are already present in fetal DNA, elucidating these aberrations derived from fetal DNA in serum of the mother could be useful biomarkers for prenatal diagnostic testing.

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## **P-Prenat-289**

### **The major sperm tail cytoskeletal protein ODF1 is essential for male fertility**

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Sperm tail associated defects have long been considered to impair sperm motility eventually resulting in infertility. Major cytoskeletal components of the sperm tail are the outer dense fibers (ODFs) that accompany the tubuli doublets on their outer side, and are responsible for passive elastic properties of the sperm tail but do not provide to active motility. One major protein component of the outer dense fibers is ODF1, a protein of 27 kDa that is conserved from mouse to man. It has recently been shown that ODF1 protein was significantly decreased in ejaculated spermatozoa of asthenozoospermic men (Chen, 2009). Reduced amount of ODF1 might thus be responsible for reduced sperm motility.

ODF1 is now recognized as a member of the small heat shock proteins (sHSPs) and therefore renamed HSPB10. Like other sHSPs ODF1 has a characteristic  $\alpha$ -crystalline domain in its C-terminal region, and a more variable N-terminal sequence containing a leucine zipper motif responsible for protein-protein interaction. The C-terminal region of ODF1 is characterized by PCX repeats. In humans, a 27-bp deletion polymorphism in this repeat region has been described that does not affect fertility. To functionally investigate ODF1 in sperm morphology and motility, we generated an Odf1 knock out mouse line. We found that deletion of ODF1 resulted in infertility and that sperm motility is considerably lower compared to wild-type or heterozygous mice. Histological and immunological investigations revealed that spermatozoa showed morphological abnormalities. Our results thus indicate that ODF1 deficiency affects the morphology of the whole spermatozoon eventually resulting in male infertility. Since ODF1 is specifically expressed in round spermatids but not in any other germ cell stages or somatic tissues our data imply that mutations in Odf1 should be regarded as an underlying cause of male infertility.

## **P-GENETIC COUNSELLING / EDUCATION / GENETIC SERVICES / PUBLIC POLICY**

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### **P-Counse-290**

#### **Evaluation of the ASPA gene in patients with Canavan Disease**

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**Background:** Canavan Disease (CD), also called Canavan-Van Bogaert-Bertrand disease, aspartoacylase deficiency or aminoacylase 2 deficiency, is an autosomal recessive degenerative disorder that causes progressive dysmyelination of neurons in the white matter. The disease is marked by early onset hydrocephalus, macrocephaly, psychomotor retardation, spongiform myelin sheath vacuolization with progressive leukodystrophy. Thus, CD belongs to the group of leukodystrophies. CD is a neurodegenerative disorder most prevalent among Ashkenazi Jews [1, 2]. Mutations in the aspartoacylase (ASPA) gene located on chromosome 17 result in undetectable activity of aspartoacylase, an enzyme catalyzing the deacetylation of N-acetyl-L-aspartate (NAA).

**Case report:** We report on a 11 year-old boy born after uneventful pregnancy. With the exception of incomplete plexus paresis he showed no abnormalities in the newborn period. He presented with severe psychomotor retardation at the age of 12 months and relative macrocephaly. Because the family history was suggestive for CD, molecular genetic testing was performed and revealed the mutation (p.Asp114Glu) in homozygous state in exon 2 of the ASPA gene. After diagnosis he received regular treatment with calcium acetate as well as acetacolamide, and disease progression remained stable until now. In the following pregnancy we performed prenatal testing, and the fetus carried the mutation in heterozygous state.

**Methods:** For mutation analysis the 6 coding exons of the ASPA gene and flanking intronic regions were sequenced directly. Deletion/duplication analysis was performed by real time PCR. In two families family-specific mutations are used for prenatal and carrier diagnostic.

**Conclusion:** Even though ASPA mutations are less prevalent in our population, CD has to be considered in severely retarded infants with hydrocephalus and leukodystrophy, especially in parents without German roots. Molecular genetic confirmation helps to differentiate CD from other leukodystrophies and is important in future prenatal diagnostics and often asked for by prospective parents. In cases where only one or no mutation is identified, biochemical measuring of N-acetylaspartic acid in the amniotic fluid can be performed. - As demonstrated in animal models, early postnatal application of glyceryltriacetate decreased brain vacuolization and improved motor performance. Apparently early treatment can improve symptoms of CD [3]. Therefore, prenatal diagnostic will be even more important in the future.

[1] Kaul R, Gao GP, Matalon R, Aloya M, Su Q, Jin M, Johnson AB, Schutgens RB, Clarke JT. Identification and expression of eight novel mutations among non-Jewish patients with Canavan disease. *Am J Hum Genet.* 1996 Jul;59(1):95-102.

[2] Elpeleg ON, Shaag A. The spectrum of mutations of the aspartoacylase gene in Canavan disease in non-Jewish patients. *J Inher Metab Dis.* 1999 Jun;22(4):531-4.

[3] Arun P, Madhavarao CN, Moffett JR, Hamilton K, Grunberg NE, Ariyannur PS, Gahl WA, Anikster Y, Mog S, Hallows WC, Denu JM, Namboodiri AM. Metabolic acetate therapy improves phenotype in the tremor rat model of Canavan disease. *J Inher Metab Dis.* 2010 Jun;33(3):195-210. Epub 2010 May 13.

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## P-Course-291

### **EuroGentest Clinical Utility Gene Cards – Connecting basic research to patient care**

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EuroGentest, an EU-funded network of excellence, is starting its second project initiative in 2011. Unit 2 “Genetic testing as part of Health Care” is focussing on the establishment of clinical utility guidelines – the Clinical Utility Gene Cards (CUGC).

CUGCs are disease-specific guidelines dealing with the clinical utility of genetic testing, meaning the ability of genetic test results to reveal information essential for the clinical setting.

Based on the ACCE (Analytical validity, Clinical validity, Clinical utility and Ethical, legal and social issues) framework, the documents present following contents: disease characteristics (name, mutational spectrum and analytical methods), test characteristics (analytical and clinical sensitivity and specificity) and clinical utility (disease management, cost effectiveness and risk assessment). Information concerning the clinical settings is divided into four sections: differential diagnostics, predictive testing, risk assessment in relatives and prenatal testing.

Each CUGC is authored by a multinational expert team; potential authors are identified based on e.g. their publication record and practical experience. Subsequent to the peer-review, the documents are published in the European Journal of Human Genetics (EJHG). EuroGentest commissions the establishment as well as the annual update of the guidelines.

Due to the concise and standardised format, CUGCs enable quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers and in the broader sense also patients. With regular revisions and inclusion of new developments and findings EuroGentest ensures that all published documents reflect the state-of-the-art.

By the end of 2014 EuroGentest is aiming at a total of 500 CUGCs, with the main focus laying on Mendelian, mostly rare, disorders. All documents are freely available on the websites of EuroGentest ([www.eurogentest.org/web/info/public/unit3/geneCards.xhtml](http://www.eurogentest.org/web/info/public/unit3/geneCards.xhtml)) and the EJHG ([www.nature.com/ejhg/](http://www.nature.com/ejhg/)). Examples of published CUGCs and the current status are presented.

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## P-Course-292

### **Quality Assessment scheme for Constitutional Molecular Karyotyping: lessons from two pilot schemes.**

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In 2008/2009 CEQA (Cytogenetics European Quality Assessment scheme) and EMQN (European Molecular Genetics Quality Network) initiated a joint pilot scheme for molecular karyotyping.

Thirty labs participated in the first scheme 2009. In this scheme a 1.7 Mb subtelomeric deletion at 20p and a 9.1 Mb interstitial duplication at 18p11.32p11.22 had to be detected. Genotyping and interpretation were considered to be of equal importance.

In the 2009 scheme 21% of the labs made significant genotyping errors. 83% of the labs failed to provide an adequate interpretation. In summary, only 3 labs (10%) fulfilled the required criteria (correct genotype and major aspects of the interpretation) in the 2009 scheme.

In the 2010 scheme a 1.3 Mb interstitial deletion of the Wolf-Hirschhorn critical region on chromosome 4 (4p16.3p16.3) was presented as a case scenario. A total of 74 labs registered to participate. As this was still a pilot scheme, we restricted the number of participants to 60. Finally, 55 labs transmitted reports. One lab failed to obtain a result.

Only one lab failed to detect the 4p deletion probably due to a sample mix-up. Three labs detected an additional second abnormality. So, in total 4/54 of the labs made a genotyping error.

All labs commented on the clinical significance of the deletion and mentioned that this deletion is associated with Wolf-Hirschhorn syndrome.

Although the quality of the reports has improved dramatically in the 2010 scheme, there are still minor errors, which can be observed in some reports. This concerns information about limitations of their testing assay, technical information (e.g. practical instead of the theoretical resolution) and the recommendation of appropriate follow-up studies (e.g. testing of parents/family members).

In summary, the submitted reports were of a much higher standard compared to last year. In 2009 many labs had still technical problems resulting in a high number of genotyping errors. This year almost all labs

provided high quality genotyping data. From the result it is obvious that molecular karyotyping has become a powerful diagnostic tool which provides informative and reliable results.

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### **P-Counse-293**

#### **An uncommon case report: Legal measures in a case with juvenile diabetes, refusal of insulin therapy and retrospective confirmation of a laypersons decision by molecular genetic diagnostics of MODY2**

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We report on family history, clinical and molecular genetic results of a family with 6 affected persons clinically diagnosed with diabetes mellitus type 1. One male family member was diagnosed at childhood to have DM1. He was treated with insulin at the age of 26 years. Under this medication a remarkable decline of his constitution was observed that became manifest by a dramatical weight loss without any changes in diet or lifestyle or the occurrence of any other pathogenic reasons. He died at the age of 29 due to a septic complication following surgical abscess eradication. His son was also diagnosed to have DM1 at the age of 2 years. Under the impression of the worsened health state that followed the insulin therapy of her husband, the boy's mother rejected such a treatment against the recommendations of the physician. This provoked the physicians to induce legal steps and notification of the youth welfare office. Besides the fact that the family did not present critical social circumstances, this resulted in some effort. However, the patients mother defied the control and organized the therapy of her son in form of a respective diet.

More than 20 years later, a molecular genetic analysis was requested for the patient to exclude or diagnose a maturity-onset diabetes of the young (MODY). Herewith we could demonstrate the occurrence of a heterozygous mutation in the GCK-gene (c.667G>A).

MODY is an autosomal dominant inherited subgroup of diabetes mellitus caused by mutations in currently known 11 different genes. Mutations in the glucokinase gene (GCK) are the molecular cause of MODY type 2. Glucokinase catalyzes glucose to glucose-6-phosphate conversion in the pancreatic beta cell and is a key regulator of insulin secretion. GCK mutations result in a higher threshold for insulin release and persistent, hyperglycemia and possibly decreased hepatic glucose uptake and glycogen synthesis. Most patients with MODY 2 can be treated with diet. Only two percent of patients with MODY 2 require or benefit from insulin therapy. Thus, molecular genetic diagnostics of MODY 2 is a crucial prerequisite for an optimal individualized therapy of diabetes mellitus.

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### **P-Counse-294**

#### **Public Policy and the Professionalization of Human Genetics after 1945 in Germany**

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„Human Genetics is both a fundamental and an applied science.[...] Because of its continued theoretical and practical interest, human genetics offers fascination and human fulfilment unparalleled by work in fields that are either primarily theoretical or entirely practical in subject matter.” [1,1]

The pathway from the first ideas in human genetics to an established field in medicine lasted a long time in Germany. In the middle of the 19th century C. Darwin and others published their opinions on evolution and selection. Now heredity gained more and more interest. Up to the 1930ies there was not much knowledge about genetics: F. Galton published about heredity and talent, and then Men-del's insights triggered the development of modern genetics. [1] Early achievements were on ABO Blood Groups and Hardy-Weinberg Law. Around 1900 the eugenics movement became strong. In Germany was also used the term “Rassenhygiene” that was associated with mystical concepts of race and the fear of degeneration of the human race. “Prominent German human geneticist identified themselves with the use of human genetics in the service of the Nazi State.” [1,16]

In 1945 Human Genetics was not established at German universities. In 1946 Karl Jasper stated that universities should develop to the best and highest level. “In the diversification of the whole the new one will be the whole, as life produces life.” [2] This leads to the point that the best performance will be a specialized one. In 1960 the Wissenschaftsrat (German Science Council) stated that new professorial chairs at universities should only be for (a) fields that were still in evolution and where it might be expected that there would be a chair in the future, (b) in special cases for the permanent support of small special fields. In medicine a chair for Genetics at every Medical faculty was regarded necessary, replenished by a chair for

anthropology. [3]The first (extraordinary) chairs were established in 1948 in Goettingen (F. Lenz) and in Kiel (Lehmann), followed by Muenster in 1951 (Verschuer). In 1960 there were only 3 institutes for Human genetics from that time on there were established up to the year 1980 21 new ones. There was a lack of scientist in human genetics in 1945. This is one reason that there was a personal continuity before and after 1945 (F. Lenz, Verschuer, Lehmann, Koch).

For the professionalization of Human Genetics in Germany were also important a German publication on Human Genetics and the foundation of a scientific association.

As conclusion can be stated:

- Professionalization of Human Genetics was supported by public policy, reflected in the Recommendations of the Wissenschaftsrat.

- There was a personal continuity of scientists dealing with human genetics up to the 1960ies.

Literature: [1] Vogel, F, A Motulsky (1986<sup>2</sup>) Human Genetics. Berlin. [2] Weber M (1991) Wissenschaft als Beruf, 8th edn., Berlin, p.11. [3] Wissenschaftsrat (ed) (1960) Empfehlungen des Wissenschaftsrates [...]. Tübingen, p.114.

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## P-Course-295

### **„Genetic Exceptionalism“: Which factors influence the view of medical professionals on the status of genetic information?**

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Introduction: Genetic Exceptionalism is a scientific concept of the late 1990s, being based on the assumption that genetic information has special qualities which require special treatment. The scientific debate abandoned exceptionalism as early as 1999, concluding that genetic data are just as sensitive as other medical, or socioeconomic data.

Research Question: In order to assess positions taken on by medical professionals at different times throughout their careers, we carried out a survey with questions regarding the so-called "genetic exceptionalism".

Methods: 1009 participants answered 30 questions on their beliefs in the (non-) exceptional status or the need to safe-guard such information by legislative steps (before 2010). Participants were medical students, MDs, geneticists, other specialists and other medical personnel who deal with genetic information. The questionnaire was distributed as a German paper-and-pencil version, as well as an identical English version online.

Results: We found no gender difference in judgement on genetic information. Participants younger than 25 years of age think of genetic information as more exceptional than their older (>25) colleagues. Students have significantly higher ratings in the exceptionalism-scale (Mean 4.04, SD 0,63; N= 309) than qualified doctors M = 3,891, SD 0,86; N = 244;  $F(1, 551) = 5.821$ ;  $p = .016$ ). The higher the level of medical education (or expertise) the less exceptional genetic information is thought to be considered (exceptional index ( $F(4, 582) = 4.077$ ;  $p = .003$ ) as well as non-exceptional-index ( $F(4, 582) = 2.74$ ;  $p = .028$ )). Gynaecologists considered genetic information to be significantly more exceptional than any other group of medical specialists. Specialists that work in human genetics centers think of genetic information as less exceptional than all others ( $F(3, 299) = 2.658$ ;  $p = .049$ ). Participants with more than one professional degree rate genetic information less exceptional than all other subjects (exceptional index:  $F(3, 1003) = 5.747$ ;  $p = .001$ ). Participants who declared interest in the fields of history, medical ethics, philosophy or genetics, rate genetic information significantly less exceptional than people who do not share those interests ( $F(1, 1005) = 7.216$ ;  $p = .007$ ). In general, English-speaking subjects judged genetic information less exceptional than German-speaking ones.

Conclusions: Judgements on genetic information seem to be subjected to a variety of culture-bound and expertise-related factors. Further studies are needed to elucidate how those factors interact.

## P-THERAPY FOR GENETIC DISEASE

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### P-Therap-296

#### **Mutational spectrum in patients with DCX-associated subcortical band heterotopia and interdisciplinary medical care**

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Heterozygous DCX mutations are the most frequent cause of subcortical band heterotopia (SBH; MIM 300067), a distinct cortical malformation with symmetric formation of a second layer of heterotopic neurons within the subcortical white matter (double cortex). The human DCX gene is located at Xq23 and codes for a microtubule-binding protein. The phenotype of heterozygous females is highly variable, grossly correlates with the thickness and extend of the heterotopic band and frequently includes chronic therapy-refractory seizures as well as cognitive and affective deficits. DCX-related disorders are inherited in an X-linked dominant manner: hemizygous male offspring will usually present with the severe phenotype of DCX-related classic lissencephaly; while heterozygous female offspring may be asymptomatic or more frequently present with clinical signs of the variable SBH phenotype.

Consecutive DCX sequence analysis of patients with SBH and classic lissencephaly between 2001 and 2010 at our center led to the identification of 62 mutation carriers (13 hemizygous males; 3 male mosaic carriers; 46 heterozygous females including 31 adult females) from 45 independent families. Prenatal diagnosis was requested for 7 pregnancies. Our data confirm for this patient cohort in frame sequence variants as the predominant mutation type (n=31/45 families), which mainly result in amino acid substitutions in the two functional domains N-DC (n=14) and C-DC (n=17).

Exemplary clinical data of two families will be presented to illustrate the specific problems in interdisciplinary medical care: In more than half of the patients seizures are refractory to common schemes of antiepileptic medication. In addition, cognitive and behavioural disabilities may significantly impair social interactions, education and professional carrier as well as the perception of health and genetic risks.

With an ongoing interdisciplinary study of a patient cohort of adult heterozygous mutation carriers with SBH we address two major objectives:

(I) A standardized neurologic ascertainment of the quality and severity of the spectrum of DCX associated seizures and their individual response to different antiepileptic schemes are applied to further characterize the DCX-associated seizure phenotype. The obtained results will be used to search for clinical parameters important for pharmacological response in order to improve antiepileptic treatment of DCX-associated seizures.

(II) The DCX-associated spectrum of cognitive as well as affective disabilities will be assessed using standardized neurocognitive protocols in order to develop new therapeutic and social support strategies to further improve quality of life parameters. The obtained data may also be relevant for our understanding of other forms of monogenic neuronal migration disorders and their gene-specific therapeutic intervention.

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### P-Therap-297

#### **In vitro evaluation of PTC124 and gentamicin as stop codon readthrough agents using a dual reporter construct**

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Nonsense mutations represent up to 15% of Fanconi anemia sequence variants. These premature termination codons (PTCs) generate mRNA isoforms with truncated reading frames. Aminoglycoside antibiotics have been shown to induce translational readthrough of PTCs which may lead to the reconstitution of – at least partially – functional proteins. However, therapeutic use of aminoglycosides is limited due to their considerable oto- and nephrotoxicity. A recently reported alternative candidate readthrough agent is PTC124 (3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl]-benzoic acid; Ataluren®). Currently, several clinical studies explore the potential of PTC124 for treatment of diseases like cystic fibrosis (CF), Duchenne muscular dystrophy (DMD) and haemophilia. Using a dual reporter assay in cell culture experiments we investigated the ability of PTC124 and gentamicin to promote ribosomal readthrough of premature termination codons representing nonsense mutations found in Fanconi anemia patients.

We established a read-out system utilizing plasmid constructs which encode the fluorescent protein AcGFP and renilla luciferase (rluc). Both genes are separated by a linker that allows for the incorporation of nonsense mutations in different sequence contexts. In the absence of in-frame stop codons a fusion protein is generated retaining both fluorescence and rluc-mediated luminescence activity. We introduced stop codon - containing DNA sequences derived from FA patients carrying nonsense mutations (TGAC, TGAT, TGAG, TAGC) in various of the Fanconi anemia genes (FANCA, FANCC, FANCE, FANCI, FANCG, FANCF, FANCD1, FANCD2, FANCD3, FANCD4, FANCD5, FANCD6, FANCD7, FANCD8, FANCD9, FANCD10, FANCD11, FANCD12, FANCD13, FANCD14, FANCD15, FANCD16, FANCD17, FANCD18, FANCD19, FANCD20, FANCD21, FANCD22, FANCD23, FANCD24, FANCD25, FANCD26, FANCD27, FANCD28, FANCD29, FANCD30, FANCD31, FANCD32, FANCD33, FANCD34, FANCD35, FANCD36, FANCD37, FANCD38, FANCD39, FANCD40, FANCD41, FANCD42, FANCD43, FANCD44, FANCD45, FANCD46, FANCD47, FANCD48, FANCD49, FANCD50, FANCD51, FANCD52, FANCD53, FANCD54, FANCD55, FANCD56, FANCD57, FANCD58, FANCD59, FANCD60, FANCD61, FANCD62, FANCD63, FANCD64, FANCD65, FANCD66, FANCD67, FANCD68, FANCD69, FANCD70, FANCD71, FANCD72, FANCD73, FANCD74, FANCD75, FANCD76, FANCD77, FANCD78, FANCD79, FANCD80, FANCD81, FANCD82, FANCD83, FANCD84, FANCD85, FANCD86, FANCD87, FANCD88, FANCD89, FANCD90, FANCD91, FANCD92, FANCD93, FANCD94, FANCD95, FANCD96, FANCD97, FANCD98, FANCD99, FANCD100). The vector design provides a tool for quantifying AcGFP fluorescence as a measure of transfection efficiency and rluc-mediated luminescence as a measure of stop codon readthrough. Transfected HEK293 cells were cultured in the presence of different concentrations of PTC124 or gentamicin; DMSO served as negative control.

Ribosomal readthrough in the presence of PTC124 was not above background levels and was independent of the dose applied. In contrast, gentamicin showed a dose-dependent statistically significant increase in stop codon readthrough being most pronounced in the case of a TAGC sequence context and least obvious in the TGAT example. Thus, the readthrough effect of PTC124 reported in studies of CF and DMD could not be verified in our experimental setting while the aminoglycoside gentamicin proved effective. Our dual reporter assay utilizing a vector construct expressing the fluorescent protein AcGFP and renilla luciferase allows for the screening of small molecule substances that are candidates for stop codon readthrough.