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Symposia

S01_01

Micromanagers of gene expression: A genetic approach to defining microRNA pathways

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microRNAs (miRNAs) are small, endogenous, regulatory RNAs that affect numerous fundamental cellular processes. miRNA pathway dysfunction has been implicated in the etiology of a number of diseases including several different cancer types and diabetes. Indeed, the intricate expression patterns of microRNAs can be used to classify cancers.

Despite their profound impact on cellular functions, little is known about the mechanisms by which miRNAs work. We have used a genetic high-throughput siRNA-based screening approach to identify cellular pathways that are involved in microRNA-directed gene silencing. By understanding the factors and pathways involved in miRNA-directed gene silencing, we will gain insight into how microRNAs work and how their expression and function is regulated. Also, we hope to identify new protein factors that can be exploited for therapeutic purposes.

S01_02

Disrupting the Pairing Between *let-7* and *Hmga2* Enhances Oncogenic Transformation

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MicroRNAs (miRNAs) are ~22-nt RNAs that can pair to sites within mRNAs to specify posttranscriptional repression of these messages. Aberrant miRNA expression can contribute to tumorigenesis, but which of the many miRNA-target relationships are relevant to this process has been unclear. We reported that chromosomal translocations that had been found previously in a wide variety of benign human tumors disrupt repression of *High Mobility Group A2* (*Hmga2*) by the *let-7* miRNA. In malignant human tumors overexpressing Hmga2 no translocations involving the Hmga2 locus have been found so far. It seems that in malignant tumors alternative splicing leads to generation of alternative 3' ends and escape from *let-7*-mediated repression. This disrupted repression promotes anchorage-independent growth, a characteristic of oncogenic transformation. Thus, losing miRNA-directed repression of an oncogene provides a mechanism for tumorigenesis, and disrupting a single miRNA-target interaction can produce an observable phenotype in mammalian cells.

S02_01

Lactase, lactose, adaptation and mutational diversity

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Persistence of lactase expression into adult life is a polymorphic trait that allows adults to consume large quantities of milk without adverse symptoms. It was first attributed to one single nucleotide polymorphism (*-13910 C>T*) in an enhancer element 13.9kb upstream of the lactase gene (*LCT*) (Enattah et al., 2002 Nature Genetics 30:233). The *-13910*T* allele occurs at very high frequency in northern Europeans as part of a very long haplotype (known as **A**), and promotes binding of the transcription factor Oct-1 (Lewinsky et al., 2005 Human Molecular Genetics 14:3945). However *-13910*T* was shown to be at very low frequency in many African milk drinking pastoralist groups where lactase persistence was reported at high frequency (Mulcare et al., 2004 Am. J. Hum. Genet. 74, 1102), and alternative explanations that could give rise to the phenotype were sought.

In recent publications we and others (Ingram et al., 2007 Human Genetics 120:779, Tishkoff et al., 2007 Nature Genetics 39:31, Enattah et al., 2008 Am. J. Hum. Genet 82, 57–72) reported new sequence variants within the upstream region, in various sub-Saharan African and Middle Eastern populations. Some of these variants were associated with lactase persistence, and occur on several different haplotype backgrounds, at least one of which

is also on a very extended haplotype. This has been interpreted as evidence for convergent evolution of the trait, with very large positive selection. Although published data now demonstrates that Oct-1 is unlikely to mediate transcriptional regulation of lactase all these sequence variants do lie within the element that enhances transcription *in vitro,* supporting the notion that they are functional and not markers of a causal change elsewhere. We have more recently identified several other allelic variants in this same region. Many of the sequence variants are highly population-specific, several are rare, and not all show an association with lactase persistence, but in our phenotype association studies several different candidate functional variants occur within a single ethnic group. However many more members of the pastoralist groups we have tested drink large quantities of fresh milk than carry lactase persistence associated mutations. Also many phenotyped as lactase non-persistent (lactose intolerant), drink copious quantities of milk. These observations will be discussed in relation to the role of selection acting on this region of the human genome, and the functional significance of the mutations will be critically examined.

S02_02

Genetics of skin, hair and eye colour

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The physical appearance of skin, hair and eye colour can vary dramatically among geographically isolated human populations. It has long been speculated that this is due to adaptive changes, but the genetic causes for this degree of phenotypic variation have remained largely unknown. Melanocytes are the cells within the cutaneous, follicular and iridial tissues that produce the melanin pigments largely responsible for these physical traits of colour. Melanin is formed via a biosynthetic pathway and packaged within a specialised subcellular compartment termed the melanosome. In the past decade my laboratory has isolated and characterised a number of human pigmentation genes to use as tools to investigate gene regulatory mechanisms operating within melanocytes, as well as allowing a combined genetic and cellular analysis of pigment variation in populations with European ancestry. These genes include the enzymes encoded by tyrosinase, tyrosinase-related protein-1 and dopachrome tautomerase (TYR, TYRP1 and DCT), the P-protein (OCA2) and the melanocortin-1 receptor (MC1R). Our studies have provided a framework to understand the genotypes associated with normal variation in pigmentary traits; moreover polymorphisms within these genes are markers for risk of development of skin cancer. Variant alleles of the MC1R gene resulting from a range of amino acid substitutions have been associated with red hair, fair skin, a high degree of freckling as well as increasing incidence of melanoma. Most recently we have found a single SNP located in a regulatory region located upstream of the OCA2 locus that determines blue-brown eye colour inheritance in Europeans. Other population studies have revealed specific polymorphisms within the MATP (SLC45A2) and NCKX5 (SLC24A5) protein coding regions associated with the degree of skin pigmentation. The identification and study of genes that have a major role in melanogenesis and melanosome formation provide an understanding to the genetic complexity of skin, hair and eye colour in humans.

S02_03

Causes of regulatory variation in the human genome

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The recent comparative analysis of the human genome has revealed a large fraction of functionally constrained non-coding DNA in mammalian genomes. However, our understanding of the function of non-coding DNA is very limited. In this talk I will present recent analysis in my group and collaborators that aims at the identification of functionally variable regulatory regions in the human genome by correlating SNPs and copy number variants with gene expression data. I will also be presenting some analysis on inference of trans regulatory interactions and evolutionary consequences of gene expression variation.

S03_01

New insights in the pathogenesis of Marfan Syndrome and related aneurysmal syndromes

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Heritable connective tissue disorders comprise a heterogeneous group of disorders that result from genetic defects affecting normal extracellular matrix assembly. Many of these diseases are associated with a significant cardiovascular risk leading to morbidity and mortality in childhood or young adulthood. Prime examples that represent important genetic models for cardiovascular pathology are the Marfan syndrome and related disorders. In these conditions, progressive dilatation of the aortic root leads to aortic dissection, often associated with precocious death. Over the last decade tremendous progress in clinical and molecular research has changed the prevailing concept of these syndromes as structural disorders of the connective tissue into diseases manifesting perturbed cytokine signaling with widespread developmental abnormalities. These insights opened new and unexpected targets for causally directed drug treatments for these aneurysm syndromes, and by extent, also for the more common non-syndromic forms of aneurysm formation, a major cause of morbidity and mortality in the Western world.

S03_02

Skeletal dysplasia; from mouse models to potential therapies

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Disruption to endochondral ossification leads to delayed and irregular bone formation and can result in a heterogeneous group of genetic disorders known as the chondrodysplasias. Two such disorders are pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED), which are characterized by dwarfism and early-onset osteoarthritis and can result from mutations in the genes encoding COMP and matrilin-3. All of the mutations identified in *COMP* and *MATN3* primarily affect conserved residues that comprise the Type III and C-terminal domains of COMP or the single A-Domain of matrilin-3.

In order to determine *in vivo* the disease mechanisms that underlie the pathophysiology of *COMP* and *MATN3* mutations we have generated three murine models of PSACH-MED by introducing specific human disease-causing mutations into the A-domain of matrilin-3 (p.V194D) and the type III (delD469) and C-terminal (T585M) domains of COMP.

Mice that are homozygous for the respective mutations develop a progressive dysplasia and have short-limbed dwarfism that is consistent in severity with the relevant human phenotype. Mutant protein is retained within the rough endoplasmic reticulum of chondrocytes and is associated with an unfolded protein response that propagates into a rER/cell stress response. Eventually there is reduced proliferation and spatially dysregulated apoptosis of chondrocytes in the cartilage growth plate, which is likely to be the cause of disrupted linear bone growth and the resulting short-limbed dwarfism in the mutant mice.

The identification and characterization of key pathways involved in the development of PSACH-MED will allow the testing of suitable therapeutic agents. Furthermore, it will have a major impact on our understanding of the disease mechanisms in other forms of chondrodysplasia and a broad spectrum of inherited connective tissue diseases.

S03_03

The ADAMTS family and the acromelic dysplasia group

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The acromelic dysplasia group includes Weill-Marchesani syndrome (WMS), geleophysic dyplasia and acromicric dysplasia. The three disorders are characterized by the association of short stature, brachydactyly and joint stiffness. WMS is distinct from the two others by the presence of eye anomalies including microspherophakia, ectopia of the lens, severe myopia and glaucoma. Despite clinical homogeneity, autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been reported in WMS. We have recently identified five distinct mutations in the fibrillin-1 (FBN1) gene in AD WMS families including three missense mutations and two in frame deletions. Using an homozygosity mapping strategy in consanguineous families from Lebanon and Saudi Arabia, we have found linkage of the AR WMS gene to chromosome 19p13.3-p13.2 and then identified mutations in the gene encoding a member of the extracellular matrix proteases, ADAMTS 10 (a disintegrin and metalloprotease with thrombospondin motifs). A total of 9 distinct mutations have been yet identified in 7 distinct families including 4 non sense and five missense mutations. The function of ADAMTS10 is yet unknown. Expression studies of ADAMTS 10 using RT-PCR, Northern blot and dot blot analyses showed that ADAMTS 10 is expressed in skin, fetal chondrocytes and fetal and adult heart. Moreover, electron microscopy and immunological studies of the skin fibroblasts of affected patients confirmed the impairment of the extracellular matrix. Finally, the observation of FBN1 mutations in the AD form of WMS and of ADAMTS10 mutations in the AR

form of WMS suggests that ADAMTS10 directly interacts with FBN1. More recently, studying consanguineous families with geleophysic dysplasia, we have mapped the disease locus gene and then identified mutations in another member of the ADAMTS family of yet unknown function. Ongoing studies will hopefully lead to the understanding of the specific role of these ADAMTS proteins in the microfibrillar network.

S04_01

EuroGentest: Report on the progress towards harmonization and improvement of the quality of the European genetic services.

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During its first three years, the NoE, with the enthusiastic help of the thousands of geneticists in the EU who contributed directly or indirectly to the aims of the Network as well as with the support of the European Society of Human Genetics, has delivered a series of products which are the instruments needed by the genetics community to improve and harmonize their services.

The NoE has become a **Trade mark** for quality European genetic services and has created an unprecedented movement in Europe towards improved quality of the services and harmonization of the service provision. Many of the activities and achievements contribute to the transfer of research results into diagnostics and create the conditions to make sure this can continue in the future so that citizens would benefit rapidly and efficiently from the daily advances in genetic research.

S04_02

Validation of methods in DNA diagnostics: an overview of EuroGentest activities

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The EuroGentest NoE (www.eurogentest.org) is focused on improvement and harmonization of the quality of genetic services in Europe. Its Unit 1 is facilitating the development of guidelines and dissemination of Quality Assurance (QA) in order to help genetic laboratories achieve and maintain laboratory accreditation according to ISO15189, including compliance with the OECD “Guidelines for QA in Molecular Genetic Testing” (www.oecd.org/sti/ biotechnology). An important part of this process is validation of molecular genetic techniques (both commercial or developed “in house”) that are routinely used in DNA diagnostics. Unit 1 “clients” comprise diagnostic laboratories (academic or private) and performs validations of established techniques, while Unit 5 is oriented towards the biotech industry and evaluates novel techniques (e.g. HRMCA, PAP assay) with respect to their suitability for diagnostic practice. Both Units closely collaborate in that Unit 5 usually transfers evaluated novel techniques to Unit 1, where “downstream” diagnostic validations are being performed. Although, general guidelines for diagnostic validation were previously stipulated by various bodies (e.g, EAL/Eurolab EAL-P11, NCCLS or COFRAC) there are no specific procedures or examples as to how the requirements of the norm are to be fulfilled. Thus, it is up to individual laboratories to establish their own strategy and scope of validation. This is both time consuming, inefficient and often leads to subjective assessments from the side of accreditation bodies. Currently, a draft for “Minimum Acceptable Standards for Analytical Validation of Molecular Genetic tests” is being prepared and we will discuss the complexity of this task, including its potential implications. Supported by EU - FP6-512148

S04_03

Prioritisation in Swedish Health Care and Impact on Provision of Clinical Genetic Service

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In any publicly financed health care system the demands on service provision overruns the public funds available through taxation/insurance or other public systems. Thus, there is a need for a prioritisation system which should be transparent and based on easily communicated criteria.

In Sweden, which has a county based but state regulated health care system, the National Board of Health and Welfare has developed a stepwise prioritisation scale, from life saving emergency room medicine to non-medical well being activities. These principles should be used to prioritise in the health care system and for the authorities when allocating resources.

The presentation will discuss the drawbacks and advantages of such a system in clinical practice for delivering laboratory and clinical genetic service.

S05_01

Understanding neurodegenerative disease processes: Protein Interaction Networks

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The main objective of our work is to understand the cell's functional organization and to link individual proteins to signalling cascades and disease processes. Therefore, we have developed an automated yeast two-hybrid (Y2H) system, which allows the high-throughput identification of human protein-protein interactions (PPIs). Using this technology, interaction networks for neurodegenerative diseases such as Huntington's disease were generated. In the last two years, we further expanded our efforts to identify human PPIs for the MAPK as well as Akt signalling pathways, ~2000 novel PPIs were identified with repeated Y2H screens. A significant fraction of these interactions was validated with functional ELISA assays and ~20 modulators of MAPK and/or Akt signalling were discovered. In addition, we have developed bioinformatic strategies in order to predict potential signalling cascades from undirected PPI data. This allowed us to analyze directed cellular pathways that transmit information from membrane receptors to transcription factors as well as to find novel signalling network motifs. Finally, we have started to hunt for Huntington's disease modifiers utilizing a computational approach. In this case, various functional genomics data sets including gene expression and PPI data were combined to prioritize the genes with relevance to Huntington's disease.

S05_02

Tissue specificity of the mitochondrial proteome: implications for phenotypic variability in mitochondriopathies

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Despite the fact that mitochondria are present in every cell and tissue, the clinical phenotypes and disease severity of patients with genetically determined mitochondrial dysfunction vary widely between recurrent headaches to a severe multi-system disorders leading to death within the first months of life. [1] Following-up on the hypothesis that mitochondria are not identical in every tissue, we have shown by 2D-electrophoresis and mass-spectrometry that mitochondria from different organs have a highly tissue-specific protein composition – in quality and quantity – that might enable them to overcome a primary defect in ATP-production or the overproduction of reactive oxygen species (ROS) in different ways (e.g. by proliferation, switching to alternative pathways or by up-regulation of lipolysis). However, in some tissues these "coping mechanisms" seem to contribute to the pathogenesis in the first place (e.g. through massive proliferation of mitochondria in the susceptible brain regions of patients with mtDNA related disease). [2] In fibroblasts of several patients with *SURF1* mutations affected with Leigh-syndrome, we found differential regulation of proteins related to the defense against reactive oxygen species and of proteins related to the pyridoxine pathway. Up-regulation of these proteins went in parallel with reduced disease severity in a subset of these patients. In conclusion: proteomic methods allow a direct view on the players involved in mitochondrial disease and help to unravel the network of agonistic and antagonistic factors that influence the clinical phenotype and may allow therapeutic intervention.

S05_03

Balance and Elasticity in the Cellular Proteome: Crucial Factors in Disease Development

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Increasing evidences suggests that the cellular proteome act in large protein-protein interaction networks. In order to study the effect of mutations on the global proteome network, we investigated a pallet of different transgenic mice or mouse embryonic stem cell lines relevant for different neurodegenerative diseases (Alzheimer's disease, Parkinsonian syndrome and Huntington disease). These disease models were cross-compared respecting protein expression profiles in different brain regions and age stages using proteomic approaches. Our results suggest that the quantitative arrangement of the cellular proteome is highly flexible and well balanced. In case of protein abnormalities due to gene mutations, the cell attempts to establish a new balance in the proteome to protect the cell from deleterious effects of gene mutation. This indicates that besides the disease-specific protein interaction pathways, a proteome-wide regulatory network of higher order exists that lends "elasticity" to the cellular proteome. According to our data, this proteomic "elasticity" seems to decrease with advancing age of the organism. When the stimulus on the cellular proteome exceeds a certain threshold of "elasticity", the proteomic balance will no longer be restored through the cellular re-balancing approach. This will cause progressively disturbed metabolic pathways, leading to disease phenotypes. In a sense, the external stimulus and internal re-balancing approach of the cellular proteome counteract upon the disease development via the "elastic" proteomic network.

S06_01

Epigenetic modifications in early mammalian embryos

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In the mammalian zygote the paternal genome undergoes rapid epigenetic reprogramming shortly after fertilization. Most dramatic changes occur in paternal genome which is actively demethylated and acquires particular histone modifications prior to the first round of replication. In our studies we investigate the possible molecular mechanisms leading to the observed epigenetic changes. Here we report on the preferential appearance of the DNA strand break marker γH2AX with the paternal pronucleus during critical stages of zygotic development. This finding may indicate that active DNA demethylation in zygotes is linked to DNA repair coupled processes. The associated possible molecular mechanisms will be discussed in light of our findings.

The general concept of active demethylation processes was challenged by reports suggesting that active paternal chromosome demethylation is absent in rabbit and ovine zygotes. To clarify this we performed comparative immunohistochemical studies on mouse, bovine and rabbit zygotes and SCNT derived rabbit one cell embryos using antibodies against 5-methyl-cytosine and two informative histone H3 modifications (H3K4me3 and H3K9me2). We observe similar dynamics of pronuclear immunofluorescence patterns in zygotes of all three species and find clear evidence for active DNA-demethylation both in rabbit zygotes and cloned one cell rabbit embryos, respectively. Our data reveal that mechanisms of epigenetic reprogramming - including DNA-demethylation of the paternal chromosomes are indeed conserved between mammalian species.

S06_02

Epigenetic modifications in stem cells

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Increasing evidence indicates that chromatin modifications are important for the establishment and maintenance of stem cell- and cell type-specific gene expression programs. One type of modification, the acetylation of histones occurs reversibly on lysine -NH3+ groups of core histones. Hyperacetylated histones are associated with active chromatin domains, whereas hypoacetylated histones are enriched in non-transcribed loci. The level of histone acetylation depends on the opposing activities of histone acetyltransferases (HATs) and deacetylases (HDACs). Previously our lab showed that altering the epigenotype of neural stem cells (NSCs) followed by transplantation enables the generation of NSC-derived hematopoietic cells (Schmittwolf et al., EMBO J, 2005). Further analyses of NSCs treated with chromatin modifying agents revealed the up-regulation of pluripotency-associated genes (Ruau et al., Stem Cells, 2008).

Despite the enormous amount of information on functional hematopoietic stem cells (HSC) biology, little is known about the epigenetic status of HSCs and their differentiated counterparts and how the epigenetic status is coupled

to HSC self-renewal and differentiation. Here we analysed the global acetylation status of HSCs and their differentiated progeny in mice. Furthermore, we address how active modulation of histone acetylation in HSCs effects self-renewal and differentiation. To directly study the functional relevance of the hyperacetylation state, bone marrow (BM) cells were treated with HDAC inhibitors. As expected, TSA and VPA treatment led to an overall increase in the acetylation status of both immature Lin- and mature Lin+ BM cells. Interestingly, the increase in acetylation status correlated with preferential death of Lin+ cells and the selective survival of Lin- cells. Furthermore, purified TSA-treated Lin- cells showed impaired proliferation and differentiation and treated cultures yielded greater numbers of progenitor cells. In repopulation studies we observe increased hematopoietic engraftment potential in HDAC inhibitor-treated compared to untreated BM cultures. Treatment of BM cells from HoxB4-/- mice revealed that the HDAC inhibitor-mediated increase in progenitor/stem cell numbers is independent of HoxB4, a critical regulator of ex vivo HSC expansion. We conclude that augmented histone acetylation levels achieved by transient HDAC inhibitor treatment increases the frequencies of cells with HSC phenotype and function in the heterogeneous pool of BM cells. In contrast, induction of hyperacetylation status in differentiated BM cells is detrimental, as evidenced by massive death of these cell types upon HDAC inhibitor treatment.

Overall, these data indicate the potentiality of chromatin modifications for the regulation of stem cells.

S06_03

Targeting epigenetic mutations in cancer

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The DNA methyltransferase inhibitors azacytidine and decitabine play a central role in epigenetic cancer therapies. Several studies have shown that decitabine is able to induce DNA demethylation in patients, but the situation is less clear for azacytidine. We have now characterized the DNA methylation changes in myelodysplastic syndrome patients undergoing azacytidine therapy. Our data demonstrate significant drug-dependent methylation changes in blood and bone marrow samples from patients. Demethylation was transient and methylation levels were restored to baseline within individual treatment cycles. Importantly, our data also indicate that demethylation responses towards azanucleosides are patient-specific. In order to identify cellular pathways relevant to drug responses we characterized the mechanisms mediating the cellular uptake of azacytidine. Using transfected cell lines as a model, we could show that concentrative nucleotide transporters mediate transmembrane transport of azacytidine. Additional data also demonstrate a functional role of these proteins in drug-induced DNA demethylation. The identification of pathways mediating the drug effects of azanucleosides will be an important aspect for the further development of epigenetic therapies.

S07_02

Side effects and consequences in clinical gene therapy

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Retroviral vectors have been successfully used for the correction of inherited immune diseases in clinical trials. However, our qualitative and quantitative in vivo monitoring of gene modified hematopoiesis in 4 clinical gene therapy trials revealed that vector induced side effects, ranging from subtle and not clinically overt effects to clonal dominance and even leukemogenesis, have to be considered to occur as ‘normal’. Our studies of vector integration site (IS) distribution (>2000 IS) before and after transplantation showed unequivocally clonal dominance in a trial of ADA-SCID and in successful CGD gene therapy. In 2 SCID-X1 trials with nearly identical clinical protocols, the outcome differed dramatically. In the french study, individual integrants in or near LMO2 were found 5 times, compared to one in the british trial. In both trials, the CD34+ cell gene expression status revealed preferred vector integration in expressed genes and gene ontology analysis showed an in vivo selection of clones with integrants in or near genes with kinase and transferase activity and genes that are involved in phosphorus metabolism. In the first successful gene therapy trial of chronic granulomatous disease (CGD), we also have observed very intensive insertional side effects resulting in activation of MDS1/EVI1, PRDM16 or SETBP1 5 months after therapy.

Our data show that integration site distribution is non random and may influence the cellular gene expression, thus leading to subtle or massive changes regarding the clonality of the hematopoietic repopulation. Incorporation of next generation sequencing technologies will help to substantially improve in depth analyses and to monitor individual and global clone contribution in a hitherto unexpected resolution. Prospective monitoring of vector

integration sites in clinical gene therapy studies is feasible, can detect possible side effects of gene therapy in real-time and may gain new insights in basic mechanisms leading to specific clonal behaviour *in vivo*.

S07_03

Advances in Genetic treatment

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What are the benefits of progress in genetics for patients? The answer of the lay person will certainly be: "gene therapy and therapeutic cloning". Our contemporaries, fascinated by these futuristic prospects, tend to ignore the growth of currently available conventional treatments and the impact of symptomatic management on quality of life and life expectancy of patients with genetic diseases. This is due to a problem of oversimplification of information presented by the media, in which fashion is more important than function and the sensational more important than objective information. In this report, I have tried to establish honestly the inventory of what is already possible. In the light of several examples, let us "render under Caesar what is Caesar's" and try to establish whether replacement of a gene (the identification of which is essential for the understanding of a disease) is truly the universal panacea for the treatment of genetic diseases it is proposed to be.

S08_01

Human Genetics of Malaria and Tuberculosis

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The availability of study groups of several thousand individuals has considerably improved the analysis of effects human genetic variants have on the course of malaria and tuberculosis (TB). For example, the classic malaria resistance factors of haemoglobin C and alpha-thalassaemia have been found not to protect against all forms of malaria but to selectively interfere with the development of distinct clinical signs of life-threatening disease. In TB, variants known to affect the function of T lymphocytes, which are considered crucial in mediating natural protection against TB, have surprisingly been found not to influence the establishment of infection but rather the severity of disease. Initial analyses of ongoing genome-wide association studies using approximately 1 Mio markers suggest that considerable numbers of genes may influence human susceptibility and resistance to malaria and TB.

S08_02

Genomics meets HIV-1

Amalio Telenti
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Infectious diseases represent a unique opportunity to evaluate the integration of several levels of genome research. During this presentation, I will describe, using HIV as the model organism, issues that may prove to be proper to infectious diseases, and unique among other fields of genomics in medicine:

1, The first genome wide association (GWA) analysis underscored the existence of GWA-significant hits that explain an important proportion of the variance of the trait (up to 10%) - well above that described in other GWAs in medicine.

2. There is a second genome involved – that of the pathogen – which leads to the interest of analysis under the premise of evolutionary genetics and genomics.

3. There has been a number of additional studies, that show the way to the next generation of analysis – that of integrative-omics. In particular, the availability of large scale genome siRNA screens and of transcriptome data that can be brought to combined analysis with GWA data.

S08_03

Mice, microbes and models of Infection

Rudi Balling

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Genetic factors have a profound effect on the development of infectious diseases. In order to study the molecular and cellular mechanisms of infection and immunity, animal models can be very useful. For a number of human infectious diseases however no adaequate animal model is available, mainly as a result of pronounced species differences, i.e. in the host-tropism of pathogens or the details of disease pathogenesis. We present studies aimed at the generation of a mouse model to test vaccines against human pathogens, i.e. HCV. For this purpose human hematopoetic stem cells and human fetal liver cells are transplanted into immune-compromised mice in order to produce animals that are chimeric for a mouse and human immune system and liver. In addition to the transplantation of human stem cells mice can be genetically modified through the introduction of human genes into the mouse genome. This allows the introduction specific HLA-human haplotypes or of human cytokine receptors and/or ligands into mice. An alternative to the genetic manipulation of the host genome is the genetic alteration of the microbial genome. We discuss studies in which insight derived from the analysis of the 3-dimensional structure of a virulence factor was used to change the host-tropism of a human bacterial pathogen.

S09_01

A sodium channel with more than one face: Linda's has red feet

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SCN9A encodes a voltage-gated sodium channel, Nav1.7, which is predominantly expressed in the peripheral nervous system. Recent genetic linkage studies have identified SCN9A, the gene that encodes the Nav1.7 sodium channel, as a key player in three inherited conditions with very different pain phenotypes. The first, primary erythermalgia (PE), autosomal dominant and characterized by severe episodic burning pain of the extremities in response to warmth and exercise, is caused by gain-of-function SCN9A mutations which enhance the activation of the Nav1.7 sodium channel, thereby producing hyperexcitability of pain-signaling neurons. The recent discovery of the genetic cause of two little-known conditions has thrown new light on the role of Nav1.7 sodium channels in pain. Paroxysmal extreme pain disorder (PEPD), which was formerly known as familial rectal pain, is characterized by paroxysmal episodes of burning pain of the eye, rectum and submandibulum accompanied by skin flushing. Positional cloning studies identified missense mutations in SCN9A in all affected family members. The mutations produce defective inactivation of Nav1.7. Lastly, an autosomal recessive disorder, formerly known as congenital indifference to pain, has recently been described as channelopathy-associated insensitivity to pain (CIP). The most distinctive feature is that individuals have a congenital absence of sense of pain. Autozygosity mapping in three Pakistani families led again to identification of SCN9A as the culprit, but in this disorder the mutations produce complete loss of functional Nav1.7 channels. These recent findings emphasize the critical role of Nav1.7 in human pain and suggest the design of tailored pharmacological options.

S09_02

Congenital insensitivity to pain

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Congenital Insensitivity to pain is an odd, very rare and disputed disorder. Affected individuals have a congenital inability to experience pain anywhere in the body despite having an apparently intact nervous system. The existence of the disorder has been debated, and many cases suspected to be undetected neuropathies. However, three groups have now reported individuals with this phenotype - especially that the peripheral nerves are normal - who have non-sense mutations in the gene SCN9A. This gene encodes a voltage gated sodium channel nav1.7 present on nociceptive neurons in mammals. The Nav1.7 channel, unusually for a Nav, has an inability to generate an action potential. The mouse knockout had reduced but not absent pain, but died at birth - a clearly different phenotype to humans. However, there can be little doubt of the importance of SCN9A in human pain, as activating mutations cause the dominant episodic pain disorders Primary Erythermalgia and paroxysmal pain Disorder. But most excitingly this discovery has the potential for new analgesic discovery of Nav1.7 blockers.

Selected Poster

SEL 1

Mutations in the Pericentrin (PCNT) gene cause primordial dwarfism

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The growth of an individual grossly depends on regulation of cell size and cell division and dysfunction of the pathways involved not only results in somatic undergrowth but contributes to a wide variety of pathological conditions.
Using positional cloning, we found in a total of 25 patients that biallelic loss-of-function mutations in the pericentrin (PCNT) gene cause microcephalic osteodysplastic primordial dwarfism type Majewski II (MOPD II, MIM 210720). Adults with this rare inherited condition belong to the shortest of the short having a height of about 100 centimeters and a brain size comparable to that of a three-month old baby, but are of near-normal intelligence. Truncal obesity and type 2 diabetes have been noted in older individuals with MOPD II and life expectancy is reduced due to a high risk of stroke secondary to cerebral vascular anomalies.
PCNT and AKAP9 share a highly related C-terminal calmodulin-binding domain and are known to mediate, in a non-compensating manner, nucleation of microtubules by anchoring the γ-tubulin ring complex, thus initiating the assembly of the mitotic spindle apparatus. We show that PCNT mutations cause absence of the protein resulting in disorganized mitotic spindles, premature sister chromatid separation and missegregation of chromosomes in patient cells. Our findings thus characterize MOPD II as a distinct clinical entity linking a key protein of the centrosome to dwarfism and a high risk of diabetes and stroke. Given that all PCNT mutations observed in MOPD II patients lead to loss of functional protein, it remains to be determined if PCNT missense variants are associated with incomplete or other distinct phenotypes.
Similarities between MOPD II individuals and the Late Pleistocene hominid fossils ("hobbits") from the island of Flores, Indonesia, suggest that these do not represent a diminutive, small-brained new species, Homo floresiensis, but are pathological modern humans.
Rauch et al., Science, in press

SEL 2

G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth

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Hypotrichosis simplex (MIM 146520 and MIM 605389) is a group of hereditary non-syndromic human alopecias that affects men and women equally. The hair loss is diffuse and progressive, and usually begins in early childhood. We mapped an autosomal-recessive form of this to chromosome 13q14.11-13q21.33, and identified homozygous truncating mutations in P2RY5, a gene which encodes an orphan G protein-coupled receptor. We analysed expression-patterns of P2RY5 in various human and mouse tissues and performed western blot and immunofluorescence analyses to characterize the protein.
Furthermore, we identified oleoyl-L-α-lysophosphatidic acid (LPA), a bioactive lipid, as being a ligand for P2Y5 in reporter gene and radioligand binding experiments. Homology and studies of signalling transduction pathways suggest that P2Y5 is a member of a subgroup of LPA receptors which also includes LPA4 and LPA5. As P2RY5 is expressed in human hair follicle cells, but LPA4 and LPA5 are not, a loss of P2Y5 function will not be compensated for, and will ultimately lead to pathological changes and hair loss.
Our study is the first to implicate a G protein-coupled receptor as being essential for and specific to the maintenance of human hair growth. With the functional characterization of the P2Y5 receptor, we identify the missing link which is required for the transmission of the LPA signal through the cell membrane in hair follicle cells. This finding may provide opportunities for new therapeutic approaches to the treatment of hair loss in humans.

SEL 3

Mutations in the a2-subunit of the v-type H+-ATPase impair Golgi function and cause a novel congenital disorder of glycosylation with cutis laxa

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Congenital disorders of glycosylation (CDG) form a growing group of hereditary diseases with variable manifestation. Hitherto known genetic defects in CDG affect glycosyltransferases, sugar transporters or proteins involved in vesicular trafficking. We investigated patients with the initial diagnoses of autosomal recessive cutis laxa type II (ARCL type II) or wrinkly skin syndrome (WSS) from several consanguineous families. The phenotype comprised congenital skin wrinkling, a large open fontanel with delayed closure, a typical facial dysmorphism, a generalised joint hyperextensability and a mental retardation of variable degree. Isoelectric focussing of serum glycoproteins revealed a CDG type 2 (CDG-II) affecting N- and O-glycosylation processes taking place in the Golgi compartment. By genetic mapping we identified a homozygous region on chromosome 12q24. Subsenquently, we detected loss of function mutations in the a2 subunit of the v-type H+-ATPase, which had been described to partially reside in the trans Golgi network. Investigations in patient cells revealed no changes of Golgi morphology and marker protein distribution in the steady state, but a significant defect in Golgi-to-ER trafficking upon treatment with brefeldin A. Thus, we were able to identify a novel genetic basis of a congenital glycosylation defect, assign a function to the a2 subunit of the v-type H+-ATPase in the Golgi-apparatus and clarify that wrinkly skin syndrome and autosomal recessive cutis laxa type 2 are allelic disorders.

SEL 4

Comparative methylation analysis of imprinted genes in miscarriages after IVF/ICSI and of spontaneous conception

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Assisted reproductive technologies (ART) interfere with very sensitive periods of development, in which the entire genome is reprogrammed and particularly vulnerable to epigenetic disturbances. To study the possible effects of ART on imprinted genes that cause developmental defects when abnormally regulated in mouse models and/or humans, we use bisulphite pyrosequencing to systematically analyze representative differentially methylated imprinting control regions in miscarriages after in vitro fertilisation (IVF) or intracytoplasmatic sperm injection (ICSI) and in miscarriages of spontaneous conception. Aneuploidies, the most frequent cause of spontaneous abortions, were excluded. In paraffin-embedded chorionic villus samples (CVS) we found quantitative methylation abnormalities of SNRPN in three of 31 and of PEG3 in two of 21 analyzed miscarriages after IVF/ICSI. One of the conspicuous samples showed abnormal methylation in both genes. In contrast, 18 respectively 9 paraffin-embedded CVS of spontaneous conception revealed normal SNRPN and PEG3 methylation levels. So far we did not find any abnormal methylation imprints in 8 frozen CVS after IVF/ICSI and in 19 frozen CVS of spontaneous conception. However, we found hypermethylation of SNRPN, PEG3, and LIT1 in one of three analyzed frozen cortex samples after IVF/ICSI and in one of 10 control brain samples. We propose that the detected methylation abnormalities are not restricted to single or a few specific genes studied, but represent a more general methylation reprogramming problem. Our preliminary data support the hypothesis that developmental defects and imprinting disorders that have been associated with IVF/ICSI, i.e. Beckwith-Wiedemann syndrome and Angelman syndrome, may at least partially be due to aberrant methylation reprogramming of imprinted genes after ART. In addition, methylation abnormalities may be an up to now underestimated cause of spontaneous abortions.

Workshops
Cytogenetics and Prenatal Diagnosis
W1 01
A high oocyte yield for ICSI is associated with an increased aneuploidy rate and polar body diagnosis of oocytes is associated with a reduced pregnancy rate

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Because the "Embryo Protection Law" does not allow aneuploidy testing of blastomeres, polar body diagnosis of oocytes is the only possibility for preimplantation genetic screening in Germany. We have used 5-color FISH for aneuploidy testing of chromosomes 13, 16, 18, 21, and 22 in a population of infertile couples undergoing ICSI treatment because of advanced maternal age and/or recurrent implantation failure. Altogether, 3.688 oocytes from 933 ICSI cycles, for which both polar bodies could be evaluated, were included in this study.
Oocytes from the first ICSI cycle displayed a dramatic increase (by 10%) in aneuploidy between women under 35 years and women of 35-40 years. The aneuploidy rate increased further (by 5%) in women over 40 years. In women with more than one ICSI cycle the maternal age-related increase in aneuploidy was less dramatic. Women with previous implantation failure(s) are already at risk of generating aneuploid oocytes. In women between 35 and 40 years, oocytes from the 2nd-4th ICSI cycle had a significantly higher aneuploidy rate than oocytes from the first cycle.
In women under 35 years and women between 35 and 40 years, oocytes from the high-yield group (>10 oocytes) had an increased likelihood for detectable aneuploidies, compared to the intermediate (6-10 oocytes) and the low-yield group (1-5 oocytes). Lower oocyte yields may indicate a more appropriate response to ovarian stimulation, allowing only the most compentent follicles and oocytes to develop. The overall high aneuploidy rate in women over 40 years appeared to be mainly due to the maternal age effect and increased only slightly with oocyte yield.
Most importantly, polar body diagnosis significantly reduced, rather than increased, the pregnancy rates in women of all age groups. The rate of spontaneous abortions was not reduced. Possible explanations are technical limitations of polar body FISH and that polar body biopsy hampers the developmental potential of the embryo.

W1 02
Quantitative methylation analysis of six imprinted loci in ICSI children born small for gestational age

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Although there is an increased frequency of low birth weight after assisted reproduction, the mechanisms underlying this association are unclear. We have proposed that some of the children conceived by intracytoplasmic sperm injection (ICSI) with low birth weight might have an epimutation (faulty methylation pattern) in one of the imprinted genes involved in fetal growth control like *LIT1*, *PEG1*, *PEG3*, *GTL2*, *H19* and *PLAGL1*. Using bisulfite DNA sequencing and Sequence-based Quantitative Methylation Analysis (SeQMA), we determined the methylation pattern of six differentially methylated regions associated with these genes in buccal smears from 19 ICSI children who were born small for gestational age (SGA) and from 29 children born after spontaneous conception (control group). We detected *de novo* hypermethylation of *LIT1* and *PEG1* in one ICSI child, whose father had oligoasthenoteratozoospermia. This epimutation probably results from an imprint erasure defect in the paternal germ line and therefore appears to be linked to the fertility problem of the father and not to IVF/ICSI.

W1 03
High-resolution aneuploidy screening by array-CGH identifies polar bodies with unbalanced translocation chromosomes and their mode of meiotic segregation

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Current estimates are that more than half of all human preimplantation embryos contain aneuploid cells. At the same time, it is still unclear whether preimplantation aneuploidy screening improves the outcome of in vitro fertilization. At present, the limited information gained by interphase-FISH or the low resolution of conventional CGH represent major drawbacks. Here, we present detailed data about increasing the performance of aneuploidy screening in polar bodies using appropriate whole genome amplification protocols and Agilent 44K Arrays. Recently, we described our strategy combining single cell amplification using GenomePlex library technology (GenomePlex® Single Cell Whole Genome Amplification Kit, Sigma-Aldrich) and detailed analysis of genomic copy number changes by high resolution array CGH (Fiegler et al., Nucleic Acids Research, 2006). Here, we extended this approach for the analysis of the haploid genome in polar bodies and demonstrate that complete karyotype information with unprecedented resolution can be derived from polar bodies. For a more detailed assessment of resolution limits, we used polar bodies from a carrier of a balanced translocation [karyotype: 46,XX,t(2;4)(p16.1;q35)]. Unbalanced meiotic segregation of the translocation chromosomes should result in distinct aneuploidies on chromosomes 2 and 4 and therefore in CGH-profiles, which can clearly be interpreted. In fact, some imbalances of the translocation chromosomes were only identified by array-CGH suggesting that significant improvement in resolution can be achieved. It is likely that the real importance of aneuploidy screening can only be established by the use of such comprehensive high-resolution approaches. Our results suggest that array-CGH is a clear future direction for preimplantation diagnosis.

W1 04

Relevance of aquired clonal chromosome aberrations in bone marrow cells of Fanconi anemia patients

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The most life-threatening early event in Fanconi anemia (FA) patients is bone marrow (BM) failure which typically develops during the first decade of life. Many FA patients acquire clonal chromosomal aberrations in BM cells. The predictive value of such clonal alterations in respect to hematopoietic function and malignant progress are not fully understood. Therefore, we initiated a systematic, prospective molecular-cytogenetic study of BM cells and peripheral blood cells of FA patients in 1996. In 2003, we reported on 18 FA patients with 3q gains who had a high incidence of MDS and AML compared to the control group of FA patients without clonal aberrations indicating that this chromosomal aberration is strongly associated with a poor prognosis. Meanwhile, 144 German FA patients participated in the study since 1996. Out of these 39 (29%) presented with clonal aberrations. The most frequent aberrations were 3q-gains in 27 of the patients with clonal aberrations (69%). Most 3q aberrations are duplications or unbalanced translocations of distal 3q to various other chromosomes not detectable by conventional cytogenetics alone. Eight patients with a 3q-gain had an additional monosomy 7, in six of these the monosomy 7 developed in the 3q aberrant clone as a secondary event.
The overall survival in the 3q group is extremely poor compared to FA patients without aberrations. None of the FA patients with 3q gains survived without bone marrow transplant. There is a significant overrepresentation of males in the group of patients with 3q-gains (19 males, 8 females) indicating a gender specific risk factor. Because of the high MDS/AML risk and the significant higher mortality of FA patients with 3q aberrations, we recommend the systematic assessment of all individual FA patients by molecular cytogenetics and interphase-FISH, in order to detect these aberrations as early as possible and to define criteria for therapeutic measures, e.g. bone marrow transplant.

W1 05

Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared to monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability

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Chromosomal aberrations have emerged as important pathogenetic and prognostic factors in plasma cell disorders. Using interphase FISH analysis, we evaluated chromosomal aberrations in a series of 75 amyloid light chain amyloidosis (AL) patients as compared to 127 patients with monoclonal gammopathy of unknown significance (MGUS). We investigated IgH translocations t(11;14), t(4;14), t(14;16) as well as gains of 1q21, 11q23, 19q13 and deletions of 8p21, 13q14 and 17p13 detecting at least one CA in 89% of the patients. Translocation t(11;14) was the most frequent aberration in AL with 47% vs. 26% in MGUS (p=0.03) and strongly associated with the lack of an intact immunoglobulin (p<0.001), thus contributing to the frequent light chain subtype in AL. Markedly, t(11;14) was recurrently found in combination with gain of 11q23 in AL (20%), but not in the MGUS group (7%; p=0.005). Other frequent aberrations in AL included deletion of 13q14 and gain of 1q21, which were shared by MGUS at comparable frequencies. The progression to MM stage I was paralleled by an increased frequency of gain of 1q21 (p=0.001) in both groups. Similar branching patterns were observed in an oncogenetic tree model indicating a common mechanism of underlying karyotypic instability in these plasma cell disorders.

Molecular Mechanisms 1

W2 01

The Opitz syndrome gene product MID1 assembles a microtubule-associated ribonucleoprotein complex

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Opitz BBB/G syndrome (OS) is a heterogenous malformation syndrome mainly characterized by hypertelorism and hypospadias. In addition, patients may present with several other defects of the ventral midline such as cleft lip and palate and congenital heart defects. The syndrome-causing gene encodes the X-linked E3 ubiquitin ligase MID1 that mediates ubiquitin-specific modification and degradation of the catalytic subunit of the translation regulator protein phosphatase 2A (PP2A). Here, we show that the MID1 protein also associates with elongation factor 1α (EF1α) and several other proteins involved in mRNA transport and translation, including RACK1, Annexin A2, Nucleophosmin and proteins of the small ribosomal subunits. Mutant MID1 proteins as found in OS patients loose the ability to interact with EF-1α. The composition of the MID1 protein complex was determined by several independent methods:
(i) Yeast two Hybrid screening and
(ii) immunofluorescence,
(iii) a biochemical approach involving affinity purification of the complex,
(iv) co-fractionation in a microtubule assembly assay and
(v) immunoprecipitation.
Moreover, we show that the cytoskeleton-bound MID1 / translation factor complex specifically associates with G- and U-rich RNAs and incorporates the MID1 mRNA, thus forming a microtubule-associated ribonucleoprotein (RNP) complex. Our data suggest a novel function of the OS gene product in directing translational control to the cytoskeleton. The dysfunction of this mechanism would lead to malfunction of microtubule-associated protein translation and to the development of OS.

W2 02

Type-2 NF1 deletions are highly unusual by virtue of the absence of non-allelic homologous recombination hotspots and an apparent preference for female mitotic recombination

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Approximately 5% of patients with neurofibromatosis type 1 (NF1) exhibit gross deletions that encompass the NF1 gene and its flanking regions. The breakpoints of the common 1.4 Mb (type-1) deletions are located within low-copy repeats (NF1-REPs) and cluster within a 3.4 kb hotspot of non-allelic homologous recombination (NAHR). Here we present the first comprehensive breakpoint analysis of type-2 deletions, a second type of recurring NF1 gene deletion. Type-2 deletions span 1.2 Mb and are characterized by breakpoints located within the SUZ12 gene and its pseudogene which closely flank the NF1-REPs. Breakpoint analysis of 13 independent type-2 deletions did not reveal any obvious hotspots of NAHR. However, an over-representation of polypyrimidine/polypurine tracts and triplex-forming sequences was noted in the breakpoint regions that could have facilitated NAHR. Intriguingly, all 13 type-2 deletions identified so far are characterized by somatic mosaicism indicating a positional preference for mitotic NAHR within the NF1 gene region. Indeed, whereas interchromosomal meiotic NAHR occurs between the NF1-REPs giving rise to type-1 deletions, NAHR during mitosis appears to occur intrachromosomally between the SUZ12 gene and its pseudogene thereby generating type-2 deletions. Such a clear distinction between the preferred sites of mitotic versus meiotic NAHR is unprecedented in any other genomic disorder induced by the local genomic architecture. Additionally, 12 of the 13 mosaic type-2 deletions were found in females. The marked female preponderance among mosaic type-2 deletions contrasts with the equal gender distribution noted for type-1 and/or atypical NF1 deletions. Although an influence of chromatin structure was strongly suspected, no gender-specific differences in the methylation pattern exhibited by the SUZ12 gene were apparent that could explain the higher rate of mitotic recombination in females.

W2 03

End-stage renal failure in WT1-associated Denys-Drash-Syndrome involves downregulation of Sulfatase 1

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Mutations of the Wilms tumor-1 (WT1) gene cause glomerulosclerosis in children (Denys-Drash syndrome; DDS) and mice and result ultimately in end-stage renal failure. The transcription factor WT1 is specifically expressed in podocytes of renal glomeruli. To identify WT1 target genes involved in glomerulosclerosis, we performed expression analysis (Affymetrix) in native glomeruli from four wild type and four Wt1 heterozygous (one non-functional allele) mice. Among the downregulated genes in glomeruli from Wt1 heterozygous mice was Sulf1, coding for a Heparan Sulfate 6-O-Endosulfatase known to participate in growth factor signaling, e.g. VEGFA. The array data were validated using quantitative RT-PCR. Sulf1 expression was also downregulated in glomerular podocyte cell lines established from patients with DDS and decreased in an immortalized renal precursor cell line (metanephric mesenchyme) after knockdown of WT1. Chromatin immunoprecipitation (ChIP) with chromatin prepared from murine kidneys could be used to demonstrate association of WT1 with the Sulf1 promoter region. In situ hybridization on E14.5 kidneys revealed that Sulf1 is expressed in a podocyte specific expression pattern during kidney development in mice. Interestingly, we could demonstrate that knockout of the Sulf1 gene in mice leads to proteinuria and to a similar ultrastructural phenotype to the one seen in human DDS patients, namely podocyte foot process effacement, thickening of the glomerular basement membrane and endotheliosis. As we had shown before in DDS, glomerulosclerosis might be linked to abnormal expression of VEGFA isoforms, and thus, impaired crosstalk between podocytes and endothelial cells. The observation that Sulf1 is expressed in metanephric mesenchyme, podocyte precursors as well as in adult podocytes may suggest that it is

involved in glomerular development and homeostasis. Its misregulation may lead to disturbed VEGFA signaling and may contribute to glomerulosclerosis.

W2 04

Therapy models for congenital ichthyosis: Managing hyperkeratosis in 3D skin systems

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In the last four years several new genes for autosomal recessive congenital ichthyosis (ARCI) have been identified. Still only little is known about the pathophysiology of this heterogeneous group of keratinisation disorders. So far treatment for ARCI is only symptomatic and often insufficient. In order to gain a patient independent, human model mimicking the barrier defect and hyperkeratosis typical for ARCI, we established different 3D skin models for our purpose. A major challenge for finding a 3D skin model for keratinisation disorders is the thickening of the stratum corneum typically seen over time without a genetic defect. To evaluate therapeutics with a skin model, it must develop a basal layer attached to the dermal layer and show a normal differentiation pattern. To stratify effects of inactivation of different genes involved in ARCI, we transfected primary keratinocytes from healthy volunteers with siRNA to knock down the expression of single genes prior to 3D model preparation. Structure, thickness of stratum corneum, immunohistochemical stainings, and quantitative analyses proved the efficiency of each knock-down. Keratinocytes from healthy donors were grown in a serum-free system until 3D model preparation. 3D skin models differed in differentiation medium, including SKDM, SKDM with TGFβ1, and KCM. We found hyperproliferation and thickening of the stratum corneum as well as detachment from the dermal layer in the model w/o knock-down when cultivated with SKDM for more than 10 and up to 21 days. Added TGFβ1 gave less prominent thickening of the stratum corneum after 7 days and well defined basal and granular layers. 3D models with KCM showed a thin, normal stratum corneum, defined basal and granular layers and constant attachment to the dermal layer. Currently immunohistochemical stainings and ultrastructural analyses are under way. The systems will be fundamental for studies of disturbed epidermal differentiation and pharmaceutical intervention.

W2 05

Interdependencies between cilia genes with hypomorphic mutations in trans: A concept for modification of disease expression

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Many genetic diseases have been linked to the dysfunction of primary cilia which occur nearly ubiquitous in the body and act as solitary cellular mechanosensory organelles. The list of clinical manifestations and affected tissues in these cilia-related disorders (ciliopathies) is broad and has been attributed to the wide expression pattern of ciliary proteins. The genetic and molecular overlap between clinical entities that were thought to be distinct, like Bardet-Biedl, Joubert, and Meckel-Gruber syndrome, is also becoming increasingly more evident. Recently, we and others identified mutations in the gene encoding the centrosomal protein CEP290 in patients of the above spectrum. However, surprisingly, comparable or even identical CEP290 mutations can cause very different phenotypes. Given the postulated network of cilia proteins, it might be legitimate to claim modifying effects by proteins interacting and/or colocalizing with CEP290 to be, at least in part, causative for some of the observed phenotypic variability, also among affected siblings of the same family. For example, in one family, the first child is alive and affected with isolated Leber congenital amaurosis, whereas the second pregnancy was terminated because of oligo-/anhydramnios and massively enlarged cystic kidneys that were later confirmed by autopsy. We provide compelling evidence that mutations of other ciliary genes in trans have an aggravating effect and contribute to the more severe clinical course of some individuals. Overall, our findings give valuable insights into the pathogenesis not only of ciliopathies, but also of the genetic basis of phenotypic variability in general.

Neurogenetics 1

W3 01

Autozygosity mapping in a large cohort of consanguineous Iranian families reveals two frequent loci for autosmal recessive mental retardation

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There is reason to believe that autosomal recessive mental retardation (ARMR) is far more common than X-linked mental retardation (XLMR), but in contrast to the latter, it has so far received comparatively little attention. In part, this is due to small family sizes and low consanguinity rates in industrialized societies, both of which have greatly hampered gene mapping and identification. Consequently, for a long time no more than three ARMR genes were known.
To shed more light on the causes of ARMR and as a prerequisite for diagnosis, counselling and therapy, we have set out in 2003 to perform systematic clinical and molecular studies in large consanguineous Iranian families with several mentally retarded children. This ongoing effort has already led us to the discovery of 12 novel ARMR loci, eight of which had a LOD score above three, and in one of them we were recently able to identify a mutation in GRIK2 (Motazacker et al., Am J Hum Genet. 2007, 81(4):792-8). Contrary to previously published observations which prima facie argued against the existence of frequently mutated genes (Najmabadi et al., Hum Genet. 2007, 121(1):43-8), investigations in our expanding cohort of more than 190 families have now led to the identification of two loci on chromosomes 5 and 19, that each show overlapping autozygosity regions of three families. Mutation analysis by sequencing these intervals in order to elucidate the underlying gene defects is ongoing and likely to reveal the first ARMR gene with increased prevalence. In addition, we report on the status of our investigations in further ARMR families. The outcome of our project will profoundly enhance the basis for proper genetic counselling and provide insight into the molecular basis of brain function.

W3 02

Transcriptional effects of mutations in the XLMR gene JARID1C

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Moderate to severe X-linked mental retardation (XLMR) affects approximately 2 in 10.000 males. Non-syndromic XLMR (NS-XLMR) in which mental retardation is the only clinically consistent feature is genetically heterogeneous and mutations have been found in >25 different genes. One of these is the transcription factor JARID1C, where mutations have been found in 13 families, 12 of which belong to the cohort of the Euro-MRX Consortium. In order to investigate transcriptional effects of JARID1C deficiency we first performed genome-wide expression analysis of mRNA from a patient cell line with a premature termination codon in JARID1C and 3 controls, using a Sentrix Human-6 Expression Beadchip together with the BeadStudio analysis software (Illumina). This investigation revealed consistent differential expression between the JARID1C deficient cell line and each of the 3 controls in 55 genes. In a second set of experiments we were able to verify the expression pattern for 24 genes by quantitative RT-PCR or Northern blot analysis using RNA from all 12 Euro-MRX patient cell lines and 5 controls. In a third set of experiments we analysed RNA from whole blood, extracted from two MR families with a missense and a truncating mutation, respectively. Our results revealed, among others, up-regulation of CMKOR1, KIAA0469 and JARID1B and show that JARID1C missense changes and nonsense mutations had an equal impact on transcription. Therefore our results suggest that mRNA expression profiling in XLMR mutation carriers might be a tool for the identification of putative pathways, which play a role in cognition. Furthermore they show that this approach could be useful for refining the molecular diagnosis of patients with disorders where mRNA expression is affected.

W3 03

A mutation in the Scyl1 gene causes neurodegeneration in the mdf-mutant mouse

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The autosomal recessive mouse mutant mdf (muscle deficient) has been described as a model for motor neuron disease. While heterozygotes are phenotypically unaffected, muscle degeneration in mdf homozygotes is progressive with first symptoms starting at 4 to 8 weeks of age. Homozygous mdf mice can easily be identified by growth retardation, posterior waddling gait, and muscular atrophy accompanied by nervous tremor. The first comprehensive patho-anatomical characterization (Blot et al.) revealed that this mutant resembles spinal muscular atrophy, a motor neuron disease caused by a progressive degeneration of anterior horn cells. The mdf locus had been initially mapped to a centromeric region of mouse chromosome 19. We have now refined the mdf locus to a 0,28 cM interval between markers D19Mit59 and D19Mit109 by genotyping 123 mdf/mdf homozygote intercross mice. We identified 48 positional candidate genes within this region, which were subjected to high throughput sequencing. We detected a homozygous single nucleotide insertion in the Scyl1 gene, as the only pathogenic mutation. Quantitative mRNA assays unveiled massive reduction of Scyl1 transcripts in skeletal muscle and brain from homozygous mdf-mice compared to wild-type (wt)-animals. Westernblot analysis using antibodies against the N-terminal and C-terminal peptide sequences were performed, detecting a ~105 kDa protein in wt-mice and showed complete absence of the protein in tissues from mdf-mice. Furthermore, we determined the normal expression pattern with in situ hybridization and found Scyl1 to be most prominently expressed in large neurons of the central nervous system, including hippocampus, and Purkinje cells in the cerebellum. Further functional analyses are ongoing. This identification of the genetic basis of the mdf mutant will pave the way to further elucidate the function of Scyl1 in these highly specialised neurons and its role in neurodegeneration in this animal model.

W3 04

Analyses of an inducible transgenic mouse model reveals the reversibility of neurodegenerative processes in SCA3

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Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene. In order to study the course of the disease, we generated an inducible transgenic mouse model using the “Tet-Off-System”. This system is based on two constructs: The promoter construct controls the expression of the so called tTA gene product. The binding of tTA to a TR element in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Doxycycline which allosterically inhibits the tTA. For the ataxin-3-responder mouse lines, a full length constructs containing an expanded repeat with the pathological length of 77 glutamine repeats was used. The use of two different promoter mouse lines with known expression in the brain (Prion protein (Prp) promoter, Ca2+/Calmoduline-dependent protein kinase II (CamKII) promoter) allows us to target the transgene expression to different brain regions. Using the CamKII promoter, the trangene is targeted mainly to the frontal brain and mice display first symptoms at the age of six months. However, when we use the Prp promoter, the transgene is widely expressed in the brain with pronounced expression in the cerebellum and the brain stem. In these mice, first motor symptoms can be detected at the age of two months. We then stopped the expression of the transgene by treatment with doxycyclin and compared treated and untreated mice at different levels: We first analysed the phenotype in behavioural studies. Afterwards we performed immunohistochemical analyses of brain sections to study the expression of the transgene and neurodegenerative processes in the brain. These analyses

revealed that turning off the pathogenic transgene at least slows down the progression of symptoms. Comparable approaches in human patients -if available- would therefore require early initiation.

W3 05

Homozygous PMS2 germline mutations in two families with early-onset haematological malignancy, brain tumours, HNPCC associated tumours, and signs of neurofibromatosis type 1

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Heterozygous germline mutations in mismatch repair (MMR) genes MLH1, PMS2, MSH2 and MSH6 cause Lynch syndrome. New studies have indicated that biallelic mutations lead to a distinctive syndrome, childhood cancer syndrome (CCS), with haematological malignancies and tumours of brain and bowel early in childhood, often associated with signs of neurofibromatosis type 1. We provide further evidence for CCS reporting on six children from two consanguineous families carrying homozygous PMS2 germline mutations. In family 1, all four children had the homozygous p.I590Xfs mutation. Two had a glioblastoma at the age of six and one of them had three additional Lynch-syndrome associated tumours at 15. Another sibling suffered from a glioblastoma at age 9, and the fourth sibling had infantile myofibromatosis at 1. In family 2, two of four siblings were homozygous for the p.G271V mutation. One had two colorectal cancers diagnosed at ages 13 and 14, the other had a Non-Hodgkin lymphoma and a colorectal cancer at ages 10 and 11, respectively. All children with malignancies had multiple café-au-lait spots. After reviewing published cases of biallelic MMR gene mutations, we provide a concise description of CCS, revealing similarities in age distribution with carriers of heterozygous MMR gene mutations.

Cancer Genetics

W4 01

Combined array-based genetic, epigenetic and transcriptional profiling suggests the presence of a precursor cell with stem cell-like features in different subtypes of aggressive B-cell lymphomas

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Gene inactivation by DNA methylation is a well-known mechanism in cancer. Here, we have applied a microarray-based DNA methylation analysis of 1505 CpGs from 807 genes (selected by their involvement in cancer) in a total of 83 aggressive B-cell lymphomas characterized by transcriptional (Affymetrix U133A) and genetic profiling (3k BAC arrays and FISH), 7 B-cell lymphoma cell lines and 10 non-malignant hematopoietic tissues. A hierarchical cluster analysis of DNA methylation profiles indicated that methylation patterns were not strictly associated with any morphologic, genetic or transcriptional features, including primary chromosomal aberrations or gene expression subgroups. Genes were subsequently classified according to their DNA methylation pattern in lymphomas and control samples. We identified sets of genes de novo methylated in all lymphoma subtypes (n=56) or in a subtype-specific manner (n=22). Remarkably, these two groups were characterized by distinct biological features. Genes de novo methylated across different genetic and pathologic lymphoma

subgroups were highly enriched for targets of the polycomb transcriptional repressor complex in stem cells (OR=8.2/p=1.7e-11) and for biological processes deregulated by DNA methylation in different cancers like signal transduction pathways. Remarkably, these genes were expressed at low levels in different series of normal hematopoietic tissues (p=5e-8 to 3e-15) but not in tissues of non-hematopoietic origin. These findings, especially the high enrichment for polycomb targets in stem cells, suggest that DNA methylation might represent an early event in lymphomagenesis. Furthermore, it suggests that aggressive B-cell lymphomas with different genetic, transcriptional and morphological background might all derive from lymphoma precursor cells with similar stem cell-like features. This study has been performed as part of the Network Project of the Deutsche Krebshilfe “Molecular Mechanisms in Malignant Lymphomas”.

W4 02

Genetic, epigenetic and expression profiling of an in vitro model for reversible differentiation in pancreatic ductal adenocarcinoma (PDAC)

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A818-6 cells, established from human PDAC-ascites, can reflect both, a malignant and a benign phenotype. Cultured under standard conditions, A818-6 cells behave like highly malignant cells. When culturing on agar the phenotype of this cell line changes dramatically into a stable benign phenotype characterized by decreased proliferation and telomerase rates. These cells grow in hollow spheres, show a clear polarity and do not induce tumours in mice. When disrupting the spheres mechanically the phenotype switches back to “malignancy”.
A reversible change from one stable phenotype to another upon environmental influence is characteristic for the involvement of epigenetic mechanisms controlling gene expression. To further characterize the two phenotypes and to analyze the impact of epigenetics we here performed several genome-wide analyses.
While array-CGH (Human Genome CGH Microarray 44K, Agilent) revealed no genetic differences between the phenotypes, we identified roughly 2000 genes differentially expressed by Affymetrix (Human Genome U133 Plus 2.0 Array) chip analysis. A 2D-gel approach provided a broad overview about differences in the proteome of the two phenotypes with up till now 30 differentially expressed proteins identified by mass spectroscopy. Interestingly, numerous genes found to be upregulated in the malignant phenotype have also been identified to be upregulated in PDAC tissue isolated from patients.
DNA methylation studies based on MeDIP- and Illumina-BeadArray analyses showed only minor changes in the “DNA- methylome” of the two A818-6 statuses, while we could identify numerous changes in the methylation pattern of K4H3 (3mK4H3, a marker for gene activity), by ChIP-on-Chip analysis. Interestingly, we identified 10 out of 328 miRNAs studied to be differentially expressed in the two phenotypes. Our results provide initial insights into the epigenetic mechanisms underlying dedifferentiation of PDAC.

W4 03

Biallelic hypomethylation of PRAME in uveal melanoma

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Uveal melanoma is the most common malignancy of the eye in adults. Approximately 50% of the patients die of liver metastases. Aberrant activation of PRAME, which belongs to the family of cancer-testis (CT) genes, is frequently observed in primary uveal melanoma. To study the epigenetic mechanisms of PRAME activation in UM we determined DNA methylation patterns of a CpG rich region located in the exon1/intron1 region of PRAME. We used direct sequencing and sequence-based quantitative methylation analysis (SeQMA) of PCR products from bisulfite treated genomic DNA from 20 uveal melanomas, from 6 uveal melanoma cell lines, from mononuclear blood cells of seven

healthy donors, and from sperm samples of two healthy donors. In addition, we determined PRAME mRNA levels by quantitative real time PCR. Mononuclear blood cells from all seven healthy donors showed almost complete methylation of the CpG rich region whereas no methylation was detected in normal sperm DNA. Altered PRAME methylation was detected in 9 of 20 primary uveal melanomas with hypomethylation in 3 samples and almost complete loss of methylation in 6 samples. As PRAME expression was only detected in hypomethylated and unmethylated tumor samples it is plausible that DNA methylation participates in regulation of PRAME expression. Two samples with loss of methylation were informative for a single nucleotide polymorphism rs113067222 located within the analysed region. In both samples heterozygosity was retained and, therefore, allele loss did not contribute to demethylation in these tumors. Hypomethylation of both alleles of PRAME in uveal melanoma may indicate that this region is actively demethylated or selectively protected from maintenance methylation during cell proliferation. Alternatively, the methylation pattern of PRAME in uveal melanomas might reflect the origin of these cells from an unmethylated progenitor.

W4 04

Disruption of Ikaros function by the CALM/AF10 fusion protein might be responsible for abortive lymphoid development in CALM/AF10 positive leukemia

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The t(10;11)(p13;q14) translocation leads to the fusion of the CALM and AF10 genes. This translocation can be found as the sole cytogenetic abnormality in acute lymphoblastic leukemia, acute myeloid leukemia and in malignant lymphomas. The expression of CALM/AF10 in primary murine bone marrow cells results in the development of an acute myeloid leukemia that is propagated by cells with lymphoid traits (Deshpande et al., Cancer Cell, 2006). Using a yeast two-hybrid screen, we identified the lymphoid regulator Ikaros as an AF10 interacting protein. Ikaros is required for normal development of lymphocytes, and aberrant expression of Ikaros isoforms was found in leukemia. In a murine model, the expression of a dominant negative isoform of Ikaros caused leukemias and lymphomas. The Ikaros interaction domain of AF10 was mapped to the leucine zipper domain of AF10, which is required for malignant transformation both by the CALM/AF10 and the MLL/AF10 fusion proteins. The interaction between AF10 and Ikaros was confirmed by GST pull down and co-immunoprecipitation. Coexpression of CALM/AF10 but not of AF10 alters the subcellular localization of Ikaros in murine fibroblasts (Greif et al., Oncogene, 2007). The transcriptional repressor activity of Ikaros is reduced by AF10. These results suggest that CALM/AF10 might interfere with normal Ikaros function, and thereby block lymphoid differentiation in CALM/AF10 positive leukemias.

W4 05

AML1/RUNX1 mutations are frequent in de novo AML with certain cytogenetics and cooperating molecular mutations differ dependent on underlying cytogenetics

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Previous reports have shown that AML1/RUNX1 mutations are associated with MDS and AML after MDS. De novo AML were poorly analyzed and cooperating mutations (coop mut) are not analyzed at all. We performed an analysis focused on de novo AML and on distinct cytogenetic subgroups. A selected group of 243 AML with normal karyotype (NK) or recurrent chromosomal imbalances were analyzed: NK (n=97); -7 (n=37), +8 (n=30), +13 (n=20), +21 (n=12), others (47). 79 of 243 patients in these selected cytogenetic subgroups showed at least one RUNX1mut. 67/79 mutations were different. 19 patients (26%) had homozygous mutations and 5 (6.8%) had two different mutations. In NK 20% had RUNX1mut, 27% in -7, 33% in +8, 90% in +13, 58% in +21. These results for the first time show that RUNX1mut are frequent in de novo AML with certain cytogenetics. All cases were also analysed for FLT3-LM, FLT3-TKD, MLL-PTD, NRAS, NPM1, JAK2 and CEBPA. NPM1mut and CEBPAmut were found to be mutually exclusive of RUNX1mut. The analysis for cooperating mutations (coop mut) was done for subgroups. NK subgroup: 29/79 carried coop mut, 18 x MLL-PTD, 15 x FLT3-LM and 3 x NRAS. -7 subgroup: No coop mut was detected in the 10 RUNX1mut cases with -7. + 8 subgroup: In 2 RUNXmut cases 3/10 coop mut were detected. In contrast nearly all

RUNXwt with +8 had an FLT3-LM, NPM1mut or NRASmut. +13 subgroup: only 3/20 had coop mut. Instead this specific cytogenetic subgroup shows a 4-fold-elevated FLT3 expression as an alternative cooperating event. Overall, MLL-PTD (62%) was found to be the most frequent coop mut for RUNX1 followed by FLT3-LM (52%) and NRAS (10%). In conclusion,
1) RUNX1mut are frequent in de novo AML with normal karyotype or single chromosomal imbalances.
2) Coop mut are frequent in some subgroups (NK and +21), rare (+13) or absent in others (-7, +8).
3) MLL-PTD plays a major role as coop mut in RUNX1mut AML.
4) some AML may reveal alternative cooperating mechanisms like overexpression of FLT3 in +13.

Disease Gene Identification 1

W5 01

Mutational hotspots in *ALOXE3* and allelic heterogeneity for mutations in *ALOX12B* underlie deficiency of the 12-lipoxygenase pathway in autosomal recessive congenital ichthyosis

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Autosomal recessive congenital ichthyosis (ARCI) represents a group of severe keratinization disorders characterized by extended clinical and genetic heterogeneity. Besides *TGM1*, several new genes underlying ARCI were identified recently, including *ABCA12*, *ichthyin*, *CYP4F22*, and the lipoxygenase genes *ALOX12B* and *ALOXE3*. Mutations in *ALOX12B* and *ALOXE3*, coding for 12R-LOX and eLOX-3 predominantly expressed in the epidermis, are the second most common cause for this rare condition. In 350 unrelated ARCI patients, mostly originating from central Europe, we found mutations in *TGM1* in one third and in *ALOX12B/ALOXE3* in another 13% of the cases. In total, we identified 18 different mutations in *ALOX12B*, including 8 novel mutations. In *ALOXE3* we found 6 different mutations including 3 novel ones. Several mutations in *ALOX12B* were private ones, and none of these was seen on more than 4 chromosomes. In contrast, two mutations in *ALOXE3*, Arg234X and Pro630Leu, were found on 11 and 15 different chromosomes, respectively. Haplotype analysis pointed out that the mutations occurred on several different genetic backgrounds. For 14 missense mutations and one in-frame deletion we demonstrated complete loss of function of the mutant protein. Still only little is known about the pathophysiology of ARCI. The epidermal lipoxygenases are subsequent members of the 12-lipoxygenase pathway metabolizing arachidonic acid in epidermal keratinocytes. We are now further analysing their involvement in the formation of the epidermal barrier using expression and functional studies in patient keratinocytes, skin equivalents, and a mouse model. The characterization of allelic heterogeneity in *ALOX12B* and two mutational hotspots in *ALOXE3* is important for analysing the epidemiology of ARCI and may point to different functional domains. Moreover, it will be implemented into the future automation of diagnostic procedures in ARCI and related disorders.

W5 02

Mutations in the Cyclin family member FAM58A cause a novel X-linked dominant disorder characterized by syndactyly, telecanthus, anogenital and renal malformations (STAR syndrome)

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We recently identified four girls with a consistent constellation of toe syndactyly, telecanthus, anogenital and renal malformations, and we propose the acronym STAR syndrome for this condition. A single mother-daughter pair had previously been reported by Green et al. with a similar combination of malformations. The authors noted that this condition was autosomal dominantly inherited and overlapping with but distinct from Townes-Brocks syndrome (OMIM #601446). Array CGH performed with DNA of one of our patients revealed a *de novo* heterozygous deletion of 37.9-50.7 kb including exon 1 of the gene *FAM58A* on Xq28, and qPCR detected a *de novo* deletion of *FAM58A* exon 5 in a second case. Point mutation analysis revealed one truncating and two splice mutations in *FAM58A* in three further cases including the family reported by Green thus confirming this disorder as a distinct recognizable X-linked dominant condition. *FAM58A* encodes a Cyclin box fold domain, and in accordance with that siRNA mediated knockdown in cultured cells revealed a proliferation defect. FAM58A interacts with SALL1 but not SALL4 as determined by co-immunoprecipitation, corresponding to the close phenotypic overlap with Townes-Brocks syndrome.

W5 03

Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia

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Many genetic diseases have been linked to the dysfunction of primary cilia which occur nearly ubiquitous in the body and act as solitary cellular mechanosensory organelles. The list of clinical manifestations and affected tissues in cilia-related disorders (ciliopathies) such as nephronophthisis is broad and has been attributed to the wide expression pattern of ciliary proteins. However, little is known about the molecular mechanisms leading to this dramatic diversity of phenotypes. We recently reported hypomorphic NPHP3 mutations in children and young adults with isolated nephronophthisis and associated hepatic fibrosis or tapeto-retinal degeneration. Here, we chose a combinatorial approach in mice and humans to define the phenotypic spectrum of NPHP3/Nphp3 mutations and the role of the nephrocystin-3 protein. We show that the pcy mutation generates a hypomorphic Nphp3 allele that is responsible for the cystic kidney disease phenotype, whereas complete loss of Nphp3-function results in situs inversus, congenital heart defects and embryonic lethality in mice. We could correlate these data with our findings in patients and show that NPHP3 mutations cause a broad clinical spectrum of early embryonic patterning defects comprising situs inversus, polydactyly, central nervous system malformations, structural heart defects, preauricular fistulas, and a wide range of congenital anomalies of the kidney and urinary tract (CAKUT).
Conclusively, NPHP3 is a gene that if mutated can result in nephronophthisis, Senior-Loken syndrome, renal-hepatic-pancreatic dysplasia, Meckel-Gruber syndrome, and embryonic lethality. Direct interaction of nephrocystin-3 with inversin suggests its role in the control of canonical and non-canonical (planar cell polarity) Wnt signaling.

W5 04

A recurrent missense mutation in the gene encoding the nuclear matrix component matrin 3 is associated with autosomal dominant distal myopathy

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Distal myopathies represent a heterogeneous group of inherited skeletal muscle disorders. One type of adult-onset, progressive autosomal dominant distal myopathy, frequently associated with dysphonia and dysphagia, has been mapped to chromosome 5q31 in a North American pedigree (vocal cord and pharyngeal weakness with distal myopathy; VCPDM). Here we report identification of a second VCPDM family of Bulgarian descent and fine mapping of the critical interval. The refined candidate region includes the MAT3 gene that encodes a protein of the nuclear matrix, a filamentous protein network in vertebrate nuclei. MAT3 is a candidate for VCPDM as mutations in the genes for the nuclear envelope proteins lamin A/C and emerin cause muscular dystrophies. Screening of MAT3 for mutations led to the identification of a non-conservative missense mutation affecting a highly conserved serine residue (S85C) in both pedigrees. Different disease related haplotype signatures were observed in the two families, providing evidence that two independent mutational events at the same position in MAT3 cause VCPDM. Our data provide evidence that the nuclear matrix is crucial for normal skeletal muscle structure and function and put VCPDM on the growing list of monogenic disorders associated with the nuclear proteome.

W5 05

Oligoasthenoteratozoospermia in dynein light chain Tcte3-3 deficient mice

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The microtubule-based motility of cilia and flagella plays a critical role in fertility and viability in mammals. The motive force to power sperm flagella is generated by dynein motors that are multi subunit complexes. They consist of heavy, intermediate and light chains, which differ in their molecular weight and function. Here we report the functional analysis of the murine dynein light chain Tcte3-3 by targeted disruption in mice. Expression studies demonstrate that human and murine Tcte3 genes are mainly expressed during male germ cell development, however, weaker expression is observed in several tissues including brain, lung and trachea.
Heterozygous Tcte3-3-deficient mice were viable and showed no malformations. Breeding of heterozygous males and females resulted in normal litter size, however, we were not able to detect homozygous Tcte3-deficent mice using standard genotype techniques. Our results indicate the presence of at least three copies of the Tcte3 gene (Tcte3-1, Tcte3-2 and Tcte3-3) in the murine genome that were highly similar and were not distinguishable by PCR analyses or by Southern blotting. Therefore, quantitative real-time PCR was established to differentiate between mice having one or two targeted alleles and Tcte3-3-/- animals were identified. Subsequent breeding of Tcte3-3-/- males with wild-type or heterozygous females resulted in no offspring. Male infertility was associated with the homozygous targeted mutation and was caused by reduced sperm motility, resulting in impaired movement of Tcte3-3-deficent spermatozoa through the female genital tract. In addition, reduction or deficiency of the Tcte3-3 gene product led to a decreased sperm number and an increased rate of apoptosis in male germ cells. Moreover, different abnormalities were observed in developing germ cells as well as in spermatozoa from the epididymis, supporting a basic role of Tcte3-3 in mammalian spermatogenesis.

W5 06

Mutations in *RAF1* (*CRAF*) cause a subtype of Noonan syndrome

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Noonan syndrome (NS) is a heterogeneous condition characterized by congenital heart defects, short stature, and typical dysmorphism. NS and clinically related disorders have been shown to be caused by activating mutations in various components of the Ras-MAPK signalling pathway. We screened the most recently identified gene for NS, *RAF1* (*CRAF*), in a cohort of 150 patients with the clinical diagnosis of NS, who had been tested negative for mutations in the genes *PTPN11*, *KRAS*, and *SOS1*. Seven different *RAF1* mutations, clustering in exons 7 and 14, were discovered in 22 individuals, including 19 sporadic patients with NS and three affected individuals of a single family. Six mutations have been described before, while we identified one novel de novo change (c.823A>G/p.R275G). This alteration affects a residue located near a cluster of amino acids known to be mutated in previously reported patients with NS. The most prevalent mutation (60%) was the c.770C>T (p.P257L) transition in exon 7. It is of note that 82% of the individuals with a *RAF1* mutation had hypertrophic cardiomyopathy (HCM). HCM was present at birth or occurred during infancy. Four affected infants died due to severe obstructive HCM, and three patients underwent cardiomyectomy at ages 6-10 years. Besides the strong association with HCM, older individuals carrying a *RAF1* mutation often had multiple pigmented naevi. Some features that are otherwise uncommon in NS were repeatedly found in our cohort of *RAF1*-mutation positive patients, such as hydronephrosis and severe dysgnathism. We conclude that patients with *RAF1* mutations display a distinct subtype of NS. This phenotype is mainly characterized by a specific pattern of cardiac involvement that is associated with significant morbidity and increased risk of lethal outcome in some cases. In summary, these findings have important implications for diagnostic strategies, prognostic considerations, and long-term follow-up.

Molecular Syndromology

W6 01

The neuro-cardio-facial-cutaneous syndromes: clinical classification and genotype phenotype correlations

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The term neuro-cardio-facial-cutaneous (NCFC) syndromes has recently been proposed for Noonan syndrome (NS) and related disorders, including cardio-facio-cutaneous syndrome (CFCS), Costello syndrome (CS), and LEOPARD syndrome (LS), as well as neurofibromatosis type 1 (NF1). This heterogeneous group of disorders is caused by constitutional deregulation of the Ras-MAPK signalling cascade as the common pathomechanism. We review clinical data from our cohort comprising more than 200 mutation-positive patients with a disorder of the NCFC spectrum (excluding NF1). Patients with mutations of PTPN11, SOS1, and RAF1 are classified as NS in the majority of cases, while individuals carrying a mutation in BRAF, MEK1, or MEK2 fall in the category of CFCS, with little overlap with NS or CS. Individuals with a HRAS mutation affecting amino acid residue p.G12 or p.G13 form the genetically and clinically very homogeneous CS group. In contrast, patients carrying a KRAS mutation show marked clinical variability. The most consistent features discriminating between molecularly proven cases of NS and CFCS is the presence of substantial mental retardation in virtually all patients with BRAF and MEK1/2 mutations. Among the NS-affected patients, the most prominent association is the one of RAF1 mutations with hypertrophic cardiomyopathy. Patients with a missense mutation of SOS1 tend to have better growth and cognitive development and may show ectodermal features similar to CFCS. We conclude that the molecular findings strongly correlate with the known clinically defined entities, thus confirming the usefulness of the established nosology. Within the groups of NS and CFC subtypes seem to emerge despite significant overlap. Particularly in infants, when the clinical discrimination between these entities is often demanding, molecular testing is useful for differential diagnosis and prognosis. The emerging genotype phenotype correlations have implications for molecular testing strategies.

W6 02

Unusual LRP5 mutations cause an atypical phenotype in a Turkish family and provide evidence that genetic variation in the signal peptide influences translation efficiency

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We performed a genome-wide linkage-analysis in a Turkish family with congenital blindness, severe muscular hypotonia and mild mental retardation. We found compound heterozygosity for two novel mutations in the wnt-co-receptor LRP5 at one of the candidate loci. The first mutation results in a 6-leucine deletion within a stretch of normally 9 leucines located in the signal peptide. The second mutation is an 8.2 kb deletion of exon 14 to 16, flanked by alu-sites, representing the first exon-spanning deletion in LRP5 described so far. Different in vitro assays showed that both receptor mutations abolish wnt-/Norrin signalling. The signal peptide mutation leads to a failure of the protein to be translocated to the ER and to improper posttranslational modification. Interestingly, also in the general population the leucine stretch is polymorphic, with rarer alleles spanning 6 to 11 leucines. Indeed, especially the shortest and longest alleles of this polymorphism exhibit a moderately reduced wnt-/Norrin signalling and are produced in the ER upon transient transfection, however at somewhat lower levels. The 8.2 kb deletion of LRP5 results in a stable RNA molecule, but in a truncated protein with impaired trafficking. Since loss-of-function mutations of LRP5 can also cause osteoporosis-pseudoglioma (OPPG-) syndrome, an autosomal-recessive disorder affecting bone and eye development, we tested 25 typical OPPG-patients by MLPA and identified two additional individuals with larger LRP5-deletions. In conclusion, these data broaden the genotypic and phenotypic spectrum of disorders caused by LRP5 dysfunction. Furthermore, we provide evidence that length variants of the signal peptide detectable in the general population may have impact on LRP5 translation efficiency. These findings are especially interesting as there is statistical evidence from association studies that genetic variation in LRP5 influences complex inherited bone mineral density.

W6 03

Hypoplasia of tibia with polysyndactyly (Werner syndrome) is allelic to preaxial polydactyly II (PPD2) and caused by a point mutation in the distant Sonic Hedgehog (SHH) cis-regulator (ZRS)

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Tibial hypoplasia, preaxial polydactyly of hands and feet and/or a five-fingered hand with absence of thumbs was first described by Werner in 1915 (MIM 188770). This condition is very variable, inherited in an autosomal dominant matter and no causative mutations have been described described so far. Here we describe a 43-year-old patient of Turkish origin with short stature [height: 135 cm (-6.4 SD)], shortening of forearms, and preaxial polydactyly of both hands. The additional fingers were surgically removed and the residual state are five-fingered hands with a triphalangeal first ray on the left hand. His femora are mildly and his lower legs are severely shortened. He had preaxial polydactyly of both feet, the supernumerary toe was surgically removed. The remaining first and his fourth toes are hypoplastic. The clinical diagnosis hypoplasia of tibia with polysyndactyly was established. Interestingly, the patient's father has a preaxial polydactyly of his right hand. We performed mutational analysis in the Sonic Hedgehog (SHH) gene and SHH regulatory region, the ZRS, located in intron 5 of the LMBR1 gene. A transition (G>A) at position 404 of the ZRS, which was previously reported as the Cuban mutation of the preaxial polydactyly type II (PPD2)-phenotype (Zguricas et al., 1999; Lettice et al., 2003), was identified in the patient and his father. The Cuban mutation was reported to be causative for the PPD2-phenotype in a family with six affected patients. One of these patients was more severely affected with additional radius and tibia dysplasia/aplasia similar to our patient. Single nucleotide substitutions in the ZRS regulatory region, also described in the Hemingway's Cats, operate as gain-of-function mutations that activate Shh expression at an ectopic embryonic site (Lettice et al., 2007). In summary, we identified the molecular cause of Werner syndrome in our patient and confirmed the previously suggested hypothesis that Werner syndrome is allelic to PPD2.

W6 04

Phenotypic spectrum of HNF1ß mutations as a common cause of cystic kidney disease: The Aachen experience

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The most common phenotype caused by an autosomal dominant mutation in the transcription factor HNF1ß is renal cysts which is frequently associated with early-onset diabetes (MODY) in the “Renal Cysts And Diabetes” (RCAD) syndrome. However, it has become apparent that there are additional phenotypic features, such as genital tract malformations, pancreatic atrophy, deranged liver function tests, and hyperuricemia. Thus, HNF1ß mutations might be considered to cause a multisystem disorder.
We analysed the HNF1ß gene in a large cohort of 102 patients with cystic kidneys by direct sequencing and MLPA. We identified 24 mutations (14 point mutations and 10 large deletions) and could further expand the phenotypic spectrum associated with HNF1ß mutations. Phenotypic variability, even within the same family, is alarming; e.g, the first pregnancy of a healthy couple was terminated because of oligo-/anhydramnios and massively enlarged cystic kidneys. Surprisingly, the identified mutation segregated paternally. A re-examination of the father showed three unilateral renal cysts, while otherwise he was found to be healthy at the age of 33 years. The father’s mother became diabetic as she advanced in years, but still does not show any renal cyst in her sixties though carrying the same HNF1ß mutation.

W6 05

Analysis of the clinical course in 231 siblings with autosomal-recessive polycystic kidney disease (ARPKD) - an important tool for the study of modifying mechanisms

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ARPKD is associated with mutations in the PKHD1 gene on chromosome 6p12. Most cases manifest peri- or neonatally with a high mortality rate in the first month of life while the clinical course of surviving patients is much more variable. We analysed the clinical course of 231 siblings with ARPKD out of 104 families. In 85 families the diagnosis was confirmed by mutational analysis and in 19 families pathoanatomically. The clinical course has been classified as “severe” (peri- or neonatal death) and “moderate” (neonatal survival). With respect to this classification affected siblings in 73% of families showed a concordant clinical course. In 47% of the families all affected siblings died after birth, while in 26% all children showed a “moderate” course. In 27% of families affected siblings showed a discordant course. If the first child died perinatally 80% of the subsequent siblings also died perinatally and did the first child belong to the “moderate” group, 26% had a “severe” course. There is neither an effect of birth order nor gender with respect to age of death, kidney and liver involvement. No patient with a “moderate” phenotype carried two truncating mutations corroborating that one missense mutation is indispensable for survival of newborns. All truncating mutations therefore can be regarded as "severe". We attempted to set up genotype-phenotype correlations and were able to categorize an increasing number of missense mutations with regard to disease severity.
Families with discordant clinical course in affected siblings with identical haplotypes are an important tool for the identification of modifying mechanisms. Since the pathogenesis of many ciliopathies displays common pathways, a great number of responsible genes in humans and animals can be regarded as potential modifiers and are subject for further research. Examples of variants which might act as modifiers will be given.
Literature: Deget et al. Clin Genet. 1995, 47:248; Bergmann et al. Kidney Int.

W6 06

Double homozygosity for truncating mutations of *AGL* and *SCN9A* mimicking neurohepatopathy syndrome

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Congenital indifference to pain (CIP, MIM #243000) is rare (<30 cases reported). We have identified a consanguineous family with the index patient displaying lesions typical of CIP. In addition, he had short stature, myopathy and liver dysfunction. This combination resembles Navajo neurohepatopathy (NNH, MIM #256810). However, no mutation was found in the NNH gene, *MPV17* . As pain perception was normal in a newborn sister with congenital liver disease, we assumed an overlap of two non-syndromic autosomal recessive conditions instead of a monogenic syndrome and performed genomewide linkage analysis. Both patients showed homozygosity by descent (HBD) for a region on chromosome 1p21.3-p13.2. Candidate gene analysis revealed a novel 5.5 kb truncating deletion encompassing exons 28 - 30 of the amylo-1,6-glucosidase gene (*AGL*), the gene for glycogen storage disease type 3 (GSD III), in homozygous state in both patients. As pain insensitivity is usually not a feature of GSD III, we critically evaluated all regions that showed HBD in the index patient only. One such region mapped to chromosome 2q24.2-q35 and contained the *SCN9A* gene. Truncating mutations of *SCN9A*, encoding a voltage-gated ion channel, have recently been shown to cause pain insensitivity. Indeed, the index patient was homozygous for a novel nonsense mutation, p.Y1421X, in *SCN9A*. As shown by this example, parental consanguinity can result in homozygosity for more than one mutation in unlinked genes. The overlap of two recessive conditions in the offspring, mimicking a single, syndromic disorder, can be a major pitfall in genetic counseling. Careful clinical investigation with comparison of affected siblings and analysis of molecular data is therefore required in patients with apparently syndromic disease born to consanguineous parents.

Molecular Mechanisms 2

W7 01

Clinical features of maternal uniparental disomy 14 are also present in patients with an epimutation and a deletion of the imprinted *DLK1/GTL2* gene cluster

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Maternal uniparental disomy 14 [upd(14)mat] is associated with a recognizable phenotype that includes pre- and postnatal growth retardation, neonatal hypotonia, feeding problems and precocious puberty. Chromosome 14 contains an imprinted gene cluster, which is regulated by a differentially methylated region (IG-DMR) between *DLK1* and *GTL2*. Here we report on six patients with clinical features of maternal upd(14)mat who show a typical methylation pattern at the IG-DMR and the *GTL2* promoter region, but biparental inheritance for chromosome 14. In five of the patients loss of paternal methylation appears to be a primary epimutation, whereas the other patient has a paternally derived deletion of ~1 Mb that includes the imprinted *DLK-GTL2* gene cluster. These findings demonstrate that the upd(14)mat phenotype is caused by altered expression of genes within this cluster.

W7 02

Comparative methylation analysis of imprinted genes in spermatogonial stem cells from adult mouse testis and embryonic stem cells

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Pluripotent stem cells, called Spermatogonial stem cells (SSCs), have been isolated from neonatal and adult mouse testis. Like embryonic stem cells (ESCs), SSCs have the potential to differentiate into various types of somatic cells and may enable important future clinical applications. SSCs are descendants of primordial germ cells (PGCs) whose development in vivo is accompanied by major epigenetic changes including DNA demethylation and imprint erasure. We have performed classical bisulphite sequencing and bisulphite pyrosequencing to comparatively analyze the methylation status of two paternally imprinted (H19, Meg3) and two maternally imprinted (Snrpn, Igf2r) gene regions in four SSC lines from adult mouse testis and two ESC lines derived from the mouse embryonic inner cell mass, each with different genetic backgrounds. In general, the four SSC lines, similar to PGCs, were moderately to strongly hypomethylated at the tested regions with the exception of H19 which exhibited strikingly heterogeneous methylation patterns. The two ESC lines were also moderately hypomethylated for Meg3 and Snrpn, but strongly differed from each other in their H19 and Igf2r methylation levels. Interestingly, one SSC and one ESC line, which both are doubly transgenic for Stra8-EGFP (to allow enrichment for germline stem cells) and ROSA26 and have already been successfully used to regenerate spermatogenesis in germ-cell-depleted mice, showed very similar moderately hypomethylated patterns for all four tested imprinted gene regions. Our data further substantiate the pluripotency of SSCs and suggest that SSCs from adult mouse testis originate from germ cells with at least largely erased methylation imprints.

W7 03

The tumor suppressors ING1 and ING2 induce cellular senescence and regulate at epigenetic level

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Cellular Senescence is a phenomenon of primary cells, which arrest irreversibly their cell cycle and change completely their morphology and gene expression. It seems to be one major pathway for prevention of cellular transformation and cancer. The role of epigenetic regulation of cellular senescence is poorly understood.
p33ING1 and p33ING2 belong to the ING-gene family that is involved in tumor suppression, DNA repair and cell cycle regulation. Their expression is increased in senescent cells. Expression of either p33ING1 or p33ING2 induces premature senescence and antisense expression of p33ING1 extends the proliferative life span. p33ING2 binds to trimethylated K4 of histone H3 suggesting that cellular senescence is controlled at epigenetic level.
Here we show that ING2 is a potent transcriptional silencer that recruits histone methyltransferase-(HMT)-activity with its C-terminus, which correlates with silencing function. ING2-mediated gene silencing is resistant to the HDAC-inhibitor TSA indicating that ING2 uses a non-HDAC class I or II pathway for gene repression. Accordingly we show that ING2 is associated with a specific HMT-activity methylating specifically H3 in vitro and in vivo. Interestingly, mutation or methylation of K9 at H3, a mark well-known for repression, abrogates histone methylation by MeCP2 but not that by the ING2 HMT-complex. Instead, the ING2 associated HMT shows an increased methylation activity if K9 is methylated. In contrast, mutation or methylation of K4, a methylation preferentially detected at active genes, led to a reduction of the ING2-associated HMT. Notably, also ING1 recruits HMT activity suggesting a more general biochemical interaction between members of p33ING-family and HMT-activity.
Taken together, the data suggest that p33ING2 is associated with HMT-activity with methylation site specificity distinct from H3K4 and K9 and regulates the onset of cellular senescence through epigenetic modulation of chromatin.

W7 04

Spermatogonial stem cells share microRNA signature with embryonic stem cells

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Spermatogonial Stem Cells (SSCs) isolated from the adult mouse testis have been shown to be pluripotent. In addition to well known pluripotency markers like Oct4, Sox2 and Nanog, SSCs express

also the two recently identified pluripotency regulators Zfp206 and Sall4. Here we substantiate further the pluripotency of SSCs in comparison to Embryonic Stem Cells (ESCs) by the results of microRNA (miRNA) analysis. miRNAs represent a recently identified class of cellular RNAs (17-24 bp) that regulate protein expression at the translational level. Recently, a set of miRNAs was described to be ESC specific in mouse, with their expression being repressed during ESC differentiation and undectable in adult organs. This set of miRNAs consists of members of miR-290 and miR-302 family. The expression profile of these miRNAs can definitely classify mouse ESCs and differentiated cells. In this study we show that identical to ESCs also SSCs from the adult mouse testis are endowed with miRNAs of both families. They are constantly expressed in cells cultured for many passages but downregulated upon loss of pluripotency. Detection of these ESC-specific miRNAs in SSCs and embryonic carcinoma cells of F9 cell line implies that they characterize generally the pluripotent state. In addition, we tested the influence of different factors that promote loss of pluripotency during time on levels of these miRNAs. Our results suggest that 290-family is more connected with Oct4 and maintenance of pluripotency than with differentiation since changes in pluripotent state are followed by similar changes in miRNA levels. In contrast, changes in pluripotent state are not followed by similar changes in levels of members of 302-family. Instead of decreasing, in most cases levels of these miRNAs increase during the first stages of in vitro differentiation. It will be studied whether overexpression or downregulation of miRNAs affect pluripotency and differentiation potential of ESCs and SSCs.

W7 05

Loss of COH1 leads to Cohen syndrome by Golgi disruption

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Cohen syndrome, caused by mutations in *COH1*, is characterised by mental retardation, postnatal microcephaly, facial dysmorphism, pigmentary retinopathy, myopia, and intermittent neutropenia. *COH1* has been reported to encode several splice variants. Recently, we found ubiquitous expression of the *COH1/Coh1* transcript including exon 28b as the predominant full-length variant in human and mouse tissues. However, human brain showed differential splicing of exon 28.
To study the functional role of COH1, we cloned the predominant *COH1* transcript and different truncated fragments into expression vectors. Transient expression showed that full-length COH1 localised to the Golgi ribbon in different human cell lines. C-terminally truncated COH1 accumulated in cytoplamic vesicles. However, N-terminal shortening of COH1 did not affect Golgi localisation and fusion of the last 315 residues of COH1 to GFP confirmed Golgi targeting function of this domain. To analyse if COH1 is important for Golgi integrity we abolished COH1 expression by siRNA knock-down in HeLa cells. Interestingly, we observed disruption of the Golgi into scattered clusters. Previously, COH1 was predicted to harbour 10 transmembrane domains. By chemical stripping of cell membrane preparations, however, we identified COH1 as a peripheral membrane protein. To get further insights into the pathomechanism we analysed fibroblasts from Cohen syndrome patients by immunofluorescence and electron microscopy. Partial fragmentation into isolated Golgi stacks emphasises the crucial role of COH1 for Golgi integrity.
We conclude that COH1 represents a novel peripheral membrane protein distributed between ER and Golgi, where it might serve as scaffold protein for the formation of protein sorting complexes. Our data are in accordance with the assumed trans-Golgi and endosomal sorting of membrane proteins by Vps13p, the yeast homologue of COH1. Further experiments are required to analyse effects of COH1 on the secretory pathway.

W7 06

BNP is a transcriptional target of the short stature homeobox gene SHOX

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Short stature due to SHOX deficiency represents a common congenital form of growth failure and is involved in the etiology of “idiopathic” short stature and the growth deficits and skeletal anomalies in Léri Weill, Langer and Turner syndrome. While much is known on the clinical and molecular aspects of SHOX haploinsufficiency, the integration of SHOX in the signalling pathways regulating bone growth is currently not defined. Here we identify NPPB encoding the natriuretic peptide BNP, a well known approved cardiac and natriuretic peptide hormone (drug), as a transcriptional target of SHOX. The ability of SHOX to transactivate the NPPB endogenous promoter was demonstrated in luciferase reporter assays using serial deletions of the NPPB promoter region. Binding of SHOX to the NPPB promoter was also demonstrated in vivo by chromatin fixation and immunoprecipitation. We also demonstrate the lack of promoter activation in two SHOX mutants from patients with Léri-Weill syndrome. In addition, immunohistochemical analysis of human growth plate sections showed for the first time a co-expression of BNP and SHOX in late proliferative and hypertrophic chondrocytes. Together these data strongly suggest that BNP represents a direct target of SHOX.

Complex Diseases

W8 01

Pseudoexfoliation Syndrome/Glaucoma is strongly associated with a common non-synonymous variant of Lysyl Oxidase-like 1 (LOXL1) resulting in reduced protein expression

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Pseudoexfoliation glaucoma (PEXG) is the most common identifiable cause of open-angle glaucoma worldwide. The underlying defect, pseudoexfoliation syndrome (PEX), is an age-related systemic disease of the extracellular matrix, characterized by pathological accumulation of abnormal fibrillar material in the anterior segment of the eye. Both population- and pedigree-based studies have shown that genetic factors contribute to its pathogenesis. Three common sequence variants in lysyl oxidase-like 1 (LOXL1) gene were recently associated with both PEX and PEXG in populations from Iceland and Sweden. In this study, we set out to confirm and extend this association in PEX and PEXG cohorts of German and Italian origin by genotyping a total of 726 unrelated PEX /PEXG patients and 418 healthy subjects. Strong association with the three LOXL1 common sequence variants was seen in both glaucoma patient groups independent of their geographic origin (rs2165241, combined OR=3.42, P=1.28x10-40; rs1048661, OR=2.43, P=2.90x10-19; rs3825942, OR=4.87, P=8.22x10-23). Similarly, the common frequent haplotype (G-G) composed of the two non-synonymous coding SNPs (rs1048661 and rs3825942) was strongly associated with disease in both cohorts (combined OR=3.58, P = 5.21 10-43). LOXL1 expression analysis in anterior segment tissues from eyes of PEX and control patients (n=16 for each group) detected LOXL1 in cornea, trabecular meshwork, iris, lens, and ciliary body of all eyes examined. Correlation analysis with individual SNP genotypes in both PEX and control eyes showed reduction of LOXL1 expression by 31% per at risk G-allele of rs1048661 (P<0.0001). In contrast, rs3825942 alleles or the haplotype did not affect LOXL1 expression level in any group. Taking into account the essential role of LOXL1 gene in elastogenesis, it is now plausible that genetic variants in LOXL1 cause features of PEX resulting from aberrant production of elastin and accumulation of fibrillar material in the eye.

W8 02

Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease

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Background: Recently, genome-wide association studies identified variants on chromosome 9p21.3 to affect the risk of coronary artery disease (CAD). We investigated the association of this locus with CAD in seven case-control studies and undertook a meta-analysis.
Methods: A single nucleotide polymorphism, rs1333049, representing the 9p21.3 locus was genotyped in seven case-control studies involving a total of 4,645 patients with myocardial infarction (MI) or CAD and 5,177 controls. The mode of inheritance was determined. A meta-analysis of the present data and previously published samples was conducted. A limited fine-mapping of the locus was performed.
Results: The risk allele (C) of the lead SNP rs1333049 was uniformly associated with CAD in each study ($p < 0.05$). In a pooled analysis the odds ratio per copy of the risk allele was 1.29 (95% CI: [1.22,1.37], p=0.0001). An autosomal additive mode of inheritance best explained the underlying association. The meta-analysis of the rs1333049 SNP in 12,004 cases and 28,949 controls increased the overall level of evidence for association with CAD to p = 6.04x10-10 (OR 1.24 [1.20,1.29]). Genotyping of 31 additional SNPs in the region identified several with highly significant association with CAD but none had predictive information beyond that of the rs1333049 SNP.
Conclusion: This broad replication provides unprecedented evidence for association between genetic variants at chromosome 9p21.3 and risk of CAD.

W8 03

A genome-wide association study for atrial fibrillation confirms association in 4q25 near the PITX2 gene and identifies additional susceptibility Loci

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Introduction: Atrial Fibrillation (AF) has 1% population prevalence. Beyond Mendelian forms, complex AF is significantly heritable (OR=3.23, p<10-3 for positive family history, Fox et al, JAMA 2004). A recent GWAS identified a susceptibility locus in 4q25 near PITX2 (Gudbjartsson et al., Nature 2007). A high-risk haplotype (MAF=0.11, allelic OR for AF=1.72, p=3.3*10-41), a low-risk haplotype (MAF=0.08, OR=1.39, p=6.9*10-11) and a common third haplotype (MAF=0.81) were observed.
Methods: We recruited 1715 AF cases (72% male, age 61.7±12.1 yr) from the German Competence Network AFNet, the LMU University Clinics Grosshadern and the Deutsches Herzzentrum Munich. 4073 probands from KORA survey S4 served as controls (49% male, age 49.2±13.9 yr). We undertook an Illumina 300k GWAS in 495 AFNet cases and 497 controls and used the remaining samples for replication.
Results: We also identified the genome-wide most significant association in the 4q25 region. In our entire sample high-risk tagSNP rs2200733 showed stronger effect size than in the Gudbjartsson et al. study (OR=2.28 (2.04-2.55) p=3.1*10-47) while it was lower with the low-risk tagSNP rs10033464 (OR=1.31 (1.14-1.49) p=8.8*10-5). In the locus we discovered an even stronger association at rs2220427 (OR = 2.85, p=1.9*10-54). Risk was higher in men (OR=2.92, p=3.1*10-37) and in persons under age 60 (OR=3.13, p=6.5*10-35). In the remaining genome we observed 72 SNP associations with p<10-4, a 2,3 fold excess over the expectation under the null hypothesis.
Conclusions: We confirmed the 4q25 complex AF susceptibility locus. Its association interacts with age and gender but appears not strong enough for primary prevention testing. We identified several additional loci which are currently undergoing replication genotyping. Whether the mechanism behind 4q25 involves the PITX2 gene and whether testing of these and other gene variants may be useful for future AF management is subject to ongoing investigations.

W8 04

A genome-wide association study in a large patient-control sample of schizophrenia originating from the German population

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Schizophrenia (SCZ) is a genetically complex neuropsychiatric disorder that affects 0.5-1.0% of the human population. Genome-wide association studies (GWAS) are expected to open new insights into the genes involved in disease development. Using chip-based technologies, hundreds of thousands of SNPs can currently be genotyped at a time, a SNP-coverage that should be dense enough to pick up linkage disequilibrium to disease-associated genetic variants, provided that at least part of the disease susceptibility is conferred by relatively common genetic variation.
491 DSM-IV-diagnosed schizophrenia patients and 1,363 controls, all of German descent, were included in this study. Genotyping was performed using Illumina's HumanHap550 BeadArrays, interrogating 561,466 SNPs per individual. Controls were derived from three different geographical regions of Germany: KORA (Augsburg region), POPGEN (Schleswig-Holstein), and UCO (Ruhr area). Stringent quality criteria were applied to minimize the number of significant SNPs that are unlikely to represent "true" disease-related differences between patients and controls (such as technical or assay problems, low minor allele frequencies, strong deviations from Hardy-Weinberg Equilibrium), and to exclude individuals that are identified as "outliers" by analysis of population substructure. We retained a final data set of 482,927 SNPs and 1,803 individuals. Our primary analysis resulted in 65 SNPs that surpassed a significance threshold of P=1.0E-04. The most significant SNP yielded an Armitage's Trend Test P value of 1.58E-07 and is located in chromosomal region 7q. A selection of SNPs is currently genotyped in independent replication samples from the German population for confirmation.

W8 05

Genetic association of Single SNPs and a LD-Haplotype at PSORS6 in Patients with early onset psoriasis and evidence for epistasis with PSORS1 risk locus

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Psoriasis is a genetically complex, chronic inflammatory skin disease. We have previously performed a genome wide linkage study in a set of psoriasis families and have identified a susceptibility locus on chromosome 19p13 (PSORS6). In a follow-up linkage disequilibrium (LD) study in an independent family based cohort we found evidence for association to two newly discovered microsatellites at this locus (D19SPS20: P < 2.7*10-2, D19SPS21: P < 5.3*10-5). An association scan in 300 trios, based on the LD structure of the region, revealed association to several single SNPs in one LD block. When we stratified this cohort for carrying the PSORS1 risk allele at the HLA-C lcous on chromosome 6p, evidence for association became much stronger at single SNP and haplotype levels (p-values between 2.0*10-4 and 9.0*10-4). In a population based replication study of 1,114 psoriasis patients and 937 control individuals, evidence for association was observed again after stratification to the PSORS1 risk allele. In both study groups, logistic regression showed evidence for interaction between the risk alleles at PSORS1 and PSORS6. The associated LD block did not comprise any known genes. Interestingly, an adjacent gene, MUC16, coding for a large glycosylated protein expressed in epithelia, could be shown to be also expressed in tissues relevant for pathogenesis of psoriasis such as skin and thymus. In summary, we confirmed and refined the susceptibility locus at PSORS6 which seems to be restricted to patients with early onset psoriasis carrying the PSORS1 risk allele.

W8 06

Elevated expression of serotonin receptor type 3 genes may contribute to irritable bowel syndrome with diarrhea

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Serotonin type 3 (5-HT3) receptor antagonists are beneficial in some but not all patients with irritable bowel syndrome and diarrhea (IBS-D). As cis-regulatory variants can play a role in the etiology of complex conditions by affecting efficiency of translation, we investigated the 5´ and 3´ untranslated region (UTR) of the 5-HT3A and 5-HT3E subunit genes. Mutation analysis was carried out in 200 patients with irritable bowel syndrome and 100 healthy controls. We found a HTR3A 5´UTR variant and a novel HTR3E 3´UTR variant associated with the IBS-D subtype. Functional studies showed that both variants lead to significant upregulation of subunit expression. In HEK293 cells, the HTR3A variant results in a higher density of 5-HT3A receptors at the cell surface compared to the wild-type control. The HTR3E variant affects a microRNA binding site and leads to a higher luciferase reporter gene expression. Both HTR3E and the miRNA co-localize in enterocytes of the mucosal cell layer of the gut epithelium as shown by in situ hybridization. We suggest that the increased expression of 5-HT3A and 5-HT3E subunits might result in a change in 5-HT3 receptor composition and/or density of 5-HT3 receptors in the epithelial cell layer of the mucosa and neurons of the enteric and central nervous system and could therefore contribute to the pathophysiology of IBS-D.

Disease Gene Identification 2

W9 01

Mutations of *CASK* cause a novel X-linked brain malformation phenotype with microcephaly and hypoplasia of the brainstem and cerebellum

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Microcephaly occurs as an isolated malformation or with associated brain anomalies such as a simplified gyral pattern or cerebellar hypoplasia. Recently, homozygous silencing of *EOMES* (*TBR2*), encoding a transcription factor of the brain-specific T-box family, was associated with microcephaly and additional brain malformations. TBR2 has a putative function in regulating cortical neurogenesis and neural identity, and plays a role in a signalling cascade upstream of TBR1, which is highly related to TBR2. We describe a novel X-linked brain malformation phenotype with postnatal microcephaly, hypoplasia of the cerebellum and brainstem, and severe developmental delay that is caused by mutations of *CASK* in Xp11.4. We mapped one breakpoint of a paracentric Xp inversion within the *CASK* gene in an affected female. By array comparative genomic hybridization we identified a heterozygous deletion of ~740 kb encompassing *CASK*, *GPR34* and *GPR82* in female patient 2 and two separated regions of copy number loss in female patient 3, including ~170 kb covering the 3□ and ~150 kb encompassing the 5□ region of *CASK*. Mutation analysis of *CASK* in 13 females and 33 males with a phenotype similar to patients 1-3 identified a heterozygous nonsense mutation (c.1915C>T/p.R639X) in a female and the hemizygous c.915G>A (p.K305) mutation altering splicing in a severely affected male who died at age 2 weeks. *CASK* encodes a multi-domain scaffolding protein that interacts with the transcription factor TBR1 and regulates expression of genes involved in

cortical development, such as *RELN*. The potential importance of the CASK-TBR1-RELN signaling cascade in brain development is highlighted by our neuropathological findings in the deceased boy, which show changes nearly identical to the *Reln* mouse mutant in the cerebellum, and overlap with the *Tbr1*$^{-/-}$ mouse in the cerebral cortex.

W9 02

Disruption of ST5 is associated with mental-retardation and multiple congenital anomalies

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We report on an 8 years old male patient with a de novo balanced translocation 46,XY,t(11;20)(p15.4;q13.2) presenting with severe mental retardation, seizures, bilateral sensorineural hearing loss, submucous cleft palate, persistent ductus Botalli, unilateral cystic kidney dysplasia and frequent infections. He also showed dysmorphic facial features including a high forehead, high arched eyebrows, hypertelorism, a small mouth, a broad nasal bridge and a bulbous nasal tip. Breakpoint mapping with FISH and sequencing of the breakpoints showed that no known genes are disrupted at 20q13.2, whereas ST5 is disrupted at 11p15.4. ST5 was identified by its ability to suppress the tumorigenicity of HeLa cells in nude mice. Additionally, it may also be involved in cytoskeletal organization, cell morphology and cell growth, in a signalling pathway leading to activation of MAPK1/ERK2 as well as to an ABL1 stimulated ERK2 inhibition. By RT-PCR from different human tissues we found, that ST5 is expressed ubiquitously and at high levels in human fetal tissues, whereas the expression in adult tissues is weaker and some tissues showed no expression. Using RNA in situ hybridisation in mouse we found that St5 is expressed highly in the frontal cortex during embryonic development. In adult mouse brain expression of St5 is weaker but ubiquitous, with higher expression in hippocampus and cerebellum. Hence we suppose that ST5 plays an important role in brain development, probably in the formation of the different cortex layers and the migration of the neurons. These findings implicate ST5 in the etiology of mental retardation, seizures and multiple congenital anomalies.

W9 03

Biallelic loss of function of the Promyelocytic Leukaemia Zinc Finger (*PLZF*) gene causes severe skeletal defects and genital hypoplasia

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Deletions of 11q23 are associated with mental retardation, craniofacial dysmorphism, microcephaly and short statue. We present a patient with similar clinical findings plus absence of thumbs, hypoplasia of radii and ulnae, additional vertebrae and ribs, retarded bone age and genital hypoplasia. Using microarray based comparative genomic hybridization and microsatellite analysis, we identified an ~8 Mbp *de novo* deletion on the paternal chromosome 11, which includes the promyelocytic leukaemia zinc finger (*PLZF*) gene.
In humans *PLZF* is one of five partners fused to the retinoic acid receptor alpha in acute promyelocytic leukaemia. *Plzf*-deficient mice show severe malformations of the vertebral and appendicular skeleton and male genital hypoplasia. Since patients with a deletion of 11q23 do not normally present with skeletal malformations and genital hypoplasia, we sequenced the maternal *PLZF* allele in our patient and identified a missense mutation (c.1849 A>G), which leads to the substitution of a highly conserved methionine to valine within the eighth zinc finger motive. The mutation was inherited from the mother, who does not have skeletal defects. *In vitro* reporter gene assays show that the mutation impairs the repressive function of PLZF. In summary, we have identified the first patient with a germline mutation of *PLZF*. Our findings as well as observations in *Plzf*-deficient mice demonstrate that PLZF is a key regulator of skeletal and male germline development. Furthermore, this case highlights the importance to search for a recessive mutation on the non-deleted allele in patients with a microdeletion and atypical clinical findings and is another example of a deletion uncovering a recessive mutation.

W9 04

Triphalangeal thumb-polysyndactyly syndrome associated with a microduplication of the long range SHH limb regulator (ZRS)

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An important player in establishing the anterior-posterior patterning of the limb is the developmental regulator gene *sonic hedgehog* (*SHH*). Previous studies have identified a long range regulator for SHH expression in the limb bud residing in a highly conserved non-coding sequence about 1 Mb upstream from the *SHH* gene itself. As shown in mice point mutations within this non-coding regulatory region designated ZRS lead to ectopic expression of *Shh* in the anterior margin of the limb bud and thus to preaxial extra digits. In humans ZRS point mutations are associated with the triphalangeal thumb and polysyndactyly (TPT-PS, OMIM #174500) phenotype.
In this study we investigated a large pedigree with a variable phenotype of TPT-PS. Although linkage to the *SHH* locus was confirmed using locus specific microsatellite marker analyses sequencing of the ZRS did not reveal point mutations. A subsequent whole genome screening for submicroscopic rearrangements by array-CGH detected a microduplication in 7q36.3 in an affected individual. A duplicated region of 588,819 bp comprising the ZRS was confirmed by quantitative real-time PCR in all affected family members. A direct sequencing strategy showed a direct tandem orientation of the duplicated segment.
In summary, we demonstrated that microduplication of the ZRS region in 7q36.3 results in a similar TPT-PS phenotype as caused by single nucleotide alterations in the limb specific SHH regulatory element. Thus, genomic duplications have to be considered as a possible mechanism which leads to the disturbance of long-range transcriptional control. The discovery of novel mechanisms of gene regulation, i.e. distant enhancers or repressors and their relevance to human disease if disrupted will continue to be a challenging task in the future.

W9 05

Microdeletions and -duplications in patients with syndromic XY gonadal dysgenesis

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46,XY gonadal dysgenesis is characterized by abnormal testicular differentiation and can occur as an isolated form or as part of a complex syndrome. Chromosome aberrations as well as mutations in genes as SRY, SF1, WT1, SOX9, DHH, DMRT1, DAX1 and ATRX altogether are causal for only a very small portion of XY gonadal dysgenesis. This prompted us to search for submicroscopic deletions and duplications by array-based comparative genomic hybridization (array CGH) in 9 patients with syndromic forms of 46,XY gonadal dysgenesis.
By using the 105K Agilent platform we were able to identify genomic imbalances in 5 from 9 analyzed patients. In one female with 46,XY gonadal dysgenesis, diaphragmatic hernia and mental retardation (MR) we identified a 97 kb deletion on chromosome 1, which encompasses a gene being expressed in the developing brain and whose corresponding protein interacts with proteins playing a pivotal role in actin cytosceletal dynamics in the testis. In another female patient with 46,XY gonadal dysgenesis, hypoplasia of maxilla and gigantism we found the deletion of only one gene on chromosome 19, which is expressed in spermatogonia and furthermore is a negative regulator of cell growth and division. In a patient with an acampomelic campomelic dysplasia, but without SOX9 mutation, we discovered a 3 Mb deletion on chromosome 17, which is localized approximately 400 kb distal of SOX9 in a regulatory region. Additionally, we detect in a male patient with hypospadia scrotalis, cryptorchism, iris coloboma and short stature a duplication of 1 MB on chromosome 15 including genes which can be deleted in Angelman/Prader-Willi syndromes. Finally, in a fifth female patient with 46,XY gonadal dysgenesis and MR we identified a gross deletion of 10 Mb on chromsosome 9 encompassing genes like DMRT1, 2 and 3, respectively. We conclude, that the technique of array-CGH can provide useful information in unexplained cases of 46,XY gonadal dysgenesis.

W9 06

Walking the interactome for prioritising candidate disease genes

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The identification of genes associated with hereditary disorders has contributed to improving medical care and to a better understanding of gene functions, interactions, and pathways. However, there are well over 1500 Mendelian disorders whose molecular basis remains unknown. At present, methods such as linkage analysis can identify the chromosomal region in which novel disease genes are located, but the regions may contain up to hundreds of candidate genes. In this work, we present a new method to prioritise candidate genes using a global network distance measure, random walk analysis (RWR), to define similarities in protein-protein interaction networks. Intuitively, the RWR algorithm calculates the similarity between two genes i and j based on the likelihood that a random walk through the interaction network starting at gene i will finish at gene j, whereby all possible paths between the two genes are taken into account. For comparison, we also implemented a similar global search algorithm based on the diffusion kernel, which conceptually performs a different type of random walk calculated by matrix exponentiation.
We tested our method on 110 disease-gene families with a total of 783 genes, and achieved an area under the ROC curve of up to 98% on simulated linkage intervals of 100 genes surrounding the disease gene, significantly outperforming previous methods based on local distance measures. We additionally tested our method on seven recently characterized genes, and acheived an enrichment score of 52 fold, compared to 37 fold for ENDEAVOUR, which has achieved the best performance among previously published methods.
Our results not only provide an improved tool for positional cloning projects, but also add weight to the assumption that phenotypically similar diseases are associated with disturbances of subnetworks within the larger protein interactome that extend beyond the disease proteins themselves.

Neurogenetics 2

W10 01

Slc4a10, a new target for the therapy of epilepsy and hydrocephaly?

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Members of the SLC4 bicarbonate transporter family are involved in solute transport and pH homeostasis. Here we report that disrupting the Slc4a10 gene, which encodes the Na+-coupled Cl-/HCO3--exchanger Slc4a10 (Ncbe), drastically reduces brain ventricle volume and protects against fatal epileptic seizures in mice. In choroid plexus epithelial cells Slc4a10 localizes to the basolateral membrane. These cells displayed a diminished recovery from an acid load in knockout mice. Slc4a10 was also expressed in neurons. Within the hippocampus the Slc4a10 protein was abundant in CA3 pyramidal cells. In the CA3 area, propionate-induced intracellular acidification and attenuation of 4-aminopyridine-induced network activity were prolonged in knockout mice. Our data indicate that Slc4a10 is involved in the control of neuronal pH and excitability and may contribute to the secretion of cerebrospinal fluid. Hence Slc4a10 is a promising pharmacological target for therapy of epilepsy or elevated intracranial pressure.

W10 02

Truncated ARHGEF9 is ssociated with epilepsy, anxiety, aggression and mental retardation and interferes with synaptic localisation of endogenous gephyrin and GABAA receptors in primary neurons

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Clustering of inhibitory GABAA and glycine receptors at synapses is thought to involve key interactions between the receptors, a ´scaffolding´ protein known as gephyrin and the RhoGEF collybistin. We report the characterisation of a balanced translocation in a female patient presenting with a disturbed sleep-wake cycle, epilepsy, increased anxiety, aggressive behavior and mental retardation. Fine mapping of the breakpoint indicated disruption of the collybistin gene (ARHGEF9) on chromosome Xq11, while the other breakpoint lies in a gene-poor region of 18q11. We show that defective collybistin transcripts are synthesized and exons 7-10 are replaced by cryptic exons from chromosomes X and 18. These mRNAs no longer encode the pleckstrin homology (PH) domain of collybistin. We demonstrate that truncated collybistin isoforms are no longer able to translocate EGFP-tagged gephyrin to submembrane microaggregates in a cellular model of gephyrin clustering. Consistent with this finding, expression of truncated collybistin proteins in cultured neurons interferes with synaptic localization of endogenous gephyrin and GABAA receptors. These results suggest that collybistin has a key role in membrane trafficking of gephyrin and selected GABAA receptor subtypes involved in epilepsy, anxiety, aggression, insomnia and learning and memory. Taken together with previous work (Harvey et al, J. Neurosci. 2004; Marco et al., J Med Genet. 2007), our results indicate that mutations in ARHGEF9 cause XLMR and suggest that different mutations may give rise to distinct clinical presentations.

W10 03

CCM2 is the core molecule of the cerebral cavernous malformation protein complex

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Cerebral cavernous malformations (CCM) are prevalent cerebrovascular lesions predisposing to chronic headaches, epilepsy, and hemorrhagic stroke. Individuals carrying an autosomal dominantly inherited mutation in one of the three cerebral cavernous malformation genes, CCM1/KRIT1, CCM2/OSM, and CCM3/PDCD10, cannot be clinically distinguished, raising the possibility that the gene products act within common molecular pathways. Apart from very few exceptions, mutations in CCM genes are truncating or the result of large genomic rearrangements. While this facilitates medical genetic counselling, this does not provide a basis for structural and functional analyses of the CCM proteins.
In this study, we demonstrate the existence of a CCM1/CCM2/CCM3 protein complex. With the help of an in-frame deletion of CCM2 exon 2 which corresponds to the naturally occurring splice variant of CCM2 on the RNA level and is predicted to result in the omission of 58 amino acids (CCM2:p.P11_K68del), we furthermore show that complex formation depends on an intact CCM2 N-terminus. The CCM2:p.P11_K68del protein could be expressed in cell culture and complexed with CCM3. However, its ability to interact with CCM1 and to form a CCM1/CCM2/CCM3 complex was lost. Via further in vitro mutagenesis, we are currently refining the novel N-terminal CCM2 domain required for CCM1 binding. Our data are in line with results obtained from an in vivo zebrafish model and demonstrate that full-length CCM2 is the essential linker strictly required for CCM1/CCM2/CCM3 complex formation.

W10 04

Niemann-Pick type C disease (NP-C) is a considerable diagnosis in juvenile and adult-onset chronic productive psychosis

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Niemann-Pick type C (NP-C) disease is an autosomal recessive neurovisceral storage disorder that is characterised by the accumulation of cholesterol, gangliosides and bis-monoacylglacerol phosphate in late endosomes/lysosomes. The wide spectrum of clinical symptoms includes hepatic and pulmonary disease and more frequently described also neuropsychiatric disorders. The disorder is caused either by mutations in the NPC1 or the NPC2 gene. The clinical manifestations of NPC disease usually appear between ages of 4 and 10 years. However, there is raising evidence that the percentage of adult manifestations in NPC disease is more frequent than up to now presumed. Nevertheless, there is only little knowledge about age of onset, first symptoms and progress in adult manifestation of NPC disease especially regarding neuropsychiatric symptoms.
We completely sequenced NPC1-gene in 189 adult Caucasian patients (age ranking between 12 and 51 years) being treated in psychiatric or neurological institutions. Most patients have been treated under the suspicion of psychiatric diseases, ataxia or epilepsy with cognitive decline. In 26 patients (14%), mutations were demonstrated within the NPC1 gene, where in 5 patients only one mutation was detectable although the filipin test demonstrated pathological cholesterol accumulation in fibroblasts. Additionally, NPC2 mutations were present in 3 cases which corresponds to about 10% of all NPC disease cases.
The high frequent of NPC disease in adult patients with neuropsychiatric symptoms makes it necessary to improve on one side the knowledge of this disease among neurologists and psychiatrists but should also suggest NPC1 testing in all cases with unclear neuropsychiatric manifestations especially where a combination of ophtalmoplegia and facial dystonia is present.

W10 05

Identification of 27 novel NKX2-1 mutations in 102 patients with broad spectrum of brain and thyroid dysfunctions

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Objective: NKX2-1, also known as TITF-1, TTF-1 or T/ebp, is a member of the homeodomain-containing NK-2 transcription factor gene family and is expressed in early development of thyroid, lung and forebrain. Targeted inactivation of Nkx2.1 in mice revealed a neonatal lethal phenotype due to lung agenesis, thyroid dysgenesis and forebrain malformations, particularly the basal ganglia and hypothalamus. To date, only few NKX2-1 mutations were identified in patients with variable congenital hypothyroidism, choreatiforme movement defect and pulmonary symptoms matching the mouse phenotype. Here we report the systematic molecular screening analysis of NKX2-1 for mutations and DNA copy number changes of 102 patients with thyroid dysfunction combined with movement disorders and pulmonary affection aiming to delineate the complete clinical spectrum of NKX2-1 deficiency.
Methods: DNA of all patients was analyzed by direct sequencing and customized high-resolution oligo array-CGH, covering the region of interest with a resolution of 200-300 bp, was applied to a subset of 48 patients.
Results: In our series 27 novel alterations were identified, comprising 9 heterozygous deletions of the NKX2-1 and adjacent regions and 18 intragenic point mutations, whereas neither a common microdeletion nor a mutational hot spot could be revealed. NKX2-1 variations were most likely to occur in patients with choreoathetosis, hypothyroidism and pulmonary dysfunction (38.5 %) and less frequently in patients with neurological and thyroid dysfunction (29.2 %) or separate choreoathetosis (25.0 %)
Conclusion: Here we describe that the 27 NKX2-1 alterations observed are highly heterogeneous. NKX2-1 deficiency does not necessarily entail the triad of neurological, thyroid and pulmonary dysfunctions, but also results in choreathetosis with or without hypothyroidism. NKX2-1 analysis can improve the diagnostic procedure in patients with movement disorders like choreathetosis who are clinically difficult to classify

W10 06

Patients with Charcot Marie Tooth neuropathy and gene targeting in mice reveal an essential role for Rho GTPase signalling in peripheral nervous system myelination

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Charcot-Marie-Tooth disease (CMT) comprises a group of clinically and genetically heterogeneous hereditary motor and sensory neuropathies (HMSNs). With an overall population prevalence of 1 in 2,500, CMT constitutes the most common inherited neuromuscular disorder. Gene mutations causing demyelinating CMT affect the development, maintenance, and function of Schwann cells which carry out myelination in the peripheral nervous system (PNS). We present twofold genetic evidence that small GTPases of the Rho family are intimately involved in the regulation of PNS myelination. First, disruption of frabin/FGD4, a guanine nucleotide exchange factor (GEF) for the Rho GTPase Cdc42, causes peripheral nerve demyelination in patients with autosomal recessive CMT (CMT4H). Secondly, inducible and Schwann-cell restricted gene targeting of Cdc42 in mice resulted in severely impaired remyelination after nerve injury. Schwann cells are highly specialised cells that need to exert a multitude of tightly regulated cellular activities such as proliferation, migration over long distances, and alignment along and ensheathing of axons. All these processes involve the temporally and spatially regulated reorganization of the actin cytoskeleton, and Rho GTPases are known to control actin dynamics.

Educational Sessions

EDU 1

Diabetes mellitus im Kindes- und Jugendalter: Klinik, Pathogenese und Frühdiagnostik einer komplexen genetischen Erkrankung

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Der Diabetes mellitus ist eine der häufigsten chronischen Erkrankungen bei Kindern und Jugendlichen und verursacht trotz verbesserter Therapiemöglichkeiten auch heute noch schwerwiegende Folgeerkrankungen. Etwa 90% aller Patienten in dieser Altersgruppe erkranken aufgrund einer selektiven Zerstörung der Insulin-produzierenden Beta-Zellen im Pankreas durch das körpereigene Immunsystem an Typ-1-Diabetes. Andere Diabetesformen treten im Kindes- und Jugendalter entsprechend seltener auf und werden im klinischen Alltag mitunter erst zu spät erkannt bzw. inadäquat therapiert (z.B. MODY und Typ-2-Diabetes). Eine optimierte, an der Pathogenese der Erkrankung orientierte genetische und/oder immunologische Frühdiagnostik ist sowohl für die richtige Klassifizierung des Diabetes bei Manifestation als auch für die Prädiktion des Diabetesrisikos bereits in der präklinischen Phase der Erkrankung von Bedeutung.

EDU 2

Linkage im Zeitalter der SNP-Chips

Christine Fischer (Heidelberg), Tom Lindner (Leipzig)

C. Fischer: Multipoint-Linkage mit SNPs: Vorrausetzungen, Informationsgehalt und statistische Power (30-40 Minuten)

T. Lindner: Linkage mit SNP-Chips: Chip-Auswahl, Analyseprogramme, Probleme und Lösungen (30-40 Minuten)

Die Kopplungsanalyse ist ein zentrales Verfahren zur Lokalisation von verantwortlichen Genen für monogene Krankheiten oder Hauptgenen bei komplexen Krankheiten. In den letzten Jahren werden für genomweite Kopplungsuntersuchungen vermehrt SNP-Chips verwendet, da sie leicht und günstig zu typisieren sowie in sehr hoher Dichte vorhanden sind und daher einen hohen Informationsgehalt besitzen. Andererseits wirft die große Menge an SNPs eine Reihe von Problemen auf. Dazu gehören rein technische Probleme der Programme wenn zu viele Marker analysiert werden müssen und sie zu dicht liegen. Zusätzlich können je nach verwendetem SNP-Chip die Marker nicht mehr im Linkage-Equilibrium sein und damit ist eine Vorrausetzung für die statistische Methode verletzt, was zu einer Erhöhung der falsch-positiven Resultate führen kann. In der Sitzung wird zunächst das Prinzip der Multipoint-Kopplungsanalyse sowie die Frage der statistischen Power in Abhängigkeit von dem genetischen Modell, der verfügbaren Stammbäume und der Markerdichte analysiert. Weitere Themen der Session sind: Welche SNP-Chips sollen verwendet werden und welche Analyseprogramme stehen zu Verfügung. Anhand etlicher Beispiele werden mögliche Probleme und Lösungen aufgezeigt.

Die EDU Sitzung wurde in Zusammenarbeit mit des Arbeitskreises Humangenetik der Gesellschaft für Medizinische Dokumentation und Statistik (GMDS) organisiert.

EDU 3

Klinik und Genetik der mentalen Retardierung

Hilger Ropers (Berlin), Anita Rauch (Erlangen)

Hilger Ropers: Genetik der geistigen Behinderung - diagnostische Möglichkeiten und Perspektiven

Die genetischen Ursachen der geistigen Behinderung sind extrem heterogen. In den letzten Jahren sind international große Anstrengungen zur Aufklärung genetischer Defekte bei Patienten und Familien mit geistiger Behinderung unternommen worden. Dieser Beitrag fasst den derzeitigen Stand der Forschung auf diesem Gebiet zusammen und zeigt die daraus resultierenden diagnostischen Möglichkeiten auf. Darüber hinaus besteht die Perspektive, dass große Forschungsverbünde durch Vernetzung von klinischen, molekularen und funktionellen Ansätzen unser Wissen auf diesem Gebiet wesentlich erweitern werden.

Anita Rauch: Klinische Variabilität häufiger erkennbarer Retardierungssyndrome

Die große klinische Variabilität, die den meisten genetischen Erkrankungen inne wohnt, erschwert häufig die Diagnosestellung. In diesem Beitrag soll an Hand molekulargenetisch gesicherter Fälle das phänotypische Spektrum häufiger Retardierungssyndrome exemplarisch dargestellt werden.

EDU 4

Genetische Ursachen der Infertilität / Genetische Diagnostik vor assistierter Reproduktion

Peter Wieacker (Münster), Dieter Meschede (Köln)

Peter Wieacker: Genetische Ursachen der Infertilität

Dieter Meschede: Genetische Diagnostik vor assistierter Reproduktion

In dieser Fortbildungsveranstaltung werden genetische Ursachen männlicher und weiblicher Infertilität erörtert. Dabei werden primär gonadale Störungen als auch Defekte der hypothalamisch-hypophysär-gonadalen Achse besprochen, die zu Störungen der Spermatogenese oder Oogenese führen. Auf der Basis klinischer und endokrinologischer Befunde werden genetisch-diagnostische Flussdiagramme vorgeschlagen.

EDU 5

Diagnostik hereditärer Netzhauterkrankungen – Klinik und Genetik

Bernhard Weber (Regensburg), Ulrich Kellner (Bonn)

Ulrich Kellner: Erbliche Netzhautdystrophien - Klinik und Differentialdiagnostik

Dargestellt wird das schrittweise Vorgehen bei der Diagnose und Differentialdiagnostik hereditärer Netzhautdystrophien. Die Kombination von Funktionsuntersuchungen (Visus, Perimetrie, Elektrophysiologie) und morphologischer Diagnostik (Ophthalmoskopie, Angiographie, Fundusautofluoreszenz, OCT) ist entscheidet für eine klinische Charakterisierung der Erkrankungen. Besonders dargestellt werden neue Methoden (multifokales ERG, Fundusautofluoreszenz, OCT), die eine Frühdiagnose erlauben, bevor auffällige Netzhautveränderungen sichtbar sind.

Bernhard Weber: Erbliche Netzhautdystrophien - Molekulare Diagnostik bei einer genetisch heterogenen Erkrankung

Die Gruppe der erblichen Netzhautdystrophien liefert eindrucksvolle Beispiele für genetische Heterogenität. So wurden bis heute für das Krankheitsbild der Retinitis Pigmentosa, einer klinisch homogenen Gruppe von peripheren Netzhautdystrophien, über 30 ursächliche Gene beschrieben. Aufgrund einer solchen Heterogenität ist eine konventionelle molekulargenetische Diagnostik mit einem enormen Zeit- und Personalaufwand und somit auch signifikanten Kosten verbunden. Mit dem Re-Sequenzierchip RetChip v1 soll eine neuere technische Entwicklung auf dem Gebiet der Arraytechnik vorgestellt werden. Diskutiert werden sollen die Vorteile und Probleme einer solchen Hochdurchsatztechnologie und die sich daraus ergebenden Konsequenzen und Erfordernisse für die humangenetische Diagnostik.

Poster

P001

Three new cases of complex rearranged small supernumerary marker chromosomes. Evidence for an underestimated entity?

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Small supernumerary marker chromosomes (sSMC) are a heterogeneous group of derivative chromosomes concerning their clinical consequences as well as their chromosomal origin and shape. Besides the sSMC present in Emanuel syndrome, i.e. der(22)t(11;22)(q23;q11), only few so-called complex sSMC were reported yet. Here we report three new cases of unique complex sSMC. One was a de novo case with a dic(13 or 21;22) and two were maternally derived: a der(18)t(8;18) and a der(13 or 21)t(13 or 21;18). In summary, among ~2400 reported sSMC cases studied for their chromosomal origin and subsequently reported, by now 22 cases with unique complex sSMC were detected. I.e. unique complex sSMC are to be expected in at least 0.9% of patients with an sSMC. However, the question is, if the percentage of this specific kind of sSMC is not underestimated. Unique complex sSMC are easy to be missed if, in case of acrocentric chromosome derived sSMC not all centromeric probes are applied, and/or if no flow sorting or microdissection followed by reverse FISH or array-CGH is performed. This can be problematic especially in prenatal diagnostics, but also concerning genotype-phenotype correlations of sSMC. In conclusion, a really comprehensive characterization of all sSMC by different probes, probe sets and approaches could enhance the detection rate of unique complex sSMC. Unique complex sSMC are especially expected in cases with a 'heterochromatic sSMC', no uniparental disomy in connection with the sSMC and, nonetheless, clinical symptoms. Here a reverse FISH or array-CGH experiment of the sSMC should be performed and might show additional chromosomal imbalances.

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P002

Using Multiplex Ligation-Dependent Probe Amplification (MLPA) assay for the detection of gene deletion in the factor IX gene

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Introduction: Hemophilia B is a recessively inherited X-linked bleeding disorder caused by mutations in the factor IX gene. Faxtor IX deficiency is characterized by spontaneous joint and muscle bleeds and easy bruising in severely affected males. Carrier females are usually asymptomatic and plasma activity of factor IX in carriers is variable. Hemophilia B is less common than hemophilia A with a frequency of approximately 1 in 25000 males worldwide.
Patient: The index case is a 1-year-old patient from Germany with diagnosed hemophilia B.
Methods: Genomic DNA was extracted from peripheral blood leukocytes followed by PCR amplification of exons including corresponding exon-intron boundaries. For detection of deletions in the factor IX gene was used the Multiplex Ligation-Dependent Probe Amplification (MLPA) SALSA P207 probemix by MRC-Holland detecting all exons of the factor IX gene. MLPA PCR products have been analysed by capillary gel electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).
Results: Sequence analysis of the factor IX gene showed no PCR products. Therefore we checked the patient for deletions in the factor IX gene with MLPA. The analysis presented a complete factor IX gene deletion in the patient as the cause of the disease.
Conclusion: Detection of large deletions by lacking of PCR amplification is often difficult especially in heterozygous carriers.
These data show that the Multiplex Ligation-Dependent Probe Amplification (MLPA) is a sensitive

technique that allows the identification of deletions of the factor IX gene and should be done in these patients suspected for hemophilia B if no mutation is detected by sequencing.

P003

Derivative chromosome der(9)t(2;9)(p25.2;q34.3) causes multiple congenital abnormalities and severe global retardation: A cryptic aberration identified by array-based comparative genomic hybridization

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The chromosome 9q subtelomere deletion syndrome (9qSTDS) is a recently described microdeletion syndrome causing a distinct facial gestalt, muscular hypotonia, mental retardation, and multiple other abnormalities. We report on a 27-year-old woman with microcephaly, typical facial features of 9qSTDS, contractions of the elbow and knee joints, severe muscular hypotonia, no ability to walk, and no speech development. Additionally she has no spontaneous defecation and no bladder control. Array-CGH revealed a cryptic 9q34.3 deletion and 2p25.2-p25.3 duplication transmitted by her mother, who was carrying a balanced translocation of chromosomes 2p and 9q. There are about 50 reported cases of deletions of the subtelomeric part of chromosome 9q, however, duplications of only the terminal part of chromosome 2p are rare. Neuroblastoma, diaphragmatic hernia, neural tube defects, broncho-pulmonary abnormalities, and congenital heart defects are conditions associated with partial trisomy 2p but only described in patients with larger duplications. To our knowledge there is only one case described with an isolated duplication as distal as in the patient reported here. This patient had exophthalmus, other dysmorphic facial features, arachnodactyly, and contractures of the elbow and interphalangeal joints but normal psychomotor development. Exophthalmus and contractures of the elbow joints are two features also seen in our patient that are not allegeable by the deletion 9q34.3 and may be due to the additional duplication of 2pter.
Our report is a hint that terminal duplications of chromosome 2p may cause no specific clinical problems. The frequently described features of partial trisomy 2p may be due to duplications of more proximal parts of 2p but not the terminal part. Nevertheless the severity of the phenotype, in particular the degree of mental retardation of our patient compared with the 9p34.3 deletion cases in the literature may be due to the duplication of 2p25.2-p25.3.

P004

Copy number variation 13q: a large interstitial deletion with benign phenotype

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Deletions of the long arm of chromosome 13 are uncommon but well-recognized associated with varying phenotypes. Common features include moderate to severe mental and growth retardation, craniofacial dysmorphisms, hand and foot anomalies, and brain, heart and kidney defects. The wide phenotypic spectrum was proposed to be related to the difference in monosomy of distinct 13q regions.
We report here a large familial interstitial 13q deletion of 14.5Mb segregating in three generations without a relevant phenotype. The index case of this family is a now 5 year old boy whose mother had amniocentesis because of polyhydramnion. The prenatal standard karyotype revealed a heterozygous interstitial deletion of chromosome 13q21.2q22. The deletion was shown to be maternally inherited. The boy was prematurely born at 34 2/7 weeks. He showed skin pigmentation anomalies following Blaschko lines and bilateral inguinal hernia. An initial neonatal hypotonia and a slightly delayed psychomotor development were caught up at the age of 1 year. No dysmorphic features or malformations were recognized. The mother of the index patient does not present any phenotype. Further family investigation revealed the maternal grandfather carrying the familial deletion without phenotypic manifestations. In order to characterize the extent of the deletion array-CGH was performed on the mother's DNA (244K Agilent Human Genome kit). The deletion comprises 14.5 Mb (13q21.1-q21.33), containing 18 genes without a known functional role in humans to date. Array-CGH

analysis of the deletion of the boy and his grandfather is ongoing.
Our data underline the importance of a phenogenetic approach of genomic copy number variations. The absence of an associated phenotype to this 13q deletion might be due to the lack of dosage-sensitive genes in the delineated region. The findings support the observation of Ballarati et al.(2007) that 13q deletions proximal the 13q32 band may cause a mild phenotype.

P005

Severe mental retardation with breathing abnormalities (Pitt-Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4

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Pitt-Hopkins syndrome (PHS) is a rare mental retardation syndrome mainly characterized by severe motor and mental retardation (MR) including absent language development, a characteristic facial gestalt, and episodes of hyperventilation. We report on a female patient with PHS showing severe MR with absent speech, pronounced muscular hypotonia, ataxia, distinctive facial features such as a coarse face, a broad nasal bridge, and a wide mouth, and hyperventilation attacks. In this patient, genomic profiling by array-based comparative genomic hybridization and FISH studies detected and confirmed a de novo 0.5 Mb deletion in 18q21.2 containing a single gene, the basic helix-loop-helix transcription factor TCF4. cDNA and genomic analyses in the patient and her parents demonstrated TCF4 haploinsufficiency as the underlying cause of the disease. Analysis of the embryonal expression pattern of the Danio rerio ortholog, tcf4, by whole mount in situ hybridization showed a highly specific expression domain in the pallium of the telencephalon during late somitogenesis, when the patterning of the zebrafish brain is advanced and neural differentiation commences, while at an earlier stage, tcf4 expression was also visible in the trunk. Later expression domains were restricted to several regions in the CNS, including continued expression in the pallium of the telencephalon, and starting expression in the diencephalon (thalamus, ventral thalamus and posterior tuberculum), the midbrain tegmentum, the hindbrain, and the branchial arches. This expression pattern correlates with the clinical phenotype. Our results indicate that haploinsufficiency of TCF4 causes PHS, and suggest that Danio rerio is a valuable model to study the molecular pathogenesis of PHS and the role of TCF4 in brain development. Knock-down of tcf4 function by antisense oligonucleotide morpholino injection into zebrafish embryos and the subsequent characterization of the morphant phenotype are currently being performed.

P006

GJB2 mutations, GJB6 mutations and karyotypes in 300 patients with sensorineural childhood deafness

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Hearing impairment is extremely common affecting 1/650 newborns. Over 100 deafness genes are known in humans, but mutations in GJB2 (gene for Connexin-26) predominate and cause 20-30% of non-syndromic childhood deafness in most populations. We searched for mutations in 300 patients with mild-to-profound sensorineural childhood deafness. More than 80% of the patients were from Germany; the others were from 12 different countries. More than 90% of patients were in the age range 0-8 years, and these all underwent age-adapted audiometric testing using subjective and objective audiometry. We identified 26 different mutations (141 mutant alleles), which represented 10 truncating (T) mutations (98 alleles) and 16 nontruncating (NT) mutations (43 alleles). Biallelic GJB2 mutations were identified in 56 patients (18.6%) and were associated with 21 different genotypes, of which 8 were classified as homozygous truncating (T/T), 5 were homozygous nontruncating (NT/NT), and 8 were compound heterozygous truncating/nontruncating (T/NT). Findings included two novel

mutations (N14D and H100Q) and one allele with a GJB6_342-kb deletion. Karyotyping was performed in all patients and revealed three most likely causative aberrations (a 13q deletion, an unbalanced translocation, and a homozygous reciprocal translocation). Three further aberrations (47,XXY and two familial reciprocal translocations) were considered to be coincidental. This study suggests that in the evaluation of childhood deafness, karyotyping may be as effective as GJB6 deletion studies. The observed frequency of 18% biallelic GJB2 mutations is within expected limits, because ~20% of our patients demonstrated syndromic, unilateral, or dominant hearing loss. Recently, truncating GJB2 mutations have been associated with more severe hearing loss than nontruncating mutations. Our correlation of the audiometric data and the genotype is pending.

P007

Familiäre primäre Arrhythmiesyndrome: Bedeutung der molekulargenetischen Diagnostik am Beispiel einer kardiologischen Spezialambulanz

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Einleitung: Die häufigsten primären, angeborenen Arrhythmiesyndrome sind das Long QT-Syndrom (LQTS), die catecholaminerge polymorphe ventrikuläre Tachykardie (CPVT) und das Brugada-Syndrom (BS). Diese können zu Synkopen und plötzlichen Herztodesfällen bei sonst zumeist gesunden Patienten führen.
Methode: Zwischen 2001 und 2006 leiteten wir bei 91 Indexpatienten mit der klinischen Diagnose LQTS, CPVT und BS eine molekulargenetische Untersuchung ein. Dabei wurden die 5 häufigsten LQTS-Krankheitsgene (KCNQ1, KCNH2, SCN5A, KCNE1 und KCNE2), das RYR2-Gen bzw. im Falle eines klinischen BS das SCN5A-Gen sequenziert. Wir untersuchten, wie häufig eine Mutation bei diesen Patienten gefunden wurde.
Ergebnisse: In 65% (59/91) der Fälle konnte eine krankheitsverursachende Mutation nachgewiesen werden (angeborenes LQTS 72% (51/71), CPVT 60% (3/5), BS 33% (5/15). In Folge konnten wir 219 potentiell betroffene Angehörige identifizieren und bei 111 (51%) Angehörigen die Mutation ausschliessen sowie bei 108 (49%) nachweisen.
Zusammenfassung: Bei 65% der Patienten mit der klinischen Diagnose angeborenes LQTS, CPVT oder BS konnte eine krankheitsverursachende Mutation gefunden werden. Die konsekutive Untersuchung von potentiell betroffenen Angehörigen identifizierte pro Familie im Durchschnitt 1.8 (0-9) weitere Mutationsträger und führte zur Möglichkeit der Meidung bekannter Trigger sowie ggf. Einleitung einer frühzeitigen Therapie und entlastete ebenso viele Angehörige

P008

A de-novo 7.6 Mb tandem duplication of 14q32.2-qter associated with primordial short stature with neurosecretory growth hormone dysfunction, distinct facial anomalies and mild developmental delay

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Duplications of the distal segment of 14q are increasingly recognized, but most patients reported were carriers of an unbalanced translocation. We here present the first case of a de novo tandem duplication partially encompassing the chromosomal bands 14q32.2 and the very distal band 14q32.3 (14q32.2-qter) refining the “14q duplication” phenotype. Molecular karyotyping using an Affymetrix 250 K Nsp SNP array revealed a maximal duplicated region of 7.6 Mb concerning chromosomal bands 14q32.2-qter, flanked by SNP_A-2294605.
Our patient, an 8 years and 3 months old girl, presented with short stature and mild developmental delay and facial anomalies such as high forehead, mild hypertelorism, broad nasel bridge, dysplastic ear helices, short philtrum, thin and “cupid bow” upper lip, broad mouth, and micrognathia. Endocrinological testing revealed a neurosecretory dysfunction and growth hormone treatment appeared effective. In accordance with the reported findings, this pure “distal 14q duplication”

phenotype is characterized by primordial short stature due to neurosecretory growth hormone dysfunction, mild developmental delay, and distinct facial dysmorphism, but no cardiovascular malformations, hypogenitalism, or cerebral MRI or EEG abnormalities, reported in more proximal duplications.

P009

A new case of Potocki-Lupski syndrome diagnosed by array-CGH

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After normal results in conventional cytogenetic analysis and molecular genetic analysis of RETT-syndrome in a one year old dystrophic girl with global developmental delay array-CGH was performed. The analysis showed a 15 clone duplication stretching over nearly 4 MB in 17p11.2. Potocki and Lupski described the syndrome in the year 2000 for the first time although the microduplication 17p11.2 in association with developmental delay and mild dysmorphic facial features has already been published by Brown in 1996. The Potocki-Lupski (PTLS) syndrome is characterised by developmental delay, language and cognitive impairment and especially by poor feeding and failure to thrive in infancy. Oral-pharyngeal dysphasia, autistic features, obstructive and central sleep apnea, structural cardiovascular abnormalities, hypermetropia, and EEG abnormalities are also quite frequent. The last has also been found in our child.
The microduplication is the reciprocal of Smith-Magenis microdeletion syndrome. Nonallelic homologous recombination between region-specific low-copy repeats (LCRs) is a major cause of DNA rearrangements associated with many genomic disorders. The proximal short arm of chromosome 17 is particularly rich in LCRs and is a regional locus for 4 genomic disorders: Charcot-Marie-Tooth type 1A, hereditary neuropathy with liability to pressure palsies, Smith-Magenis syndrome, and the duplication 17p11.2 syndrome. Array CGH was performed as BAC array with Bluegnome cytochips version 2.0 with a resolution of about 850 kb. The result was confirmed by FISH with a probe for Smith-Magenis syndrome. Testing of the healthy parents gave normal results.
Because of the rather mild phenotype on the one side and the limitations of conventional chromosomal analysis on the other side PTLS might be an underdiagnosed syndrome that will become better known with the enhanced use of array diagnostics.

P010

Silver-Russel-Syndrome: A follow-up case report

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Silver-Russell syndrome (SRS) is a heterogeneous congenital disorder characterized by intrauterine growth retardation, postnatal growth failure and typical dysmorphic features like body asymmetry, prominent forehead, triangular face and fifth finger clinodactyly. The majority of cases are sporadic, in 10% of cases a maternal uniparental disomy of chromosome 7 (UPD7) has been detected. The most recent findings suggest that imprinting defects within the 11p15 region play a role in more than 30% of SRS patients. Chromosomal anomalies, familial autosomal dominant and X-linked forms have also been reported.
A follow-up report of the clinical outcome of a 2-year-old female infant is presented. The parents were non-consanguineous and clinically normal. At birth, her length was 50 cm (25th-50th percentile), weight 2,830 kg (10th-25th percentile), and head circumference 35 cm (50th-75th percentile). At 6 weeks of age dysplasia of the hip was diagnosed and body asymmetry was observed on the occasion of routine paediatric examination. Also excessive sweating was described. The parents were referred to genetic counselling with their daughter at 10 months of age. She showed SRS-compatible features as triangular and asymmetric face, broad forehead, mild retrognathia and right hemihypotrophy, with a lower limb length difference of 3 cm. The diagnosis of Silver-Russell syndrome was suspected. A molecular genetic analysis of ICR1 and maternal UPD7 was performed and an epigenetic mutation in 11p15 affecting the telomeric imprinting domain (ICR1) was detected.
A marked intrauterine growth retardation was not present in this case and the molecular confirmation

of the clinical tentative diagnosis in untypical cases can be useful for a better management and surveillance of the affected patient. This case supports the hypothesis that dysregulation of genes at 11p15 region are involved in SRS.

P011

Atypical deletion 22q11.2 in a girl with small stature, microcephaly and developmental delay revealed by oligo-array CGH

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The 22q11.2 microdeletion syndrome is one of the most frequently occurring genomic disorders associated with a wide clinical variability, including e.g. DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome. About 90% of the patients carry a typical ~3Mb deletion in the region 22q11.2. A subset of patients carries only a 1.5Mb proximally nested deletion, which is also known as the primary DiGeorge critical region (DGCR). Rarely, patients with other alterations in this region including atypical deletions, translocations or TBX1 mutations were reported. Here we present a 6 ½ year old girl with small stature, dystrophy, microcephaly and developmental delay. She showed characteristic facial dysmorphisms including upslanting palpebral fissures and hypotelorism. She further had a patent ductus arteriosus and was treated for duodenal atresia shortly after birth. Ophthalmologic investigation showed alternating strabismus and hyperopia. A radiograph of the left hand revealed a retarded bone age. Standard chromosome analysis in the girl was normal. Using oligo-array CGH, we detected a de novo 1.16 Mb deletion in the region 22q11.21q11.22. This atypical deletion maps distal to the common ~3Mb deletion in 22q11.2 microdeletion syndrome and includes 12 known genes. Still, our patient shows some of the unspecific features of VCFS, including small stature, microcephaly and developmental delay. The deletion detected in our patient appears to partially overlap with the deleted region in the patients reported by Rauch et al. (2005) and Saitta et al (1999). Those patients show similar facial dysmorphisms to our patient, including upslanting palpebral fissures, a high nasal bridge and a thin upper lip. Further studies are necessary to define the phenotype of those atypical deletions 22q11.2.

P012

A patient with fibrodysplasia ossificans progressiva (FOP) and detection of a de novo 617G>A mutation in the ACVR1 gene

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Fibrodysplasia ossificans progressiva (FOP; OMIM 135100) is a rare genetic disorder with autosomal dominant transmission. FOP is the most severe disorder of heterotopic ossification and results in postnatal formation of an ectopic skeleton. Heterotopic ossification in FOP begins in childhood and can be induced most often by trauma. The ossification is episodic and progressive, leading to extra-articular ankylosis of all major joints of the axial and appendicular skeleton, rendering movement impossible. A specific congenital deformation of the great toes, a bilateral hallux valgus deformation, is common. Recently, Shore et al. (2006) mapped FOP to chromosome 2q23-24 by linkage analysis and identified a heterozygous mutation (617G>A; R206H) in the glycine-serine (GS) activation domain of ACVR1, encoding a BMP type I receptor. Our patient (a girl born 10.04.2003) has congenital bilateral hallux valgus deformation. Medical history at the age of 6 month revealed retarded motor development and a cervicocephal syndrome. In progression, neurological examinations revealed central coordination malfunction and functional disturbance of the motion system.
With age of 1 7/12 years the patient was examined in our genetic department. We had the clinical suspicion of FOP which was confirmed by appearance of first post-traumatic ossified lesions on the head and sternoclavicular region at the age of about 2 years.
Since only the 617G>A mutation has been found in FOP so far we investigated whether ACVR1 617G>A mutation is causative for FOP in our patient. We identified the 617G>A mutation in our patient by restriction endonuclease digestion of a PCR-fragment of ACVR1 exon 6. Thus the detection of the ACVR1 617G>A mutation confirms the clinical diagnosis FOP in our patient. The presence of the mutation hot spot facilitates molecular diagnosis in clinical practice.

P013

The wide phenotypic variability of the de Grouchy II-syndrome

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We report on a girl who is the second child (one elder healthy sister) of a non-consanguineous healthy couple. She was born after 38 weeks of pregnancy with normal birth-weight (3200g), length (52 cm) and head circumference (33 cm). At six months failure to thrive and developmental delay resulted in further investigations. At that time a microcephaly and a “syndromical appearance” was described. At presentation she was 14 months old. Length and weight were on the 3rd centile, head circumference was below the 3rd centile. She had facial anomalies including flat back of the head, low frontal hairline, midface hypoplasia, hypertelorism, thick lips with downturned corners of mouth, low set ears. Furthermore she had nystagmus and showed a retardation of developmental milestones.The EEG was normal. The cytogenetic analysis revealed a deletion of the long arm of chromosome 18, (46,XX,del(18)(q21)). The breakpoint was characterized by FISH (MALT+, BCL2-). The karyotype of the mother was normal, a blood sample of the father was not available. Deletions of 18q are described as de Grouchy II-syndrome. The most common deletion extends from region 18q21 to qter. Most cases described with this deletion exhibit additional features as seizures, dysmyelination, palatal abnormalities (high or cleft palate), hearing impairment (caused by aural atresia), skeletal defects (scoliosis), limb anomalies (clinodactyly, tapering fingers, foot deformities), abnormalities of the external genitalia, cardiac anomalies, immunodeficiency (IgA-deficiency) and hypotonia. The relative mild symptoms in our patient illustrates the broad range variability of the phenotype associated with deletions of 18q. Even so we have to consider, that differences of the accurate size of the deletion could possibly in part explain the phenotypic differences.

P014

Array-CGH for the identification of constitutional copy number changes

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Up to date, we have analyzed 98 patients with a conspicuous phenotype by array-CGH. We employed different array platforms, including 1 Mb or 8k large insert clone arrays (kindly provided by B. Radlwimmer and P. Lichter, DKFZ, Heidelberg) and the commercially available 44K oligoarray from Agilent.
Out of 88 patients with an apparently normal karyotype array-CGH identified copy number changes in 12 cases (=14%). Findings include an unbalanced translocation of the subtelomeric regions of 12p and 15q, de novo deletions on chromosomes 2, 4, 5, 9, 10, 17, 22 and duplications on chromosomes 12 and 19. Subsequent analyses usually include evaluation of the parental genomes and verification either by FISH or RT-PCR. Some of the imbalances involved known genes explaining features of the patients´ phenotype. For example, an especial interesting case represented a de novo deletion of 0.8 Mb on chromosome 17p13.1, harboring the tumor suppressor gene p53, which was identified in a girl with developmental delay. Furthermore, we found that in this patient the breakpoint disrupted the transcriptional control of the GUCY2D gene, which likely causes the amaurosis of the patient.
We also analyzed 10 patients with abnormal karyotypes. In 3 patients with a de novo alteration array-CGH did not demonstrate imbalances at the breakpoints or in any other region of the genome. In the 7 other cases, array-CGH fine-mapped the respective breakpoints and/or revealed that the rearrangement was more complex than expected from banding analysis.
At present, it is unclear whether special features of a patient’s phenotype allow the prediction whether array-CGH will indeed detect a disease associated imbalance. To address this question, we used a scoring system, which was originally proposed by de Vries and colleagues for subtelomere screening (2001). However, we did not observe a correlation between phenotype classification based on this score or the likelihood to detect an imbalance.

P015

Characterization of cardiovascular manifestations in patients with Williams syndrome

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Congenital cardiac abnormalities (CCA)are the marker's feature of Williams syndrome (WS, MIM 194050) phenotype. 25 patients with suspected WS (heart defects, mental/growth delay, dysmorphiñ craniofacial signs, normal GTG karyotype) were examined by FISH. Microdeletion 7q11.23 was detected in 60% cases. We analyzed clinical data of 15 patients with confirmed WS (9 male, 6 girls, age ranged 1 month - 22 years) and presented cardiovascular abnormalities. 90% of cardiac defects were detected at birth or during first year of life (in one case manifested of heart "murmur" echocardiography was not performed). Isolated heart defect was detected in 3 patients: supravalvular aortic stenosis (SVAS); atrium septal defect (ASD); coarctation of aorta (CoA). Multiply heart malformations were registered in 11/15 patients and presented following combinations:
- SVAS, pulmonary arterial stenosis (PAS), CoA, foramen ovale (FO);
- SVAS, PAS;
- SVAS, mitral valve prolapse (MVP);
- PAS, CoA
- ASD, CoA, FO, aortic, mitral valves dysfunction;
- ventricular septal defect (VSD), CoA;
- PAS, ASD;
- PAS, FO;
- VSD, FO;
- FO, additional chorder of left ventricule;
- VSD, aortic, mitral valves fibroses

Our patients showed a wide spectrum of heart abnormalities, multiply defects were predominant lesion (72.3% cases). The most common malformations were coarctation of aorta and pulmonary arterial stenosis (30%). Supravalvular aortic stenosis (typical heart defect of WS) was identified in 26% cases. Septal and aortic/ mitral valves defect registered in 20% patients. Follow up study and comparison of CCA spectrum with literature data will be presented.

P016

Report of a second patient with Left-Ventricular Non-Compaction (LVNC) and monosomy 1p36

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Hemizygosity for the most distal chromosomal band on the short arm of chromosome 1, monosomy 1p36, represents the most common human terminal deletion and is characterized by psychomotor retardation, eye/vision problems, seizures, growth delay, dysmorphic craniofacial features, and behavioral problems. Cardiac abnormalities such as dilated cardiomyopathy and structural cardiac defects occur in 44% of patients with monosomy 1p36, but only one patient with left-ventricular non-compaction (LVNC) has been published so far (Thienpont et al., 2007). LVNC is a cardiomyopathy characterized by deep trabeculations in the ventricular wall.
Here, we report on a two-month-old girl with LVNC, ventricular septal defect, hypothyroidism, large anterior fontanel, cleft palate, right sided choanal stenosis, and facial dysmorphism consisting of deep-set eyes, up slanting palpebral fissures, small mouth, low-set ears, and frontal bossing. She is the first child of non-consanguineous German parents and was born by Caesarean section after 35 weeks of gestation with normal measurements [weight 2400 g (-0.2 SD), length 46.5 cm (-0.3 SD), and OFC 34.5 cm (+1.2 SD)]. The mother and several family members suffer from autosomal dominant polycystic kidney disease.
Because of the craniofacial dysmorphism in combination with LVNC we suspected a monosomy 1p36. Routine karyotyping using G-banding analysis at a resolution of ~450 bands per haploid genome was indicative of a deletion 1p36. The deletion was confirmed by FISH and by MLPA. Parental karyotypes including FISH studies showed normal results. The size of the deletion was determined to be 4.8 Mbp by SNP array analysis (Affymetrix 250K Styl array; Dr. Klein-Hitpass, IFZ, Essen).
In conclusion, the finding of a second patient with LVNC and monosomy 1p36 suggests that LVNC is more common in monosomy 1p36 than previously reported.

P017

Hyperparathyroidism-Jaw Tumor syndrome (HPT-JT) in a 30 year old woman

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Hyperparathyroidism-jaw tumor syndrome (HPT-JT) is characterized by primary hyperparathyroidism, the mandible and maxilla affecting ossifying fibromas, renal cysts and solid tumors. The underlying cause of the HPT-JT syndrome are mutations in the HRPT2 gene, which encodes for the protein parafibromin. The HRPT2 gene acts as a tumor suppressor gene. Inactivating germline mutations are found in patients with HPT-JT syndrome.
Here we describe a 30 year old woman who presented with a giant cell granuloma of the right mandible at the age of 19 years. One year after surgical removement of the tumor recurrence of the giant cell granuloma occurred. Serum calcium (3,65 mmol/l) and parathormon levels (398 ng/l) were elevated. The patient was diagnosed for primary hyperparathyreoidism as a result of a parathyroid adenoma. After surgical removement of the parathyreoid gland with the adenoma the serum calcium and parathormon levels went normal. The mutation analysis of the HRPT2 gene revealed a heterozygous novel deletion of two nuclotides in exon 16 (c.1432_1433delCT).This mutation leads to a frameshift and a premature stop codon. It is likely that this mutation leads to a functional loss of the parafibromin.
The identification of HRPT2 carriers is useful for early diagnosis, detection and removal of malignant parathyroid tumors and associated malignancies.

P018

Setleis syndrome - evidence for autosomal dominant inheritance in a five generation family

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Setleis syndrome (bitemporal forceps marks syndrome, OMIM 227260) is a rare syndrome characterized by bitemporal skin depressions and additional abnormalities. Both autosomal dominant and autosomal recessive modes of inheritance have been reported. A gene locus is still unknown.
We present a five-generation German family with bitemporal focal dermal dysplasia, discoloration in the temporal areas of the skin and distichiasis of the upper lids. In addition, two of the twelve affected patients had congenital nystagmus, and two male patients had hypertrichosis of the trunk and extremities. Body measurements and psychomotor development were normal in all patients.
Neither congenital nystagmus nor hypertrichosis have previously been reported in patients with Setleis syndrome. The mode of inheritance in our family provides further evidence for the existence of an autosomal dominant form of Setleis syndrome. Linkage analysis in this large family may reveal a gene locus and will eventually contribute to the elucidation of the causative gene defect

P019

Incidence of dural ectasia in patients with genetically proven Marfan syndrome

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Marfan syndrome is an autosomal dominant disorder involving different organ systems. Marfan syndrome type 1 (MFS1) is caused by mutations in the FBN1-gene.
The phenotype is described by the Ghent nosology which classifies the clinical manifestations in major and minor criteria. Dural ectasia is one of the major criteria for Marfan syndrome but it is rarely tested for. Dural ectasia is described as widening of the spinal canal, scalloping of the vertebral body posteriorly, increased thinning of the cortex of pedicles and laminae, widening of the neural foramina or the presence of a meningocele.
The frequency of dural ectasia among patients with an established mutation was not examined so far. The aim of the study was to show that dural ectasia is an important criterion for fulfilling the Ghent Nosolgy.
60 patients with identified mutations in the FBN1-gene were examined for dural ectasia. 47 of the 60 patients (78%) showed the criterion of dural ectasia.
28 of the 60 patients (47%) fulfilled the criteria of the Ghent Nosolgy without having been tested for

dural ectasia. After testing for dural ectasia 19 of 32 patients (59%) with the suspicion of having Marfan syndrome fulfilled the Ghent Nosolgy when the diagnosis of dural ectasia was made.

P020

A maternally derived 5Mb deletion on chromosome 21q21.2-q21.3 in a patient with a Silver-Russel phenotype and mental retardation

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A 7 year-old boy with pre- and postnatal growth retardation and slight facial dysmorphisms consistent with the Silver-Russel syndrome (SRS) is presented. Additionally, the patient shows a mild mental retardation and severely restricted speech skills. Chromosome analysis revealed a normal karyotype (46,XY) and screening for subtelomeric rearrangements was also negative. Testing for a SRS mutation (UPD chromosome 7, hypomethylation/duplication ICR1 chromosome 11p15) as well as testing for a Noonan syndrome mutation (PTPN11 gene) revealed no mutation. Using array-based comparative genomic hybridization (aCGH, Agilent, 244K chip), we identified a 5Mb deletion on chromosome 21q21.2-q21.3. Analyses of both parents showed that this deletion was also present in the unaffected mother, whereas aCGH analysis of the father was normal. This deletion is maternally inherited and was also detected in the boy´s unaffected grandmother and uncle by using quantitative PCR. Therefore, we suggest that this deletion could represent a copy number variation (CNV) without phenotypic consequences. Nonetheless, the coincidence of such an unusually large CNV and the phenotype of the boy is striking. Hence, we hypothesize that the CNV might have unmasked a paternally inherited hemizygous mutation. The deleted region contains 13 genes. Only one of these genes, the amyloid precursor protein gene (APP), is connected to human disease. Mutations in this gene are related to early onset forms of Alzheimers disease and are inherited in an autosomal dominant fashion. Mutations in both alleles of the human APP gene were not described so far. We started sequencing of the APP gene in the affected boy and his unaffected father. In addition, further characterisation of the remaining 12 genes in this region is in progress.

P021

EEC syndrome without C

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The EEC syndrome comprises the cardinal features of ectrodactyly, ectodermal dysplasia, and cleft lip/palate. Inheritance is autosomal dominant with wide intra- and interfamilial variability of clinical expression. In 1988, Wallis [Clin Genet 34:252-7] reported on a family with variable degree of ectrodactyly and ectodermal dysplasia but without cleft lip or palate and discussed the possibility of a distinct subtype of EEC syndrome. Here we report on a second family with similar findings. Symptoms in the male index patient are a split hand on the left, lacrimal duct stenosis, unilateral preauricular fistula, dry skin, and oligodontia with 20 permanent teeth missing. Deciduous dentition was normal. His maternal half-brother has eczematous skin changes on the back and nine permanent teeth missing. Two primary teeth were fused and eruption of permanent teeth was delayed. Symptoms in the mother of the boys are dry skin, unilateral preauricular fistula, generalised sparse and localized absent scalp hair, oligodontia with 24 permanent teeth missing and incomplete deciduous dentition. One brother and one sister of the mother as well as her mother and maternal grand mother are all affected by abnormal dentition and oligodontia. None of the affected individuals has cleft lip or palate. With respect to the known clinical variability the diagnosis EEC syndrome seems reasonable. However, since mutational analysis of TP63 gene in our family gave normal results and genetic heterogeneity in EEC syndrome is considered we wondering whether there might really exists a distinct subtype without cleft lip/palate as suggested by Wallis. As in their family, also in our family no male-to-male transmission was observed which could be of counselling interest. However, further pedigree analyses of other TP63-negative EEC families without cleft lip/palate are necessary to answer this question.

P022

Characterization of five novel large deletions causing hereditary hemorrhagic telangiectasia

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Using quantitative Real Time PCR (QRT-PCR) molecular genetic analysis was carried out for endoglin (ENG) and activin A receptor type II-like kinase 1 (ACVRL1/ALK1) gene rearrangements in a group of 45 clinically confirmed HHT families with negative direct sequencing results. We detected 5 large novel deletions, four in the ALK1 gene and one in the ENG gene. In two of the families, the whole ALK1 gene was deleted. One of these two deletions spanned at least 216kb and included five neighbouring genes (LOC728503, ANKRD33, ACVR1B, GRASP, and NR4A1). The lack of additional symptoms in the patient carrying this large deletion indicates that heterozygous loss of these five genes has no obvious phenotypical effect. To our knowledge, this is the first report on whole ALK1 gene deletions in HHT patients. We re-screened our 45 families for large rearrangements using the Multiplex Ligation-dependent Probe Amplification (MLPA) method. No discrepancies between the results of QRT-PCR and MLPA were found. Our present work proves QRT-PCR as a reliable and sensitive method. Thus, our study supports that screening for large rearrangements should be considered to improve the genetic analysis in HHT patients with no apparent mutations in ALK1 and ENG using direct sequencing.

P023

A case of trisomy 8 mosaicism

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Mosaic-trisomy 8 is a rare, but well known chromosome aberration. We report on a 5 year old boy with distinct clinical signs and mild development delay. He is the first child of a healthy couple, born after uneventful pregnancy besides mild oligohydramnion. Weight 4160g, length 55cm; head circumference 36cm. The boy presented with hypoplasia of corpus callosumm,cavernoma of gyrus cinguli, supernumerary kindney, hyperopia and strabismus, lumbal hemivertebrae, kyphoscoliosis, 13.th rib pair, cafe au lait spots, hemangiomas, deep longitudinal plantar furrows. The face is expressionless with prominent forehead, mild hypertelorism, coarse nose, everted and thick lower lip, high palate. The psychomotoric development is retarded. Karyotype analysis and subtelomer analysis from lymphocytes revealed a normal male karyotype, 46,XY. FISH-analysis from buccal smear with a centromer specific probe showed mosaic trisomy 8 in 10% of the analysed cells. The risk for various haematological disorders are discussed.

P024

X-linked Alport syndrome due to a novel mutation in the COL4A5 gene

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We report on a 33 year old woman who asked for counselling for ADPKD. Her father developed end stage renal failure (ESRF) at the age of 56 years. His kidneys showed multiple cysts with compression of the parenchyma and ADPKD was diagnosed. Our proposita had bilateral renal cysts, no hypertension and a pathological urinary sediment with acantocytes, microhematuria and a slight proteinuria since childhood. Similar findings were present in her sister. Kidney biopsy showed thin basement membrane nephropathy and a focal loss of expression of the alpha-chain of collagen IV compatible with Alport syndrome. Sequence analysis of the COL4A5-gene identified a novel heterozygous missense mutation leading to a substitution of glycine by serin in codon 328. Since Gly-X-Y-repeats are essential for the formation of the triple-helix structure of the collagenous domain of the alpha-IV-chain it is very likely to be the causative mutation. Glycine substitutions located 5` are suggested to result in a relatively mild phenotype with a mean age of ESRF above 30 years in males. This is in accordance with our family. In between, a boy child was born who inherited the mutation. Up to now (9 month) no proteinuria or hematuria are present. Our case illustrates that secondary cystic transformation of the kidneys in X-linked Alport syndrome may imitate ADPKD. Careful evaluation of the initial symptoms of the affected individual and pedigree information are necessary for the correct diagnosis.

P025

Refinement of the GINGF3 locus for hereditary gingival fibromatosis

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Hereditary gingival fibromatosis (HGF) is a rare, benign disorder characterized by slowly progressive fibrous overgrowth of the gingiva. To date, four gene loci have been mapped for autosomal dominant non-syndromic HGF. A single SOS1 (son of sevenless one) gene mutation has been identified at the GINGF1 (gingival fibromatosis 1) locus so far. We identified a family with 9 affected individuals and tested for linkage to the known loci by polymorphic microsatellite marker analysis. A maximal multipoint logarithm of the odds (LOD) score of 3.91 was obtained with marker D2S390 ($\theta = 0$) at the GINGF3 locus on chromosome 2p23.3-p22.3, and linkage to other known loci was excluded. The linked interval was flanked by markers D2S220 and D2S352. The GINGF3 locus was thus refined to a 6.56-cM, 8.28-Mb region. Our data suggest that the GINGF3 locus might account for a higher percentage of HGF cases.

P026

Two novel MYCN mutations in sporadic and familial cases with Feingold syndrome including monozygotic twins

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Feingold syndrome is a developmental disorder with considerable phenotypic variability and caused by mutations in the MYCN gene. 14 different mutations have been described up to now, 12 of which were found in exon 3. Only one mutation was detected in exon 2, and this was shown to result in a milder phenotype without developmental delay. Here we report two novel mutations in MYCN. The first one is a de novo frameshift mutation in exon 2 associated with microcephaly, developmental delay, typical dysmorphic features but no intestinal atresia. In the familial case, a heterozygous missense mutation was found, replacing yet another conserved arginine residue in the helix-loop-helix domain conserved in MYCN family members. The mutation was first found in a pair of monozygotic twins who were unequally affected by motor and speech delay with only relative microcephaly, limb malformations, doudenal atresia and hearing loss. The mother had only clinodactyly of 5th fingers, her brother is mentally retarded and has only one kidney, clinodactyly of Vth fingers, and syndactyly 4-5 of toes. The father of the mother has only syndactyly 4-5 of toes. Our results show, that mutations in exon 2 of MYCN can also be associated with a severe developmental outcome, whereas missense mutations in exon 3 show striking phenotypic variability, arguing for a major influence of genetic or environmental modifying factors.

P027

Experiences of a routine diagnostic laboratory with oligo-based array CGH in the diagnosis of mental retardation combined with epilepsy

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Array CGH is a powerful diagnostic tool for the detection of submicroscopic chromosomal imbalances. Such imbalances are regarded as an important cause of congenital mental retardation. Recent publications show that chromosomal aberrations can also be associated with different types of seizure disorders. In one study 38% of patients with epilepsy and congenital abnormalities had chromosomal aberrations that were likely to be causative for the phenotype.
Oligo-based array CGH is implemented in our genetic service in order to reach a resolution for screening the whole genome that extends far beyond routine cytogenetic analysis. We perform array CGH using 44k or 105k 60mer oligonucleotide arrays (Agilent Technologies) with a resolution of about 100kb or 40kb, respectively. Usually the indication for array CGH analysis in our laboratory is

syndromal mental retardation. Since epilepsy is often combined with mental retardation, we found some cases with chromosomal imbalances and epilepsy in our patient cohort. Selected cases will be presented.
Epilepsy is a genetically poorly characterized neurological disorder with many different genes involved, a lot of them still unknown. Therefore array CGH can be a valuable tool for the genetic analysis of patients with seizure disorders and for the identification of new genes associated with epilepsy.

P028

Frequency of large genomic deletions and genotype-phenotype update in 80 families with Juvenile Polyposis Syndrome (JPS)

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Background: Germline point mutations in the SMAD4 and BMPR1A genes have been reported in around 40% of patients with juvenile polyposis syndrome (JPS); the frequency of large genomic deletions in the SMAD4 and BMPR1A genes was unknown.
Methods: Mutation and phenotype analysis was used in 80 unrelated patients of whom 65 met the clinical criteria for JPS (typical JPS) and 15 were suspected to have JPS.
Results: By direct sequencing of the two genes, point mutations were identified in 30 patients (46% of typical JPS). Using MLPA, large genomic deletions were found in 14% of all patients with typical JPS (6 deletions in SMAD4 and 3 deletions in BMPR1A). Mutation analysis of the PTEN gene in the remaining 41 mutation negative cases uncovered a point mutation in 2 patients (5%). SMAD4 mutation carriers had a significantly higher frequency of gastric polyposis (73%) than did patients with BMPR1A mutations (8%) ($p<0.001$); all 7 cases of gastric cancer occurred in families with SMAD4 mutations. SMAD4 mutation carriers with gastric polyps were significantly older at gastroscopy than those without ($p<0.001$). In 22% of the 23 unrelated SMAD4 mutation carriers, hereditary hemorrhagic telangiectasia (HHT) was also diagnosed clinically. The documented histologic findings encompassed a wide distribution of different polyp types, comparable with that described in hereditary mixed polyposis syndromes (HMPS).
Conclusions: Screening for large deletions raised the mutation detection rate to 60% in the 65 patients with typical JPS. A strong genotype-phenotype correlation for gastric polyposis, gastric cancer, and HHT was identified, which should have implications for counselling and surveillance. Histopathological results in hamartomatous polyposis syndromes must be critically interpreted.

P029

Microcephaly and congenital heart defect due to 8p23.1 microdeletion and review of the literature

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Deletions of chromosome region 8p23.1 lead to microcephaly, mental retardation, congenital heart defects and genitourinary anomalies. We present two patients with a microdeletion in this region detected by microarray CGH (Agilent Oligo CGH array 105K and 244K respectively). Patient 1 is a two-year-old girl with microcephaly, a flat occiput, bitemporal narrowing, developmental delay, a complex heart defect (Ebstein anomaly, ventricular and atrial septal defect and persistent left superior vena cava), left duplex kidney and gastroesophageal reflux. Parents complain about the hyperactive, impulsive behaviour. Patient 2 is a 14-year-old boy of consanguineous Turkish parents. He also has microcephaly, mental retardation, hyperactive impulsive behavior during childhood, sleep

disturbances, a congenital heart defect (atrial septal defect and persistent left superior vena cava), glandular hypospadia and high arched palate. Both patients share minor facial anomalies including synophrys, long prominent eyebrows, thin upper lip and smooth philtrum. The interstitial deletion is spanning in patient 1 from 7.26 to 12.08 Mb (size: 4.8 Mb) and in patient 2 from 8.14 to 11.90 Mb (size: 3.8 Mb) on chromosome 8p23.1. Both deletions comprise the GATA4 gene. This zinc finger transcription factor is involved in heart formation during embryonic development. Haploinsufficiency of GATA4 has been implicated to be the cause of congenital heart defects in this deletion syndrome. The multitude of case reports in the literature suggests this microdeletion to be a major genetic cause in patients with congenital heart defects and microcephaly, although reliable epidemiological data is still lacking.

P030

Screening of 296 individuals with mental retardation for subtelomeric rearrangements by quantitative PCR

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According to the literature, subtelomeric rearrangements are causative for unspecific mental retardation in 0,5-16,5% of the patients (Rooms et al. 2005). For subtelomeric screening, we established a method using quantitative PCR (qPCR).
We reviewed the clinical data and qPCR results of 296 screened individuals that were referred to our Institute due to unspecific mental retardation with or without dysmorphisms during January 2003 and April 2007. All patients included in the study had a normal karyotype. A clinical score for each patient was calculated according to the de Vries checklist (de Vries et al. 2001).
In 17 of our 296 patients (5,74 %) we detected subtelomeric rearrangements. Among these 17 patients 3 subtelomeric rearrangements were classified as polymorphisms because they were also detected in the patient's phenotypically normal mother or father.
In 6 patients (2,03 %), the subtelomeric rearrangement occurred de novo. These aberrations were classified as causative for the phenotype. In 8 cases a differentiation was not possible as parents were not available for screening. If these aberrations could be verified as causative, the rate of pathologic subtelomeric deletions and/or duplications would rise to 4,73 %. All subtelomeric aberrations were verified by FISH. Therefore, an approach with qPCR can be considered as a rapid and versatile method for the detection of subtelomeric rearrangements.
Two out of the six patients with a causative subtelomeric aberration had a de Vries score of 0 points, the mean score of all included patients was 2,55 points.
Our data indicate that the deVries score is not suitable in all cases for a preselection of mentally retarded patients for subtelomeric screening.

P031

Autosomal recessive cutis laxa due to a homozygous mutation in the Fibulin-4 gene

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Cutis laxa is characterized by redundant, inelastic skin with deep wrinkling. In addition a variable systemic involvement occurs frequently including elastic tissue abnormalities in blood vessels, lung and heart valves. Cutis laxa is found in a heterogenous group of acquired and genetic disorders. Inherited forms are very rare. Autosomal dominant and recessive as well as X-linked inheritance patterns were described. Mutations in Fibulin-4 and Fibulin-5 were found to be causative for autosomal recessive forms in few cases. All known cutis laxa genes play an important role for elastic fiber formation. Fibulins show various interactions and are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular extracellular matrix structures.
We identified a homozygous missense mutation (p.C267Y) in the Fibulin-4 gene in a patient with cutis laxa. The female proband was the first child of healthy consanguineous parents of Iraqi descent with non contributing family history. Pregnancy was remarkable for fetal overgrowth determined by ultrasound examinations and oligohydramnios noted at 32 weeks of gestation, which led to Caesarean section two weeks later. The newborn girl showed extreme bradycardia and died within a few hours. Apart from overgrowth, cutis laxa, arachnodactyly of hands and feet with contractures of the third to

fifth fingers, medial rotation of feet, spina bifida of the os sacrum and facial dysmorphic features were noted.
Autopsy showed collapsed lungs with hypoplastic diaphragm and signs of cervical soft tissue bleedings due to fragility of vessels. Histologic examination showed fragmentation of elastic fibers with formation of cystic cavities in the medial layer of the aorta and central lung vessels.
Our observation extends the phenotypic spectrum of Fibulin-4 mutations by overgrowth and arachnodactyly.

P032

Phenotypic spectrum and novel mutations in patients with Cohen syndrome

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Cohen syndrome is characterised by mental retardation, postnatal microcephaly, facial dysmorphism, pigmentary retinopathy, myopia, and neutropenia. Mutations in COH1 have been found in ethnically diverse cohorts of patients. We carried out mutation analysis in 12 novel patients with Cohen syndrome from 9 families. In this series, we identified 13 different COH1 mutations, 12 are novel, including 6 frameshift mutations, 4 nonsense mutations, 2 splice site mutations, and one codon deletion. Eleven of these novel mutations, including one confirmed splice mutation, are predicted to result in a premature stop of protein synthesis causing truncated COH1 protein or in degradation of COH1 mRNA by NMD. The second splice site mutation is also expected to result in a premature stop or a larger deletion through exon skipping or activation of a cryptic splice site. Consistent with previously reported data, the clinical presentation in this series of patients was highly variable. Mental retardation of varying degree, myopia, retinopathy (in patients older than 5 years), and typical facial aspect were present in all of them. The majority had microcephaly, truncal obesity, and short stature. While mild neutropenia was exclusively documented in Caucasian patients, our data confirm that patients from outside Europe were often found to have a normal neutrophil count. While abnormal ocular findings confined to the posterior segment were frequent, anomalies of the anterior segment such as subcapsular cataracts and lens subluxation were rarely reported in patients aged over 40 years and were found in affected siblings included in this study. Kyphosis/scoliosis present in 3/12 patients as well as syndactyly observed in 2/12 patients were not frequent findings in Cohen syndrome. The clinical information and the results of the molecular analysis of COH1 on patients with Cohen syndrome, presented here, enabled us to provide evidence of extended clinical and mutational spectra.

P033

Idiopathic epilepsy: Diagnostic of the SCN1A gene

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Idiopathic epilepsies represent ~40% of the epilepsy spectrum, ~2% of which follow a monogenetic inheritance. In the largest group of people with non-Mendelian form of epilepsy, the genetic influence in risk might consist mainly of complex disease genes. Even in epilepsies in which a causative gene exists, the pattern of inheritance and its analysis is sophisticated. In some epilepsies genes/mutations identified so far raise risk dramatically, but penetrance is incomplete with even asymptomatic family members or mutations in different genes have been found to cause the same phenotype. So far, about ~12 genes for □single gene□ epilepsies have been identified, mostly encoding ion channels joining the epilepsy phenotype to the channelopathies. Ion channels are critical for neuronal excitability and, therefore for the delicate balances that maintain electric stability in the CNS.
Here, we present the diagnostic of the SCN1A gene in 18 children suffering from GEFS+ type 2 (n=3), and SMEI (n=15). Both phenotypes belong to the spectrum of GEFS+. In ~15% of familial cases, GEFS+1/2 is inherited in a monogenic autosomal dominant pattern of inheritance, for the vast majority a complex inheritance is evident. Whereas GEFS+2 is a relatively mild form of the GEFS+ spectrum and therapeutic strategies work well, SMEI (Dravet syndrome) is the most severe phenotype with a

poor outcome. In 33-100% mutations (different studies) in the SCNA1 gene in SMEI are de novo. In this study, we did not detect mutations by DGGE, sequencing and MLPA analysis in our GEFS+2 patients, but in 4/15 SMEI cases (all de novo) including small deletions, truncating and missense mutations, mostly located in the pore and voltage sensitive region of the SCN1A protein.
The molecular genetic diagnostic of epilepsies particularly in children is challenging, but will facilitate the early diagnosis, prognosis and also therapeutic strategy.

P034

CHANDS: a rare ectodermal dysplasia-subtype

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We report on two siblings, a girl and a boy, affected by CHANDS (= Curly Hair-Ankyloblepharon-Nail Dysplasia-Syndrome), a rare subtype of ectodermal dysplasia. The nine years old girl exhibits a surgically corrected bilateral cleft lip-palate, curly scalp and sparse body hair, a broadened nasal bridge, a narrow jaw with conical teeth, alveolar synechies, hyperpigmentation and hyperkeratosis, increased palmar and plantar sweating, dystrophic nails but no heart or genitourinary anomalies. Eyelid-adhesions at birth and vaginal-adhesions in babyhood were reported by the mother. Mental development is normal. Moleculargenetic analysis in the so far known AEC-causative p63-gene was performed for the girl for the purpose of differential diagnostics and showed no mutation. The four years old boy has a milder phenotype than his sister as the cleft lip-palate is only unilateral. All the other symptoms are similar. The mother exhibits commissural lip pits suggesting autosomal dominant inheritance with variable expressivity. So far CHANDS has been thought to be most likely inherited in an autosomal-recessive pattern.

P035

Interstitial 7q21.3 deletion including the SHFM1 locus in a patient without split hand/foot malformation

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We report on a newborn girl with low birth measurements (birth at term, 2670g, 47cm, OFC 32cm, all below 3rd cent.), minor facial dysmorphism (upslanting palpebral fissures, small dysplastic ears with overfolded helices), a simian crease on her left hand, and a partial longitudinal nail aplasia of the radial part of her right index finger but otherwise normal hands and feet (including normal X-ray of the right hand). GTG-banding showed a complex translocation between chromosomes 2, 18 and 20 that seemed cytogenetically balanced: 46,XX,t(2;18;20)(q21.3;q11.2;p11.2). To clarify whether submicroscopic deletions or duplications in the breakpoint regions could be responsible for her phenotypic changes, a genome-wide oligo array CGH analysis was performed (Agilent Human Genome CGH Microarray 244A). This revealed normal results for the breakpoint regions. Surprisingly an interstitial deletion on the long arm of chromosome 7 in 7q21.3 was found (confirmed by qPCR). Translocation and deletion could be excluded in her mother, the father is not available for investigation. The detected deletion comprises 2.38 to 2.46 Mb in size and contains approximately 11 genes, among them the SHFM1 locus with the candidate genes for split hand/foot malformation type1 (SHFM1, DLX5 and DLX6). So far, all but one patient (Tzschach et al., 2007) with a deletion in this region had split hand/foot malformation which contributed to mapping of the SHFM1 locus. Because of a normal hand/foot phenotype in his patient, Tzschach proposed a region 300kb further distal to the locus might be responsible for SHFM1 phenotype. Although the deletion in our patient comprises the SHFM1 locus including the critical region proposed by Tzschach and spans even another 400kb further distal, she has no split hand/foot phenotype. The absence of limb malformations despite haploinsufficiency of the deleted SHFM1 candidate genes might either be caused by reduced penetrance or due to other factors that remain to be solved.

P036

Omani type spondyloepiphyseal dysplasia caused by a new missense mutation in CHST3

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We report a large inbred kindred with a spondyloepiphyseal dysplasia from Turkey with short stature, severe kyphoscoliosis, mild heart defects, brachydactyly with shortening of the metacarpals 3 and 4, and normal intelligence. A genome wide scan using the 250K Affymetrix SNP chip revealed a single locus for homozygosity on chromosome 10q23. This interval includes an obvious candidate, Chondroitin 6-O-sulfotransferase-1 (C6ST-1) gene(CHST3), previously shown to be mutated in Spondyloepiphyseal Dysplasia (SED) Omani type. SED Omani type is a novel type of chondrodysplasia characterized by severe progressive kyphoscoliosis, short stature, premature arthritits, and contractures. Focusing on CHST3, we amplified the coding region of the CHST3 and identified a homozygous missense mutation (T141M) in the exon 3 of the CHST3 gene in all three of the affected members of the family. Using recombinant C6ST-1, it could be shown that the identified missense mutation reduced the activity of C6ST-1 to 24-29%. This is the second description of SED Omani type further supporting this skeletal dysplasia as a distinct clinical entity. The clinical features of the here described family are somewhat different from the previously described cases making the initial diagnosis difficult. The differences in clinical outcome are likely to be due to differences in the nature of the mutation. The original SED Omani mutation resulted in a complete loss of function whereas T141M appears to have residual function. The phenotypical differences of the two families with different CHST3 missense mutations are discussed.

P037

Oligonucleotide array-CGH in postnatal cytogenetics - Kiel experiences

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High-resolution array-CGH (aCGH) allows the detection of small, cytogenetically unresolveable chromosome imbalances in euchromatic chromosomal segments. We have performed aCGH for the detection of constitutional imbalances in 129 patients with normal karyotype and developmental delay and/or various malformations, dysmorphisms, or complicated epilepsy of unknown etiology using initially 44K and later on 105K or 244K oligonucleotide arrays (Agilent). In 23/129 patients euchromatic imbalances have been detected (~18%). In 21 of these 23 patients, the imbalance has already been verified by FISH. In 7/16 cases, in which parental samples were available, the detected imbalance has been transmitted maternally or paternally (3 deletions: 0.4 Mb-1.9 Mb; 4 duplications: 1.3 Mb-5.3 Mb). Overall, in 14/129 patients (~11%) a de novo aberration or an aberration consistent with an already known phenotype could be detected: one unbalanced translocation, two duplications (sizes 2,8 and 3,9 Mb, respectively) and 11 deletions (size range: 0,6 Mb-7,7 Mb). In addition to the 129 patients, 19 patients were analysed to further characterize a chromosomal aberration detected by conventional cytogenetics. In 9 of these cases (~47%), including 5 complex translocations, 2 ring chromosomes and each one interstitial deletion and add(X)-case, the imbalance could be characterized in more detail using aCGH. In 8 of the 19 samples no euchromatic deletion or duplication could be observed by aCGH using whole genomic DNA. Hybridization of microdissected chromosome material onto a 105 K array allowed fine mapping of a supernumerary ring chromosome 19 present as mosaic. In two patients with a cytogenetically visible disorder aCGH revealed an additional aberration on a different chromosome. In summary, aCGH and array-hybridization of microdissected chromosomes are highly informative methods for the detection and characterization of chromosomal aberrations complementing postnatal cytogenetics.

P038

Identification of a rare duplication 13q by oligo-array-CGH in a male with mild dysmorphic signs

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We report about a 50 year old man with mild mental retardation, cataract, dysmorphy of the oral cavity with malformation of the teeth. Conventional cytogenetic analysis first showed a normal male karyotype. Because of the phenotype we investigated the patient by 105k oligo-array-CGH. This analysis revealed an 11 Mb interstitial duplication of the region 13q14.1->13q21.1. Retrospective we could diagnose this duplication by comprehensive high resolution G-banding. We confirmed the duplication by FISH analysis with DNA probe LSI 13q14 (Vysis) containing the Retinoblastoma gene locus (RB1, 13q14.2). The reported chromosomal aberration is rare described in the literature. Duplication of exactly this specific segment has not been reported in the literature yet. Most patients having a duplication including the region 13q14 showed a smaller duplication than our patient. All those patients described up to now present normal intelligence and minor dysmorphic features. We demonstrate patients with similar duplication segments in comparison of their phenotype. This case shows the importance of searching for interstitial chromosomal imbalances in patients with mild mental retardation and conspicuous phenotype by array-CGH.

P039

A novel frameshift mutation of TRPS1 in a family with tricho-rhino-phalangeal syndrome type I

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The tricho-rhino-phalangeal syndrome (TRPS) is a rare autosomal dominantly inherited disorder. Three types have been described. TRPS I is characterized by thin and slowly growing hair, a pear-shaped nose, a long flat philtrum, short stature, brachyphalangy with deformation of fingers and cone-shaped epiphyses.
The gene TRPS1 consists of 7 exons and encodes a zinc-finger transcription factor. Mutation analyses have revealed high heterogeneity.
We report on a new frameshift mutation in the TRPS1 gene in two sisters and their mother from a family with TRPS I. The mother and her two children had slowly growing and sparse scalp hair with recessed fronto-temporal hairlines, medially thick eyebrows, a bulbous tip of the nose, a flat broad philtrum, thin upper lip, and shortening of phalanges. In the mother and the older daughter radiographs showed cone-shaped epiphyses. Height of the mother is 155 cm.
Cytogenetic analyses revealed normal female karyotypes 46,XX in all three patients. Molecular investigations identified a 2-bp deletion in exon 5 of the TRPS1 gene: c.2479_2480delCT, with consequent frameshift from codon 827. Due to a premature stop codon at the novel amino acid position 829 protein biosynthesis results most likely in a truncated protein (p.Leu827LysfsX3). The predicted gene product lacks the two nuclear localization signals as well as the C-terminal GATA-binding and IKAROS-like zinc fingers, which are necessary for normal function of the TRPS1 protein. Therefore a disease causing effect of this mutation can be postulated.

P040

X-linked Bruton agammaglobulinaemia in a girl with atypical Turner-Syndrom

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A 6 year old girl with the suspected diagnosis of rare autosomal-recessive agammaglobulinaemia was referred for cytogenetic diagnostic because she showed additional dysmorphic features.
Conventional cytogenetic analysis showed an atypical Turner syndrome with a Turner karotype (45,X) in 15 out of 16 analysed metaphases and one cell with a tiny ringchromosome X instead of the second X-chromosome. FISH analysis confirmed the mosaic (94 % of the cells showed only one signal for the X-centromere, 6 % of the cells contained 2 signals, in accordance with the portion of cells with the ring chromosome). The ring contains the complete short arm of the X-chromosome including the subtelomeric region, the bigger part of the long arm is lost with the breakpoint approximately in the distal region of band Xq22. It was suggested, that the girl may not be affected with the rare recessive

agammaglobulinaemia but with the more common Bruton agammaglobulinaemia.
The locus of the Bruton Agammaglobulinaemia is localized in the proximal region of Xq22. Obviously the retained X-chromosome contains a non functional allele of the gene while the ring chromosome contains a functional gene. This is in accordance with the girl having a weaker form of the disease. The diagnosis of Bruton agammaglobulinaemia could finally be confirmed by detecting the heterozygous mutation c.1025C>T (p.Arg288Trp) in exon 10 of BTK gene.

P041

Multi-copy amplification of the exon 2 of the dystrophin gene in a Duchenne muscular dystrophy patient: A new approach by oligo-array-CGH

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The Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are the most common inherited muscle disorders. The prevalence of DMD is generally estimated at 1:3500 male births.
Dystrophin gene deletions are found in 55% of patients with BMD and 65% of patients with DMD. Point mutations account for appr. 30% of mutations, while duplications can be found in 5-10% of DMD patients.
MLPA (multiplex ligation-dependent probe amplification) is now widely used as a reliable method to detect both deletions and duplications of dystrophin exons. Here we describe a 5 year old male patient from lithuania with suspected Duchenne muscular dystrophy. CPK test revealed a value of >10,000 iu/l.
Analysis of the dystrophin gene by multiplex PCR excluded deletions.
To detect duplications MLPA testing was performed. MLPA showed a single amplification of the exon 2 of the dystrophin gene. Surprisingly, calculation of the MLPA data revealed the presence of 4 to 5 copies of the exon 2. To validate this finding quantitative real-time PCR was performed giving also evidence for 4 to 5 copies of exon 2. MLPA testing of the patients mother demonstrated that the amplification was de novo.
To identify the exact range of the amplification a new approach by oligo-array-CGH was conceived. In short, a customer-designed 44k Oligo-Array (Agilent) was created with probes spanning the exon 2 of the dystrophin gene. In a next step the array-CGH data will provide a basis for the design of PCR systems for exact determination of the amplification limits.

P042

Phenotypic variability and pathogenesis of fetuses with neural tube defects (NTD) in the Meckel Anatomical Collections at the University of Halle, Germany

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The purpose of our paper is to re-evaluate the preparations of the Meckel Anatomical Collections with NTD and to discuss the terminology of NTD during and after the Meckel period. MRI, CT scanning and comparative genomic hybridization (CGH) were used to investigate these fetuses. In 1826, when Johann Friedrich Meckel the Younger (1781-1833) described hemicephalia, he had studied this condition for over 15 years and had assembled a collection of 24 specimens, which display this disorder to varying degrees concerning the preservation of the cranial vault and the cerebral hemispheres. Hemicephalia -today known as anencephaly- is classified as a NTD, and is almost always associated with malformations of the neuro- and viscerocrania. Based on the classification by closure site, proposed by Nakatsu et al. (2004), hemicephalia is a form of upper neural tube closure defect (NTD). Meckel was interested in malformations of both the cranium and the external parts of the head. Of the 24 hemicephalic fetuses discussed by Meckel, ten skeletons, two skulls, two integuments and two specimens preserved in alcohol are still available for study today. Various types

of NTD have been described, ranging from craniorachischisis totalis, craniorachischisis, holocranium, merocranium, encephaloceles, iniencephaly, and spina bifida to microforms with sacrococcygeal dysgenesis. The etiology is extremely heterogeneous and involves gene-gene, gene-environment and gene-nutrient interactions. However, it should be noted that the presence of the NTD fetuses in the Meckel anatomical collection merely reflects their lethality, rather than their prevalence. Here, we present essential material upon which Meckel and his medical students based their descriptions. Moreover, our re-evaluation of 88 preparations led to the discovery of a wide range of NTD forms. In the present paper, we discuss the fetuses with NTD under consideration of the pathogenetic mechanisms of neural tube closure.

P043

Weaver-like phenotype in a girl with partial trisomy 4p and partial monosomy 8p due to a cryptic translocation 46,XX.ish der (8)t(4;8)(p16;p23.1)

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In recent years, subtelomeric rearrangements have been identified as a major cause of idiopathic mental retardation and mental retardation syndromes. We report about a female patient with an overgrowth syndrome, particularly with features of Weaver syndrome. She was born after an uneventful pregnancy at the 37th week of gestation, birth weight was 3060 g (50-75%), length 51 cm (75-90%), CFC 32 cm (25%). After the first year her somatic data crossed the 97th percentile. Eruption of the teeth started at 4 months. At the age of 5 2/12 years her hight was 122 cm (>97%), weight 30,7 kg (>97), the CFC was 53 cm (90-97%). She had tick hair, mild hypertelorism, small palpebral fissures, coarse low pitched voice, highly arched palate, large ears, deep-set nails, prominent finger pads, hirsutism, generalized obesity, metatarsus adductus. Her psychomotor development was retarded corresponding to an age of 3-3 ½ years. Bone age was retarded by 1 ½ years. Metaphyses of the distal femora were not flared. Cranial MRT was normal. Chromosome analysis showed a normal female karyotype 46,XX. However, subtelomeric probes revealed a cryptic rearrangement resulting in partial trisomy 4p and partial monosomy 8p (46,XX.ish der (8)t(4;8)(p16;p23.1). NSD1 deletions and mutations were excluded. The parents showed a normal karyotype. We discuss the symptoms of the patient within the context of the underlying subtelomeric abnormality.

P044

Pierson syndrome caused by homozygosity for a LAMB2 mutation due to a paternal uniparental isodisomy of chromosome 3

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Pierson Syndrome (OMIM609049) is characterized by severe congenital nephrotic syndrome, a complex ocular maldevelopment with microcoria as the most prominent clinical feature, and variable neurodevelopmental deficiencies. This rare autosomal recessive disorder is caused by loss-of-function mutations in the LAMB2 gene. LAMB2 is located in 3p21 and encodes laminin beta 2 which is expressed in basal laminae of renal glomeruli, ocular structures, and the neuromuscular synapse, corresponding to the phenotype.
We report on a male newborn with typical features of Pierson syndrome, who was born to healthy non-consanguineous German parents. By routine chromosome examination a normal male karyotype (46, XY) was recorded. Direct sequencing of the LAMB2 gene revealed a homozygous mutation affecting the exon 10 splice donor (c.1405+1G>A). Investigation of parental DNAs, however, showed that the mutation was present in heterozygous state only in the father. By genotyping of microsatellite markers covering the entire chromosome 3, we found that the patient was homozygous at all loci and negative for the maternal allele, thus proving paternal uniparental isodisomy. Uniparental disomy (UPD) for chromosomes not undergoing genomic imprinting may be asymptomatic unless it unmasks a recessive disorder. Uniparental disomy of chromosome 3 has been reported in a few instances. Since the present patient exhibited no symptoms that could not be attributed to Pierson syndrome, we conclude that his phenotype is exclusively due to the homozygous LAMB2 mutation. Unusual genetic

mechanisms have to be considered if rare autosomal recessive conditions with very low carrier frequencies occur in children of non-consanguineous parents.

P045

Array-CGH investigations in patients with unclear syndromic nephropathies identifies a microdeletion in Xq22.3-q23 in a female patient with Alport Syndrome, mental retardation, and focal epilepsy

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Molecular cytogenetic techniques such as array-based CGH have been instrumental in the identification of microimbalances associated with syndromic phenotypes. In this study, patients with unclear syndromic nephropathies are being analyzed. So far, we investigated ten patients with unexplained syndromic nephropathies and additional clinical features, such as mental retardation, heart defects or growth abnormalities. Array-CGH analysis was performed with a whole-genome array with 8000 large insert clones providing an average resolution of <0.5Mb. In one 16-year old female patient that presented with microhematuria, glomerular proteinuria (0,5g/day), midface hypoplasia, mental retardation including severe speech impairment, senso-neuronal hearing loss, and focal epilepsy, we detected a microdeletion in chromosomal bands Xq22.3-q23. This deletion was verified by FISH, found to be de novo, uniallelic and 3.3Mb in size. A kidney biopsy was performed. Electron microscopy showed splitting of the lamina densa and a thin basal membrane, which is diagnostic for Alport syndrome. By cranial magnetic resonance and diffusion tension imaging, a severe neuronal migration disorder with double cortex formation and pronounced reduction of the fronto-occipital tract system was detected. In summary, in ten patients with unclear syndromic nephropathies, we identified a female with a contiguous gene syndrome at Xq22.3-q23. The microdeletion includes the X-linked Alport syndrome gene COL4A5, the mental retardation genes FACL4 and PAK3, and the X-chromosomal lissencephaly gene DCX associated with double cortex formation in girls, mental retardation and epilepsy. Thus, the phenotype observed in our female patient combines features of the AMME-complex (Alport syndrome, mental retardation, midface hypoplasia, elliptocytosis) with X-linked lissencephaly.

P046

Case report of a newborn with congenital manifestations of Loeys-Dietz-syndrome including severe musculoskeletal deformities

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After an uneventful pregnancy a boy was born with bilateral hyperextensive knee dislocation and clubfeet, contractures of the fingers and arachnodactyly. Postpartal ultrasound of the heart and cerebrum exhibited pulmonary stenosis and insufficiency as well as periventricular cysts. Ocular manifestations were excluded at that point. Artificial respiratory support was necessary for ten days. Three weeks after birth the findings on the pulmonary valve had disappeared, but elongation and dilation of the ascending aorta were found. At this point neonatal Marfan or Loeys-Dietz syndrome as well as congenital contractural arachnodactyly and Larsen syndrome were considered as possible diagnoses. Molecular testing was initiated starting with FBN1-gene, where no mutation was found. Genetic tests were not covered by the private health insurance, stating that “Marfan syndrome itself has no pathogenous value and cannot be treated causally, therefore molecular tests are irrelevant for therapy and concern only individual planning of life.” Thus further molecular testing could not be performed.
Reevaluation at the age of seven months revealed hypertelorism, macrocrania with frontal bossing and tendency to dolichocephaly, intermittent blue sclerae, retrognathia and an atrophic scar. In May 2007, Yetman et al. published a paper on neonatal manifestations of Loeys-Dietz syndrome,

resembling Larsen or Beals syndrome. With the analogy to this description, Loeys-Dietz syndrome was strongly suggested in our patient, although the characteristic symptom of tortuous arteries was only confirmed at the age of eleven months. Subsequently molecular confirmation was indispensable for further surgical approaches, and by testing of TGFBR2 gene the mutation R528H was detected. This mutation has repeatedly been described in association with typical Loeys-Dietz syndrome (type I) by Loeys et al., 2006. Prior to the boy's first birthday the aortic dilation (31mm) required cardiovascular surgery.

P047

Mild phenotypes in four patients with Cornelia de Lange syndrome (CDLS) due to novel splice site mutations in the NIPBL gene

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Cornelia de Lange syndrome (CDLS) is a multiple congenital anomaly syndrome characterized by a distinctive face with synophrys, reduction defects of the upper limbs, pre- and postnatal growth deficiency, microcephaly, mental retardation and behaviour problems. Both missense and nonsense mutations as well as small insertions and deletions within the NIPBL gene have been associated with CDLS. Phenotypes range from considerably severe to rather mild forms. We present four patients showing only mild features (synophrys, microcephaly, short stature) of CDLS. In these patients we identified four novel heterozygous base substitutions within intronic regions [IVS10+1, G>T; IVS19+4, A>G; IVS27-15, A>G; IVS36, del(-)13-(-)8]. By RT-PCR analyses we could show that all four substitutions result in aberrant NIPBL transcripts. By comparing the intensity of RT-PCR products of the wild type with the effected transcripts all aberrant products were clearly less intensive than the wild type. These unequal proportions might indicate that all four mutations provoke alternative splicing events for the mutated allele but do not completely eliminate the pre-mRNA splicing as described for the wild type allele. Since in all cases the aberrant transcripts were much lower expressed than the wild type, these hypomorphic alleles could explain the mild phenotypes of the patients.

P048

Extracolonic tumour spectrum in MUTYH-associated polyposis (MAP)

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MUTYH-associated polyposis (MAP) is an autosomal recessive adenomatous polyposis of the colorectum which is caused by germline mutations in the base excision repair gene MUTYH. MAP is associated with a colorectal cancer lifetime risk of up to 100%, comparable to familial adenomatous polyposis (FAP). To date, no systematic evaluation of extracolonic MAP manifestations has been published. Here we report on the extracolonic tumour spectrum in a large cohort of MAP patients, based on medical records and anamnestic information. The study is a collaborative European trial including 280 cases (185 index patients, 95 affected relatives). The median age at evaluation was 50 years (range 17-84). In 17% of the patients duodenal polyposis was reported; 29% had any kind of extracolonic lesion, 17% of these had at least one extracolonic malignancy:

Tumour	Frequency	Age at diagnosis
Breast cancer	7.6% (9/119 females)	45-78
Ovarian cancer	2.5% (3/119 females)	45, 56
Endometrial cancer	1.7% (2/119 females)	47, 54
Benign endometrial tumours	3.5% (4/119 females)	32-57
Skin cancer	4.6% (13/280)	30-71

Benign skin tumours	11.8% (33/280)	15-74
Lipoma	2.9% (8/280)	30-65
Gastric cancer	1.1% (3/280)	17-48
Duodenal cancer	0.7% (2/280)	56, 65

The preliminary analysis of the data indicates a slightly increased overall incidence of extraintestinal malignancies compared to the lifetime risk of the general population. Although no predominant tumour was observed, gynaecological malignancies (endometrium, ovary) and precursor lesions (endometrial hyperplasia) as well as duodenal cancer and cutaneous tumours might be more frequent in MAP patients compared to the general population. The tumour spectrum is wide with an overlap to HNPCC. The risk of breast cancer is close to the age-related female population risk.

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P049

Unusual clinical findings in a 4 year old girl with Fanconi Anemia complicated by hepatoblastoma

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Fanconi Anemia (FA) is a rare autosomal recessive condition associated with an abnormal response to DNA damage. Apart of the well-known susceptibility to pancytopenia, leukemia, and solid tumours, several phenotypic anomalies may suggest the correct diagnosis. We report on a 4 years old girl with a delayed diagnosis of FA due to an unusual clinical pattern. She was born at term and noted to be growth-retarded: weight 1900g (-4.32 SD), length 44cm (-3.16 SD), OFC 31cm (-3.62 SD). Prenatal sonography had shown polyhydramnios, mild internal hydrocephalus but amniocentesis detected no cytogenetic anomalies. Clinical investigation revealed anal atresia, bilateral hip dysplasia, bilateral vesicoureteral reflux, and microcephaly. No limb anomalies were detected. Mild hypothyroidism was treated with levothyroxine. At age 13 months, reinvestigation of the karyogram showed, apart of a normal constitutive karyotype, several unrelated chromosomal rearrangements. Regarding possible chromosomal breakage syndromes further investigation was recommended, but unfortunately postponed. At age 32 mo severe growth failure was diagnosed (73,2cm, -6.53 SD) and GH substitution therapy was initiated. The response to this therapy was unexpectedly high (13,6cm in the 1styear). At the age of 51 mo a large intrahepatic calcified liver tumor was detected. Alpha feto protein (AFP) was extremely high (103512µg/l), and biopsy confirmed the diagnosis of hepatoblastoma. Reexamination by a clinical geneticist suggested FA, which was finally proven by DEB-induced increased breakage rates and reunion figures. Conclusion. This case report seems to us of interest due to some unusual findings:
(1) the observation of hepatoblastoma which has not been reported so far in FA
(2) the unusual high response to growth hormone treatment
(3) the lack of first ray anomaly and
(4) the overlapping features to the VACTERL-H association suggesting that similar cases be tested for chromosomal breakage syndromes.

P050

Array CGH detection of genomic imbalances in 333 patients with unresolved retardation syndrome

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Genomic imbalances are a major cause of mental retardation and developmental delay in patients with congenital and developmental abnormalities. The detection rate of these imbalances using chromosome banding is approximately 5%. Small cryptic deletions in regions of known microdeletion syndromes as well as subtelomeric deletions and duplications are found in another 5-8% of these cases. An increasing rate of formerly underdiagnosed microduplications in regions of microdeletion syndromes has been detected recently using interphase FISH or MLPA studies giving rise to new microduplication syndromes. With the advent of whole genome array-CGH analysis, the number

especially of interstitial genomic imbalances increased dramatically. Here we present our results of array-CGH investigations in 333 cases of patients with retardation syndromes and dysmorphic features using the Cytochip v2 BAC array (BlueGnome, Cambridge). Among these cases we found 50 genomic imbalances (15%). Seven (2,1%) of the genomic imbalances were telomeric (3 deletions, 4 duplications) and 35 interstitial (10,5%). Of these interstitial imbalances 19 were >1 Mb in size (average 3,5) whereas 16 were smaller or equal to 1 Mb in size. Four aberrations included terminal deletion/duplication events (three de novo, one inherited from a balanced father). 8 cases (2,4%) were patients with known microdeletion or microduplication syndromes (two microdeletions 22q11.2, two microdeletions 17p11.2, two microdeletions 15q11.2q13, two microduplications 17p11.2). FISH has not been performed prior to the array CGH, as no obvious indication of the referrals existed. All of the detected gains and losses have been confirmed by either FISH or MLPA. A small 0,8 Mb deletion in the long arm of chromosome 2 (2q22.3) lead to the molecular genetically confirmed diagnosis of Mowat-Wilson syndrome. To summarize, array CGH is a very powerful tool to detect genomic imbalances in at up to 15% of previously unresolved cases.

P051

Identification and in-silico analysis of 14 novel GJB1, MPZ and PMP22 mutations

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Unrelated Austrian patients with Charcot-Marie-Tooth disease (CMT; n=250) were examined for the duplication on chromosome 17p11.2-p12 and for mutations in the GJB1, MPZ and PMP22 genes. The 17p11.2-p12 duplication was present in 79 patients (31.6%). In the remaining 171 CMT patients screening of the GJB1, MPZ and PMP22 genes revealed 28 mutations, 14 of which were novel (p.T4K, p.R32G, p.I52fs, p.M93K, p.E146A, p.S198A, p.S277fs in GJB1, p.I30S, p.Y33F, p.C50G, p.Q100X, p.R185fs, p.D224Y in MPZ and p.M111T in PMP22). The 17p11.2 duplication, GJB1, MPZ and PMP22 mutation frequencies were similar to those described in other studies of CMT patient collectives with different ethnical backgrounds. All missense mutations concerned highly conserved residues according to a ClustalW analysis, and were found to segregate with the disease in available families or occurred de-novo. We also used the PANTHER program, which calculates an evolutionary conservation score for missense variants, to predict the functional consequences of published and novel mutations, and polymorphisms in MPZ and PMP22. Notably, data regarding segregation in families, and investigation of control samples were not available for up to 50% of mutations in the CMT database. The PANTHER program scored all known polymorphisms as such, and ~90% of published mutations were classified as likely deleterious. Mutations either associated with axonal CMT or thought to result in loss-of-function were less likely to be classified as deleterious.

P052

Cutaneous angiomas and neurofibromatosis type I in a patient with microdeletion 17q11.2

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Vascular malformations represent a rare abnormality in the phenotypic spectrum of neurofibromatosis type I (NF1). The observation that angiomas in general occur more common in NF1 affected persons than in unaffected relatives was underlined by a cohort study and in very few case reports (Wertelecki et al., 1988).
We report on an 8 months old female infant affected with NF1 and a severe manifestation of cutaneous angiomas as well as a single liver angioma. We diagnosed NF1 due to the appearance of seven café-au-lait naevi (> 5 mm) and a discreet freckling in the axillar region. Angiomas appeared shortly after birth at various locations (chin, lower flank, thigh), and showed an accelerated growth behaviour. Besides innumerable small angiomas there were four large malformations up to 9 cm in diameter with a peduncle-like or broadly based connection to the skin.
Molecular cytogenetic analysis revealed a de novo microdeletion including the NF1 gene. No further

aberrations were detected in conventional cytogenetic analysis. As the child had Noonan-like facial features mutations in PTPN11, KRAS and SOS1 were excluded. Our report shows severe vascular malformations in a child with NF1 having a microdeletion 17q11.2. We hypothesize that the underlying mechanism for development of angiomas is similar to the development of neurofibromas, meaning that a second somatic hit of the NF1 gene leads to increased proliferative activity of the affected tissue. The difference in frequency between neurofibromas and angiomas may be either due to a tissue-specific preponderance for mutational vulnerability or due to another unknown genomic hit. Although, it is known that disturbance in the NF1-associated signalling cascade (MAPK) can result in misregulation of vascular factors, the underlying molecular mechanism for the development of angiomas in the child reported here remains to be elucidated.

P053

CGH-Array analysis in 5 patients with Filippi Syndrome reveals 2 different deletions on chromosome 2q: A putative locus?

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Here we report on an additional patient with Filippi syndrome. This is a rare autosomal recessive disorder, named after a medical geneticist who described three sibs with pre- and postnatal growth retardation, facial dysmorphic stigmata, mental retardation, microcephaly, and toe syndactyly (involving at least toes 2-3) Finger syndactylies were present in 2 of 3 sibs. Our patient had low normal birth measurements for length and weight, but had a head circumference on the 3. percentile. Until the age of 7 months, however, she had developed a micro- and brachycephaly (-3,1 SD) and was dystrophic (-2,5 SD). Due to severe feeding difficulties, nourishing a nasogastric tube was necessary. She also had syndactylies of the toes 2-5 on the left and 2-3 and 4-5 on the right side. The pattern of anomalies resembles the symptoms and signs of the patients in the original publication of Filippi (1985).
We performed a CGH-Array (44K chip, Agilent) analysis on DNA of our patient and found an 8,4 Mb deletion on 2q31. CGH analysis of 3 other patients (105K chip, Agilent) previously described in the literature did not reveal any abnormality. Interestingly, a further patient with Fillipppi - syndrome - like features (presented by Strenge et al during the GfH Congress in Bonn, 2007) had a 12,1Mb deletion on 2q22.3-24.1 (105K chip, Agilent). Since the 2 found deletions are not overlapping, we discuss the clinical similarities and differences between these two patients. We hope that further studies will help to pinpoint the mutant gene.

P054

Duplication of 20q13.2-q13.33 and partial deletion in 18q23 in a second patient with mental retardation and dysmorphic signs resulting from a submicroscopic unbalanced translocation: Clinical, cytogenetic and Array-CGH data

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We report on a 2 4/12-year-old boy with developmental delay (not yet independently walking, severe speech delay), hypotonia, umbilical hernia, cranio-facial dysmorphism (triangular face, telecanthus, strabismus, deep set eyes, prominent nose, deeply grooved philtrum, highly arched palate, malformed ears), and pigmentary anomalies of skin and hair. Brain magnetic resonance tomography showed a hypoplasia of the corpus callosum. Cardiologic examination revealed a mild pulmonary valve stenosis. A normal karyotype was found by cytogenetic analysis of lymphocytes and skin fibroblasts. By Array-CGH analysis using a whole genome oligonucleotide chip (35k OpArray V4 70mer Oligonucleotid Chip; Operon, Köln) we detected a deletion of chromosomal material originating from 18q23 as well as a duplication of chromosomal material from 20q13.2-q13.33. The deletion spans approx. 2 Mb and the duplication approx. 8 Mb of genomic material. Both aberrations were confirmed by FISH analysis, which, furthermore, revealed an unbalanced translocation between the long arms of chromosomes 18 and 20 [der(18)t(18;20)(q23;q13.2)]. Analysis of the parental chromosomes showed a normal

karyotype for the mother and father of our patient. However, the FISH analysis on metaphase spreads of the father revealed that he is the carrier of the balanced translocation t(18;20)(q23;q13.2).
The terminal deletion of the long arm of chromosome 18 causes one of the more common deletion syndromes. The majority of these deletions span larger regions compared to our patient. The literature review revealed one citation (Herens et al. 1990) with a similar constellation t(18;20)(q23.2;q13.2)mat resulting in a partial trisomy 20q. The symptoms of our patient will be discussed together with a review of the rare cases showing a partial duplication 20q13.

P055

Czech dysplasia: Clinical and molecular delineation of a novel COL2A1 disorder

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Czech dysplasia (OMIM 609162) is a novel type II collagen disorder which is phenotypically distinct from other COL2A1-related diseases. Czech dysplasia is characterized by early-onset progressive pseudorheumatoid arthritis, short third and fourth metatarsals, mild platyspondyly, normal height, and the absence of ophthalmological problems or cleft palate. The disorder is caused by a specific missense mutation (R275C, c.823C>T) in the triple helical domain of the COL2A1 gene. We report a large German family consisting of 11 patients who not only suffered from the typical features mentioned above, but who also had sensorineural hearing loss, a problem that has hitherto not been considered as a major feature of Czech dysplasia. Mutation analysis revealed the COL2A1 c.823C>T (R275C) mutation in all patients. This finding provides further evidence that Czech dysplasia is caused exclusively by the R275C mutation, which is a unique situation among the COL2A1 disorders, and indicates that this amino acid change leads to a specific structural alteration of type II collagen. The clinical and radiological data of this family and previously reported patients with the R275C mutation demonstrate a remarkably uniform manifestation of the pathological features and add hearing loss to the list of major problems of Czech dysplasia.

P056

Non-fixation of the intestine in a boy with tricho-rhino-phalangeal syndrome type II: Are the TRP syndromes more than hair and skeleton disorders?

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The hallmarks of the tricho-rhino-phalangeal syndromes (TRPS) are sparse scalp hair, a bulbous tip of the nose and cone-shaped epiphyses of the phalanges. In addition, patients with the TRPS type II, also known as Langer-Giedion syndrome, have multiple cartilaginous exostoses. All patients with TRPS II are hemizygous for at least the 2.8 Mb interval from *TRPS1* through *EXT1* in 8q24.1, which also includes several other genes/loci. Here, we report on a boy with TRPS II and a 9 Mb interstitial deletion, which included the *TRPS1* - *EXT1*-interval. The boy died at the age of nearly five years of heart failure. Upon autopsy, the duodenum was found heavily dilated. The small intestine was attached to the abdominal wall by only a 3-cm stalk, which was completely rotated leading to crushing of all vessels and the decline of the intestinal wall. The mesentery of the large intestine wasn´t fixed either. Anamnestic, the boy had complained about stomach ache only a few times during life but ailments always meliorated spontaneously. Ultrasound examinations of the abdomen had been inconspicuous.
Comparable malformations of the gastrointestinal tract in patients with TRPS II have so far only been described in a female fetus with an interstitial deletion in 8q23 (Ariel *et al*. 1994). However, in the internet forum of the American TRPS support group, the mothers of three girls with TRPS II report on Ladd operations performed to correct the intestinal malrotation in their babies. In addition to these life threatening intestinal anomalies, several malformations of the urogenital tract in patients with TRPS have been described (Kozlowski *et al*. 1977, Partington *et al*. 1991, Wilson *et al*. 1983, Ramos *et al*. 1992, Fryns 1997, Graybeal *et al*. 2005, and pers. comm.) Thus, malformations of inner organs may constitute another, even though rare component of the TRP syndromes.

P057

A 4.8 Mb microdeletion in 10q23 encompassing PTEN and BMPR1A in a child with juvenile polyposis of infancy

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Background: Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder characterised by multiple juvenile polyps of the gastrointestinal tract and caused by germline mutations in the SMAD4 or BMPR1A genes. Few reports on cases of JPS with a very severe course and early onset of gastrointestinal symptoms have been published; this condition has been designated as juvenile polyposis of infancy. Recently, large genomic deletions of the contiguous genes BMPR1A and PTEN in chromosomal band 10q23 were identified as the underlying cause in some cases. Most of these patients exhibit extraintestinal symptoms typically seen in PTEN-hamartoma tumour syndrome.
Patient and results: We present an 11 year old boy diagnosed with colorectal polyposis at 2 years of age due to rectal bleeding and anaemia. At colonoscopy, 10 polyps were observed; during the following years multiple juvenile polyps in the stomach, small bowel, and colon were seen. At 7 years of age an invagination was treated. In addition, congenital macrocephaly, a subcutaneous lipoma, hypospadia, and pigmentation of the glans penis were observed. Psychomotor and language development were delayed. Family history was normal.
Screening for large genomic deletions using MLPA revealed a heterozygous germline deletion including the BMPR1A and PTEN genes, pointing to a loss of at least 1.2 Mb. By use of array-CGH the mutation was characterised as a 4.8 Mb deletion in 10q23.1-q23.31. Compared with published deletions our case showed an overlap of 1.8 Mb. Haplotype analysis suggests a paternal origin.
Conclusions: Severe infantile juvenile polyposis accompanied by extraintestinal abnormalities suggestive of PTEN mutations is a distinct phenotype caused by 10q23 microdeletions including both BMPR1A and PTEN in a substantial number of cases. However, clinical variability was described. To date, all known cases occurred de novo.

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P058

Barber-Say and Ablepharon-Macrostomia syndromes presumably represent one disorder

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Barber-Say syndrome (BSS, OMIM 209885) and Ablepharon-Macrostomia syndrome (AMS, OMIM 200110) are recognizable multiple congenital malformation syndromes which share similar patterns of organ involvement, including dermatological abnormalities, eye malformations, abnormal external ears and mild to moderate hearing loss, and genital malformations. Despite the extensive phenotypic overlap, the two syndromes have always been considered distinct disorders. Distinguishing features include ablepharon in AMS versus ectropion in BSS and marked hypertrichosis in Barber-Say syndrome. Genital abnormalities are much more severe in AMS. Here we report on a 7 year old girl with unequivocal features of the Barber-Say syndrome (marked hypertrichosis, redundant dry skin, macrostomia, thin lips, cup shaped ears, mild hearing impairment (>3 kHz, 25 to 35 db), hypoplastic nipples, and severely abnormal external genitalia. Additionally, our patient demonstrated a microblepharon. The karyotype was normal (46,XX) and CGH array analysis has been initiated. Ablepharon (i.e. absence of eyelids) and its milder variant, microblepharon, are typical features of the Ablepharon-Macrostomia syndrome. There has been no previous report of microblepharon in BSS. Thus, our case is the first demonstrating typical features of both, BSS and AMS. Previously, several authors have discussed whether BSS and AMS could represent different manifestations of one syndrome, and our observation is in support of this hypothesis.

P059

A 7,7 Mb deletion of 16p11.2-p12.2 in a 13 years old boy with severe speech delay

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We report on a boy who was born with low birth weight (2210g) after 38 weeks of an uneventful pregnancy as the third child of healthy parents. The postnatal period was complicated by hypotonia and poor sucking. Motor development was delayed. Crawling started at 18 months, walking at 23 months. At the age of three years, there was no speech development. Both, conductive and mild inner ear hearing loss was detected. Hearing aids were applied but speech development did not improve. Chromosome analysis with a resolution of 450 bands revealed an aberration on the short arm of chromosome 16 (46,XY,der(16)). Both parents had a normal karyotype.
Examination at the age of 13 years revealed mildly dysmorphic features: a long face with frontal upsweep of the hair, deep set eyes, a long nose with bulbous nasal tip, short and smooth philtrum, small mouth and clinodactyly. Mental retardation was moderate but the expressive language was severely impaired. He learned to interact with his parents by sign language. The behaviour was introverted. Microarray using the Sentrix® HumanHap 550 Genotyping BeadChip (Illumina) performed to clarify the chromosomal aberration on 16p. We appointed a 7,7Mb deletion with breakpoints at 16p11.1and 16p12.2 (chr16:21,512,681-29.223.380). Our case confirmed the findings in four patients with deletion at 16p11.2-p12.2, recently reported by Ballif et al. (2007). Dysmorphic features, feeding difficulties, significant delay in speech development, and recurrent ear infections seem to be frequent signs of this deletion syndrome.

P060

22q13 deletion syndrome coincidentally identified by Array-CGH analysis in a patient with global developmental delay and minor dysmorphic features

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The 22q13 deletion syndrome is characterized by global developmental delay, hypotonia, absent or delayed speech, autistic-like behaviour, and dysmorphic facial features. The 22q13 monosomy is observed with simple chromosomal de novo deletions of varying sizes, unbalanced translocations, and ring chromosomes. Mosaic cases have also been described. Even though there have been a number of reports on this syndrome and a distinct phenotype has been described, prevalence of the condition is still unknown and it seems to be underdiagnosed; most cases are detected by FISH when the velocardiofacial syndrome is suspected or by array comparative genomic hybridization (aCGH) as part of a screening examination for mental retardation.
We report on a 2-year-old boy presenting with global developmental delay, delayed speech, minor dysmorphic facial features including mild ptosis, median sparing of eyebrows, and pointed chin, large and dysplastic ears, dermal sinus, large hands and feet, toe nail anomalies, and mild scoliosis. Cytogenetic examination (GTG banding at 550 band resolution) revealed a normal male karyotype 46, XY. By Array-CGH analysis using the OpArray V4 70mer oligonucleotide chip (Operon, Köln) a de novo deletion representing 7.3 Mb of genomic sequence within chromosomal bands 22q13.2-q13.33 was detected. Subsequent FISH experiments confirmed the deletion in this region. Thus, the 22q13.3 deletion syndrome should be considered as a differential diagnosis in patients with global developmental delay.

P061

Phenotypic diagnosis of von Willebrand Disease (vWD) and the value of genetic testing in two cases

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A 30-year-old patient with atypical von Willebrand disease (vWD) had, despite markedly decreased vWF parameters no severe bleeding symptoms during the last years. Agarose gel electrophoresis revealed an unusual smeary pattern despite the presence of all vWF oligomers. Genetic analysis of vWF gene revealed compound heterozygous point mutations leading to amino acid changes p.R760C

(responsible for a secretory defect) and p.R854Q (a reported mutation resulting in decreased FVIII binding). The combination of p.R760C and a vWD type 2N mutation had been reported previously and named vWD subtype 2P (Casonato et al., Blood 101, 151, 2003). We conclude that such atypical vWD patients, as demonstrated in our case, may respond well to desmopressin.
A 32-year-old female patient with a phenotypically well characterized vWD type 2A sufferered from mucocutaneous bleedings since childhood. Significant laboratory findings were decreased vWF parametes with an abnormal multimer profile. PCR and DNA-sequencing were performed for the patient´s entire vWF gene in order to ascertain a possible compound heterozygous state. Mutation screening revealed two genetic alterations in a compound heterozygous state. These alterations included c.4883T>C which induces a p.I1628T substitution, and c.4430C>T which predicts a novel p.T1477I substitution. The patient´s child had similar vWF parameters as the mother. However, only the p.I1628T mutation was present in the child. Previously, neither functional effects of the mutation p.I1628T with respect to ADAMTS13-dependent proteolysis of vWF in vitro (Blood, 107, 2339, 2006) nor structural changes in a homology model for the A2 domain (J Mol Model, 10, 259, 2004) were observed.These data suggest that the vWD phenotype in our patient is exclusively due to the p.I1628T mutation.
Our data of the two cases support the value of genetic testing in selected vWD patients.

P062

A 16.7 Mb deletion on 18q21.2-q22.2 in a patient with dysmyelination, muscular hypotonia and mental retardation

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Partial deletions of the long arm of chromosome 18 produce a highly variable phenotype. Mental retardation, short stature, foot deformities, midface hypoplasia, hypotonia, hearing impairment, strabismus, atretic or stenotic external auditory canals, and genito-urinary malformations are the most commonly reported features. We report on a 14 months old boy with muscular hypotonia, brachycephaly, dysmorphic facial phenotype (frontal bossing, broad nasal bridge), tapering fingers, short feet, poor differentiation of gray and white matter in brain MRI and mental retardation. Extensive neuro-metabolic investigations showed normal results. Cytogenetic analysis revealed an unbalanced de novo translocation t(16;18)(q22;q21.2) with a deletion of 16.7 Mb of the long arm of chromosome 18q21.2-q22.2 as determined by array-CGH.
The interstitial 18q deletions are rare and vary in size, but proximal breakpoints are most commonly within bands 18q21.2 to q22.2. Investigations of the individuals with interstitial 18q deletions have further narrowed the critical areas for certain features. Abnormal cerebral myelination was reported to be associated with a small deleted region at 18q23 [Linnankivi et al., 2006]. In our patient the typical white matter abnormalities are present without haploinsufficiency of the assumed region. Typical brain MRI findings with abnormal myelination may lead to suspicion of a neuro-metabolic cause. In these cases, if also some of the other clinical features are fulfilled, the diagnosis of 18q deletion may be established using first 18q subtelomeric FISH and, secondly, molecular analysis.

P063

Interstitial duplication of chromosome 3p14.2~14.3 creates a specific phenotype with mental retardation, facial dysmorphism, cerebral and skeletal anomalies

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A boy with a karyotype 47,XYY had been referred for genetic diagnostics because of moderate mental retardation and skeletal anomalies. A talipes equinovarus had been surgically corrected.
Visible anomalies were short fingers, a simian crease, bilateral club feet, clinodactyly with the 2nd toes overcrossing the 3rd toes, and a funnel chest. An inguinal hernia and maldecensus testes had been operated. Features included epicanthic folds, down-slanting palpebral fissures, bushy eyebrows, protruding lips, a long, beaked nose, low set, anteverted ears, and low frontal and posterior hairlines. His growth had been normal. No severe impairment of his general health or any developmental

regression had occurred. Hearing and visual impairment as well as cardiac and renal malformations had been ruled out.
MRI-scan of the skull revealed demyelinisation of the left dorsal parahippocampic gyrus and an abnormal configuration of the posterior cranial fossa.
The parents were nonconsangouineous. The boy had a healthy older brother. All had a regular karyotype and facial features distinct from the patient.
Genetic analysis included FISH subtelomeric screening, sequencing for PTPN11 and SOS1 mutations, and repeated karyotyping. Eventually, duplication in the short arm of chromosome 3 was detected. This was narrowed down to the region 3p14.2~14.3 by reverse FISH analysis.
We are strongly convinced that this duplication is the cause of the features, while the additional Y chromosome is an innocent but irritating concomitant finding.
Our case shows striking facial similarity to case 1 described by Antonini et al in 2002, who had a duplication 3p12-21. This boy also had congenital bilateral clubfeet. The region duplicated contains the Filamin B gene which is in involved in joint formation and whose triplicate dose is probably the reason for the talipes and the clinodactyly. We presume that duplication 3p14.2~14.3 creates a very specific phenotype.

P064

MKS3 mutations in a family overlapping Joubert and Meckel-Gruber syndrome

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Meckel-Gruber syndrome (MKS) is an autosomal recessive multisystemic disorder characterized by occipital encephalocele, postaxial polydactyly, cystic kidneys, and ductal plate malformation (congenital hepatic fibrosis with hyperplastic biliary ducts). Survival beyond birth is unusual with the vast majority of cases dying in utero. MKS is considered to be the most frequent syndromic cause of neural tube defects. Recently, direct genetic testing has become feasible by the identification of four different MKS genes for which we all offer linkage analysis and sequencing. MKS proteins colocalize at the primary cilium putting MKS on the list of ciliary disorders (ciliopathies). Interestingly, MKS3 was recently shown to be causative for some patients with Joubert syndrome (JBTS) too. Like MKS, Joubert syndrome is a genetically heterogeneous, autosomal recessive ciliopathy. However, in contrast to MKS, JBTS patients usually survive and present with a mid-hindbrain malformation (molar tooth sign = cerebellar vermis hypo-/aplasia, thick maloriented superior cerebellar peduncles, and abnormally deep interpeduncular fossa), developmental delay, and further “cerebellar” features. Other variables include retinal dystrophy and renal anomalies. Here we compile clinical and genetic data of all MKS3 cases described so far and present own unpublished families which further expand the associated phenotypic spectrum. Among these cases is a compound heterozygous fetus with a novel MKS3 splice mutation that provides striking evidence for the clinical and genetic overlap between Meckel-Gruber and Joubert syndrome.

P065

CHARGE syndrome: Report of 14 novel mutations and a familial case caused by a large *CHD7* duplication

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CHARGE syndrome (OMIM#214800) is an autosomal dominant malformation syndrome characterized by choanal atresia or cleft lip/palate, ocular colobomas, cardiovascular malformations, retardation of growth, ear anomalies and deafness. To date more than 100 *CHD7* mutations have been described in CHARGE syndrome patients. Here we describe 15 novel CHD7 mutations found in 15 German patients referred for molecular confirmation of the suspected clinical diagnosis of CHARGE syndrome. Of the novel mutations, 8 are frameshift, 2 nonsense, 3 splice and 1 missense mutations. We also describe a family in which one unaffected man fathered three children with CHARGE syndrome with two different women. None of the parents showed any minor sign of CHARGE syndrome. All affected children shared a duplication of 57,6-63,3 kb spanning from intron 5 to exon 31 of the *CHD7* gene

detected by qPCR, oligo array CGH and MLPA. The parents were negative for the duplication. Targeted array CGH for precise mapping of the duplication is under way.

P066

Centromere activity in dicentric small supernumerary marker chromosomes

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Dicentric small supernumerary marker chromosomes (sSMC) constitute about 70% of this rare chromosomal disorder. According to the current theories in a dicentric chromosome one of the two centromeres is inactivated. However, up to present only singular studies are available concerning their centromeric activity. Here we present the first pilot study for a more systematic approach. 22 cases with dicentric sSMC were studied by two CENP-specific antibodies. CENP-B stained active and inactive, CENP-C only the active centromeres. sSMC were derived from #14 (4x), #15 (14x), #18 (1x) and #22 (3x). Surprisingly, neither the expected, nor uniform results were obtained. Overall, 7/22 'dicentric' sSMC showed only one signal immunohistochemistry, as the break and fusion point was within or adjacent to the alpha-satellite region of the corresponding chromosome. 3/22 dicentric sSMC had one active and one inactive centromere, as to be expected. One case with al large sSMC - an inv dup(15)(q13) - had two active centromeres in all analysable 13 cells. The remaining 10 cases showed sSMC with only one or two active centromeres. An example for the discordance of the results is shown exemplarily for the four studied cases with an inv dup(14)(q11.1) in Tab. 1. It is discussed that the centromeric activity also is correlated with heterochromatization of the centromere-near region. Thus, the obtained results might be an explanation for divergent clinical outcomes correlated with genetically similar sSMC. The obtained results need to be investigated on more dicentric sSMC, however, first evidence on epigenetics involved here might be provided already by the present study.

Case	1 active cen	2 active cen
072	80%	20%
100	100%	100%
115	80%	20%
176	12%	88%

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P067

A new probe set for the characterization of centromere-near rearrangements

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The centromere-near region of the human genome has not yet studied systematically by means of molecular cytogenetics. In the last 5 years two probe sets suitable for FISH (fluorescence in situ hybridization) were established: subcentromere-specific (subcenM-FISH, Starke et al., 2003, Hum Genet 114:51-67) and a combination of subcentromeric and subtelomeric probes (subCTM-FISH - Gross et al., 2006, Cytogenet Genome Res 112:67-75). However, both probe sets were only available chromosome-specific, i.e. information on the centromere-near region could only be obtained for one chromosome in a single experiment. Here we present a new probe set suitable to obtain information on previously cryptic centromere-near rearrangements for all 24 human chromosomes after one FISH experiment. This all-chromosomes directed subcentromeric probe set (= ACM-FISH) is introduced and the possibilities of its application are presented and discussed.

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P068

The hierarchically organized splitting of chromosomal bands for all human chromosomes

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Here we present for the first time the biological nature of hierarchically organized splitting of chromosomal bands for all human chromosomes. To do this, multicolor banding (MCB) probe-sets for all human chromosomes were hybridized to normal metaphase spreads of GTG-band levels at ~850, ~550, ~400 and ~300. The hierarchically organized splitting of bands into sub-bands was analyzed by comparing the disappearance or appearance of MCB-pseudo-color bands of the four different band-levels according to Lehrer et al. 2004 (Cytogenet Genome Res 105:25-28). In summary, it is a general process that only Giemsa-dark bands split into dark and light sub-bands, as this has now been demonstrated previously by chromosome stretching. Thus, the concept of chromosomal region-specific protein swelling gets even more evidence (Claussen et al., 2002, Cytogenet Genome Res 98:136-146). Moreover, we could show that the biological band splitting is in >50% of the subbands different than implemented by the ISCN nomenclature. This finding may be an explanation why mapping data from the human genome project do not always fit to the cytogenetic gene mapping data. Overall, the present study enables a better understanding of the chromosome architecture.

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P069

Another case of a small supernumerary marker chromosome only stainable by DNA derived from itself

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Small supernumerary marker chromosomes (sSMC) are a heterogeneous group concerning their chromosomal origin, their shape and structure and their clinical effects. Among sSMC-carriers about 70% show clinical signs. Only one sSMC cases stainable exclusivly by DNA derived from itself is reported by now (Mackie Ogilvie et al. 2001, Cytogenet Cell Genet 92:69-73). Here we present a second case with this characteristic. However, molecular cytogenetic comparison of both sSMC showed that they are not based on the amplification of similar DNA. To elucidate the origin of both sSMC we applied a new approach. We did characterization of sSMC by flow-sorting and subsequent cDNA-library construction followed by sequencing of the cDNA. The latter part of the analyses is presently in progress. However, from the first results we could conclude that in the sequence of our sSMC we found homology to transposon Mer1 type with a size of 135 bp. Thus, the sSMC would be constituted, at least in parts by a very frequently present repetitive unit of the human genome.

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P070

Molecular definition of high resolution multicolor banding (MCB) probes - first within the human DNA-sequence anchored FISH-banding probe set

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Fluorescence in situ hybridization (FISH) banding approaches are standard for the exact characterization of simple, complex and even cryptic chromosomal aberrations within the human genome since half a decade. The most frequently applied FISH-banding is the so-called multicolor-banding approach, also abbreviated as m-band, MCB, or in its whole genomic variant multitude MCB (mMCB). MCB allows the differentiation of chromosome region specific areas at the GTG band and

sub-band level and is based on region specific microdissection libraries, producing changing fluorescence intensity ratios along the chromosomes. The latter are used to assign different pseudo colors to specific chromosomal regions. Here we present the first BAC array-CGH mapped, comprehensive, genome wide human MCB probe set. All 169 region specific microdissection libraries were characterized in detail for their size and the regions of overlaps. In summary, the unique possibilities of the MCB technique to characterize chromosomal breakpoints in one FISH-experiment are now complemented by the feature of being anchored within the human DNA-sequence on BAC level.

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P071

Small supernumerary marker chromosomes (sSMC) in patients with a karyotype 45,X/46,X,+mar - 17 new cases and a review of the literature

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Small supernumerary marker chromosomes (sSMC) can appear in a numerically normal 'basic karyotype', but also in numerically abnormal one like in a 'Turner-syndrome karyotype' (=sSMCT). Here we present 17 new cases with such a karyotype mos 45X/46,X,+mar. Moreover we reviewed all available 512 cytogenetically similar cases from the literature and supply for the first time data on occurrence, shapes and subgroups of this rare cytogenetic entity. sSMCT are very rare in the common population (1:100000) - however, they can be observed 45 and even 60 times more frequent in infertile and (develop)mentally retarded patients, respectively. Even though sSMCT derive in >99% of the cases from one of the gonosomes, there are also exceptional reports on sSMCT derived from one of the autosomes. The majority of sSMCT(X) form ring-chromosomes, while most sSMCT(Y) are inverted duplicated / isodicentric ones. Albeit > 500 sSMCT are reported, only a minority of them has been characterized in detail for chromosomal breakpoints. Thus, more cases with detailed (molecular) cytogenetic marker chromosome characterization are needed to learn about formation and effects of an sSMCT.

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P072

Analysis of fragile sites and breakpoints in Fanconi anemia patients

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Fanconi anemia (FA) is a rare, autosomal recessively inherited chromosome breakage syndrome leading to early onset cancers and a cellular hypersensitivity to DNA-crosslinking agents. FA can be caused by different defects in the pathway responsible for DNA double strand breakage repair. So far, Mitomycin C induced chromosomal breaks in metaphases of FA-lymphocytes were examined exclusively on the cytogenetic level. These banding cytogenetic data suggests a co-localization of FA-breakpoint regions with known fragile sites (FS). FS are chromosomal regions showing a high incidence of gaps and breaks in metaphase chromosomes of healthy individuals. They are thought to develop due to partial inhibition of DNA synthesis and have taken on novel significance as sites of sensitivity to replication stress and preferential regions of rearrangements in tumor cells. Here, we present the first molecular cytogenetic investigation of FA associated breakpoints in comparison to FS. Hints on possible common induction mechanism resulting in breaks and gaps within the same sequence are expected. So far, we already were able to demonstrate that of 30 studied FS about 2/3 co-localize with evolutionary conserved breakpoints (Mrasek et al., unpublished data). Applying the corresponding FS breakpoint-spanning BAC clones on metaphase spreads of FA patients a possible co-localization can easily be revealed. The identification and molecular cytogenetic comparison of these breakpoint regions may lead to further findings concerning the origin of chromosomal instability

and reasons of the malignant transformation of karyotpes.

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P073

Prove of molecular co-localization of fragile sites and evolutionary conserved breakpoints

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Fragile sites (FS) are specific loci that preferentially exhibit gaps and breaks on metaphase chromosomes, following partial inhibition of DNA synthesis that can be induced by different culture conditions. A possible association of FS, evolutionary conserved breakpoints (bp) and cancer associated bp is discussed since Dutrillaux (1979) suggested a common mechanism for their formation. Nevertheless, only few FS are already investigated on a molecular level to test this hypothesis. In contrast, evolutionary conserved bp are well defined for great apes, especially for the chimpanzee, since the release of its complete genomic sequence. Thus, here we selected BAC clones for these evolutionary conserved bp regions and applied them for direct comparison on aphidicolin induced FS. 33 molecular characterized evolutionary bp are reported in great apes that are caused by macro rearrangements. 14 of them are localized in the same cytogenetic region as FS. After analyzing 8 of these regions on a molecular cytogenetic level four of them were mapped within a common FS. Additionally, a number of micro rearrangements were published for chimpanzee. Focusing on these 25 submicroscopic bp we could show a molecular co-localization with 16 common FS by breakpoint spanning / flanking BAC clones (e.g. FRA2H (2q32.1), FRA2J (2q37) or FRA4C (4q31)). Comparison of evolutionary conserved bp with chromosomal changes in cell lines of tumor patients did not show such concordance. In summary, this suggests a remarkable role of FS in connection with development of evolutionary fixed chromosomal rearrangements, but not in tumor development. Nevertheless, as not all evolutionary conserved bp have this feature others may be driven by different sequence properties like repeats or segmental duplications.

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P074

Mosaic ring chromosome 17 in an eight-year-old girl with microcephaly, short stature, epilepsy and mild developmental delay - review of the literature

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Ring chromosome 17 is a known but rare chromosomal aberration. The phenotype is variable. The main features include mental retardation, microcephaly, seizures and café-au-lait spots (Carpenter et al., 1981; Chudley et al., 1982; Shashi et al., 2003). Some cases of ring 17 include a deletion of the Miller-Dieker region on 17p13.3 and reveal a severe phenotype with lissencephaly, mental retardation, typical facial features and shortened life expectancy (Dobyns et al., 1983; Stratton et al., 1984; Sharief et al., 1991).
Here we report a detailed clinical and cytogenetic investigation of an eight-year-old girl with a high-grade mosaicism of ring chromosome 17. Interestingly, chromosomal imbalances were not detectable by array-CGH. She presented with a mild developmental delay consisting mainly of cognitive impairment, growth delay, microcephaly, epilepsy and café-au-lait spots. Neither dysmorphic features, nor congenital anomalies were found. Conventional cytogenetics of cultured lymphocytes were performed twice and revealed the following karyotype: 46,XX,r(17)(p13q25)/46,XX. Ring chromosome 17 was present in 85-93% of cells. In the fibroblast cultures an aberrant clone with ring chromosome 17 in 48% of cells was observed. Array-CGH analysis (Agilent, 105 K) failed to detect any imbalances. The patient's mother revealed a ring chromosome 17 in 2% of analysed blood cells. We discuss the

phenotype-genotype correlations and the possible reasons of the negative array-CGH data. A review of the literature is provided.

P075

Case report of a partial trisomy 21 without phenotypic relevance

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We present cytogenetic and molecular data of a prenatally diagnosed partial trisomy of chromosome 21 without phenotypic relevance for the carrier. The index patient was detected prenatally: an amniocentesis was performed due to parental desire, even though ultrasound abnormality could be excluded. In the short-tandem-repeat analysis (STR) two conspicuous markers in the region 21q21.1 were detected, of which one was tri-allelic and one had a suspicious peak-ratio. Furthermore, all ten markers covering the region 21q21.2 to 21q22.3 showed a normal signal pattern. Prenatal cytogenetic analysis revealed an aberrant chromosome 21. In combination with the results from STR analysis this finding was interpreted as an insertion resulting in a karyotype 46,XX,der(21)?ins(21;21)(q22.1?3;q21.1q21.?1).
To further delineate the karyotype, FISH analyses applying different locus specific probes were performed. The results showed an inverted duplication of the region 21q21.1→q21.2 resulting in the karyotype 46,XX,der(21)ins(21;21)(pter→q22.1?3::q21.2→q21.1::q22.1?3→qter). The Down-critical region was not involved and the size of the duplication is about 5 Mb compassing mainly pseudogenes.
Cytogenetic analyses of the parents revealed that the father carried the identical chromosomal imbalance like his unborn child. The father has not shown any phenotypic abnormality before the age of 45. We therefore expect that the unborn child will also be phenotypically inconspicuous. In conclusion, a duplication of a 5 Mb region in 21q21.1-q21.2 seems not to be associated with phenotypical consequences and could be considered as another euchromatic variant region (Barber, 2005, J Med Genet 42, 609-629).

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P076

Interstitial deletion of chromosome 7p: molecularcytogenetic characterization and phenotype

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Structural changes of chromosomes that are not published at a certain point of evaluation in the literature are one of the challenges in prenatal diagnostics. Here we present a deletion (7)(p21.1p22) previously not described and comment on the molecularcytogenetic and pre- and perinatal clinical findings. Amniocentesis was performed after 30 weeks of pregnancy because of abnormal ultrasound findings and advanced maternal age. The fetus was small for gestational age, showed an ASD I and a cleft palate. The conventional chromosome analysis of the cultivated amniocytes revealed in all cells analysed a male karyotype with an abnormal banding pattern of the short arm of one chromosome 7. FISH analyses confirmed that no other chromosomal material was involved in the aberrant chromosome 7. The subtelomeric region of 7p was present at the position expected. Microdissection analyses resulted in an interstitial deletion of the band 7p21.1 up to 7p22, which leaded to a partial monosomy 7. Chromosome analyses of both parents revealed normal chromosome complements. The patient was born after 40 weeks of pregnancy by spontaneous vaginal delivery. The dystrophic boy presented conspicuous facies with hypertelorism, microcephaly, cleft lip, analatresia and slight hypospadia. The echocardiogram indicated a large atrium septum defect (ASD) and a suspected mitralcleft. Further development will show whether other clinical signs will be expressed that are found to be associated with deletions of parts of the critical chromosomal region.

P077

De novo t(14;15)(q12;q11.2) resulting in a microdeletion 15pter-q11.2 proximal to the SNRPN gene and duplication 14pter-q12

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We report on a 18 months old female patient with short stature, mild developmental delay and aberrant right subclavian artery (arteria lusoria). Minor features were a prominent forehead, an absent right anthelix, a crease of the right ear lobule, and a small and thin 3rd toe. Conventional cytogenetics showed abnormal banding subcentromeric on the long arm of chromosome 15. A normal karyotype was described in both parents. FISH on metaphases of the patient with a locus specific probe for the Prader-Willi- / Angelman-Syndrome Critical Region (PWS-AS) showed normal signals on both homologues and FISH with a centromere probe for the acrocentric chromosomes 14/22 showed a signal on the aberrant chromosome (therefore designated derivative chromosome 14 or 22). MS-MLPA (Methylation-Specific Multiplex Ligation-Dependent Probe Amplification) using a kit specific for the PWS-AS critical region (MRC Holland) showed an ~50% peak size reduction of eight probes proximal to the SNRPN gene. By array CGH (NimbleGen®) the ~3 Mb microdeletion could be confirmed and in addition, by detecting a ~10 Mb duplication of the proximal long arm of chromosome 14, the derivative chromosome could be described as der(14)t(14;15)(q12;q11.2). To our knowledge, a microdeletion limited to the PWS-AS region proximal to the SNRPN gene has been reported only once (Murthy SK et al. Cytogenet Genome Res 2007;116:135-140). In the described family the index patient suffered from severe developmental delay and cleft palate while his father with the same microdeletion was diagnosed only with mild developmental delay. To our opinion, based on the clinical features described in this family and the clinical features of our patient, it is not possible to conclude that the 15q microdeletion is causative of at least some of these features. Only the description of more cases with a similar 15q microdeletion will allow for a better genotype-phenotype correlation.

P078

Balanced reciprocal whole-arm translocations forming pseudodicentric chromosomes: Two unrelated phenotypically normal cases and literature review

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Whole-arm translocations (WATs) result from centromere-centromere or centromere-telomere fusion of two chromosomes, followed by a reciprocal exchange of entire arm. The whole-arm is fused by its centromeric edge with the centromere or telomere of a receptor chromosome and may result in a pseudodicentric chromosome. Constitutional WATs are rather rare and can be identified in either a balanced or unbalanced status. Although most of the balanced ones are found in phenotypically normal subjects, some are detected due to the carrier's reproductive failure or other clinical findings. We report on two different unrelated WATs, namely t(1;14)(q12;p11) in a 33-years-old woman with reproductive problems and t(X;7)(q28;p11.1) in a 31-years-old healthy woman, latter found in the course of ovum donation examination. Results of FISH analysis were: translocation t(1;14): the pericentromere 1q12 beta-satellite II/III probe gave a strong signal on both the normal chromosome 1 and the der(1q14q) as well as a weak signal on the der(1p14p); the centromere 14/22 probe gave signals on both the normal and the der(1q14q). The derivative chromosome 14, thus, presented with a hybrid 1/14 centromere. FISH on translocation t(X;7): the centromere-7 alphoid probe gave a strong signal on both the normal chromosome 7 and the der(Xpq7q) as well as a weak signal on the der(7p); the Xq subtelomere probe gave two signals on both the normal X and the der(Xqtel7p). The derivative chromosome X, thus, presented as pseudodicentric, carrying centromere X and a major part of centromere 7 fused with Xq subtelomere. The authors discuss a mechanism of mispairing and nonhomologous recombination favored by the analogous repetitive sequences for the deriving of such chromosomal aberrations, in these cases between satellite DNA III of chromosome 1q12 and 14p11, and respectively centromere 7 alphoid DNA and subtelomere Xq DNA. The literature review for WATs resulting in pseudodicentric chromosomes is provided.

P079

Rare pericentric inversion of chromosome 4 in parent and rec(4) in child with Wolf-Hirschhorn syndrome: diagnostics by multicolor banding FISH

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We presented the characterization of pericentric inversion of sizeable part of chromosome 4 in father, and heritance of rearranged chromosome in the form of recombinant chromosome 4 in 6-months old malformed child having Wolf-Hirschhorn syndrome phenotype. The conventional cytogenetic analysis using GTG-banding was performed in both of healthy parents and in proband. The abnormal chromosome 4 was found in father, his karyotype was 46,XY,der(4)add(4)(p15),add(4)(q33). Karyotype of proband was described as 46,XY,add(4)(p15).
High resolution method of multicolor banding (MCB) FISH [Liehr et al., Int J Mol Med 9: 335-339, 2002] using corresponding probe set for chromosome 4 demonstrated intrachromosomal aberration. Analysis revealed the pericentric inversion with breakpoints in subterminal segments of p and q arms of chromosome 4 in father's karyotype - ish inv(4)(p15.2q33). As MCB demonstrated, the syndromal chromosomal disease in proband was determined by imbalanced karyotype due to recombinant chromosome 4: rec(4)dup(4q)inv(4)(p15.2q33). Meiotic events resulted in duplication of terminal segment of 4q33. Consequently, partial monosomy of noninvolved into inversion distal segment 4p15 is responsible for Wolf-Hirschhorn syndrome phenotype of proband. Final step of fluorescent in situ hybridization using LSI Wolf-Hirschhorn syndrome region probe (LSI WHS SO, CEP 4 SG, Vysis) confirmed the deletion of critical locus 4p16.3 in recombinant chromosome 4 of malformed boy. Analysis of meiotic fate of aberrant chromosome 4 showed that conjugation and crossing over take place in big pericentric zone involved into inversion. That explains high probability of recombinant chromosome 4 formation during gametogenesis in father, balanced carrier of inv(4). Understanding of mechanisms of chromosomal reorganization in meiosis could contribute to the prenatal diagnostics and genetic counseling of the family and consanguineous relatives.

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P080

De novo deletion del(6)(q23.1q23.2) in a patient with alalia, mental retardation, microcephaly, and short stature

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We report on the first case of a small de novo interstitial deletion of chromosomal region 6q23.1-23.2. The patient presented with microcephaly, short stature, amblyacousia, abnormal behaviour, and alalia. His motor development was slightly delayed and his facial aspect was largely normal. The deletion was detected by GTG-banding and confirmed by hybridisation of locus specific BAC probes (ish del(6)(q23.1q23.2)(RP-324N14-,RP3-346G2+)). The deleted region comprises approximately 30 genes. Alalia may be the after effect of the hearing problems and mental retardation. However, amblyacousia was corrected by hearing aids in our patient and alalia is rare in patients with comparable cognitive skills. Therefore, the deletion of this particular chromosomal region may interfere with speech development.
To the best of our knowledge no information about comparable cases exist. Indeed, only one patient was described so far with a deletion comprising as well chromosomal band 6q23.2. (del(6)(q23.1q24.2). However, the karyotype of this patient was not confirmed by molecular cytogenetic techniques. The phenotype of this patient overlaps concerning the unspecific symptoms as microcephaly, short stature, and mental retardation, but does not show the more specific symptoms of alalia and amblyacousia.

P081

De novo translocation (21;Y) with loss of SYBL1 (PAR2) in a boy with cardiac defect and developmental delay

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Translocations between the Y chromosome and an autosome occur in about 1:2000 newborns. In approximately 70% of the cases the translocation involves an acrocentric chromosome with a translocation of the Yq heterochromatic sequences onto the p-arm of an acrocentric chromosome. Frequently, the translocation is familiar and does not cause a phenotype.
Here, we report on a 4 months old boy who was born after an uneventful pregnancy at 37th week of gestation. During hospitalisation due to developmental retardation, a large VSD and ASD and dysmorphic features were diagnosed. A cytogenetic analysis using GTG-, QFQ- and C-banding techniques revealed a pseudodicentric 21;Y- translocation chromosome with the Y-centromere inactive in all 50 metaphases, karyotype 45,X,psu dic(21;Y)(p11.2;qter). FISH with probes DYZ3, D13/21Z1 and LSI21q22 gave signals in consistent positions on the translocation chromosome. The father´s karyotype was normal.
MLPA for subtelomeric regions was normal for all but the PAR2 region at the Xq/Yq telomere. For this region, a heterozygous deletion was detected.
We will discuss the phenotypic, cytogenetic and molecular findings with reference to a review of the literature.

P082

Detection and monitoring of clonal chromosome aberrations in Fanconi anemia patients

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Fanconi anemia (FA) patients have a high risk for bone marrow failure, aplastic anemia (AA), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and, later in life, for epithelial malignancies. Acquired clonal chromosomal aberrations in bone marrow cells, such as gains of the chromosomal segment 3q26q29 and monosomy 7 are strongly associated with a poor prognosis and represent an adverse risk factor in FA. Therefore, routine cytogenetic analyses of bone marrow cells once per year should be offered as an integral part of medical care (FARF: Standards of Clinical Care; www.fanconi.org). For the sensitive detection of clonal aberrations in bone marrow cells, conventional cytogenetics, CGH, and FISH are utilized. In 2003, we demonstrated that these clonal aberrations could also be detected in peripheral blood mononuclear cells (PBMCs) by interphase FISH. The advantage of screening PBMCs is that many FA patients who decline bone marrow aspirations accept regular blood withdrawal as a less invasive procedure. Here, we report on the high sensitivity and specificity of interphase FISH to screen for aberrant cells in PBMCs. Furthermore, we present data on the rapid increase of cells with 3q gains and monosomy 7 in bone marrow cells and in PBMCs of several FA patients. No transient appearance of 3q gains or of monosomy 7, as described for other clonal aberrations in FA patients, was noticed. Our data indicate either a high proliferative advantage or an increased survival of bone marrow cells carrying these aberrations. Using a self-capturing automated fluorescence microscope allows high throughput analyses of many FA patients at regular time intervals ensuring the early detection of adverse aberrations. Their occurrence suggest a very strong clinical care precaution: the finding of any chromosomal abnormalities, especially the abnormalities of chromosomes 3 and 7, warrant very close clinical follow-up, as they may signal the development of MDS or AML.

P083

A 10,9 Mb interstitial deletion of chromosome 13q without phenotypic effect

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Chromosome 13q deletions are associated with widely varying phenotypes, but the clinical picture includes in almost every case mental and growth retardation, craniofacial dysmorphisms, and/or malformations.
Several attempts have been made to link monosomy of distinct 13q intervals with specific clinical features, but there is still no consensus on possible correlations. Even if more proximal deletions tended to have fewer major anomalies, with the exception of retinoblastoma, the complete lack of

clinical signs together with a visible euchromatic deletion of 13q presents a remarkable finding. We report on a Nigerian 32-years old woman with normal phenotype and intelligence. Chromosome analysis was done due to the history of recurrent abortions followed by reproductive loss, and revealed an interstitial deletion of chromosome 13q. To confirm the unbalanced status and to estimate the exact deletion breakpoints and size, further characterisation was carried out by FISH and array-CGH. These investigations revealed a deletion ranging form band 13q21.1 to 13q21.32, comprising a loss of nearly 10,9 Mb euchromatic material.
To the best of our knowledge this is the second report of a deletion of band 13q21 compatible with a normal phenotype. This repeated observation indicates that this specific deletion in 13q21 might be added to the growing list of euchromatic duplications and deletions, e.g. euchromatic variants, that seem to be phenotypically neutral. Previous studies emphasized that benign euchromatic variants are underestimated and often not published. This kind of genomic alteration represents a diagnostic dilemma, especially for the interpretation of results obtained by more recent techniques like array-CGH or within prenatal diagnostic.

P084

Conventional chromosome analysis in mature B-cell leukemias using DSP30 and IL2 stimulation

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Conventional chromosome analysis (CCA) plays a key role in the diagnosis and prognosis of mature B-cell leukemias. However, this analysis is frequently hampered by the difficulty of obtaining tumor metaphases, which is caused either by low infiltration and/or low proliferative activity of the tumor cells in vitro. To increase the informativity of CCA, several mitogens have been used in the past like TPA, LSI or PHA. Here, we have studied the effect of DSP30 (an immunstimulatory CpG-oligonucleotide) and IL2 (Decker et al., 2000). A cohort of 320 consecutively ascertained patients affected with mature B-cell leukemias, including typical B-cell chronic lymphocytic leukemia (CLL), immunocytoma and other mature B-cell leukemias (e.g. HCL, PLL and atypical CLL) was studied. All cases were subjected to CCA by R-banding and interphase FISH with probes for 11q22~23 (ATM, FDX), 12q13 (CHOP), 13q14 (RB1, D13S319, D13S25), 14q32 (IGH) and 17p13 (TP53).
After stimulation with DSP30 and IL2, we obtained metaphases in 293 cases (92%). From those, 152 cases (52%) showed clonally aberrant karyotypes and displayed a total number of 321 alterations by CCA (median per case 1, range=1-9). The percetage of cases with clonal aberrations by CCA at diagnosis was 50% in typical CLL, 50% in immunocytoma and 66% in other mature B-cell leukemias. Recurrent gains were trisomies of chromosomes 3, 5, 7, 18 and 19. With regard to structural changes, 47 cases showed only unbalanced, 14 cases only balanced and 12 cases both balanced and unbalanced aberrations. In 35 and 26 cases, alterations were detected either by interphase FISH or by CCA, respectively. These findings highlight the importance of interphase FISH to detect cytogenetically cryptic alterations, and the necessity of CCA to obtain a whole picture of the karyotype. Therefore, interphase FISH and CCA with DSP30 and IL2 should be used in combination for a more informative cytogenetic analysis of mature B-cell leukemias.

P085

Characterization of a microdeletion 3q22.3 in blepharophimosis-ptosis-epicanthus inversus (BPES) by FISH, MLPA and Array-CGH analysis

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Mutations in the forkhead transcription factor gene 2 (FOXL2) cause blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) type I (with infertility in females) and type II (without infertility in females). Deletions of the FOXL2 gene in patients with BPES are rare. In addition, large deletions of the chromosome region 3q22.3 haboring the FOXL2 gene were observed in patients with more complex mental retardation-malformation phenotypes.
We report on the molecular cytogenetic and molecular genetic characterization of a microdeletion of subband 3q22.3 in a 2 1/2-months-old female newborn with BPES. She shows the typical facial

dysmorphism with blepharophimosis, ptosis and epicanthus inversus. Additional features were simple, low-set ears, a flat nasal bridge and a short neck. Cytogenetic analysis at 550 band level revealed a female karyotype. The appearance of chromosome band 3q22 was suspicious for a microdeletion. FISH analysis with BAC probes for the chromosome region 3q22.1->3q24 confirmed the presence of a microdeletion proximal to the FOXL2 gene, but not distal. Using the MLPA kit PO54 (including primers of the FOXL2 and ATR genes in 3q22.3 and 3q23), we showed that the FOXL2 gene, but not the ATR gene, was deleted. The proximal breakpoint of the microdeletion was mapped between 135,1 Mb and 135,5 Mb in 3q22.1 and the distal breakpoint between the FOXL2 gene (140,1 Mb) and 140,2 Mb in 3q22.3 by employing array-CGH analysis with an 40k oligo chip (OpArray V4, Operon).
In conclusion, this microdeletion 3q22.1->3q22.3 is approximately 4.6 - 5.1 Mb of size. The deletion of the FOXL2 gene correlates with the BPES phenotype. If any of the other deleted genes may have contributed to the phenotype of the patient remains unknown.

P086

Complete feto-placental discordance for a non-mosaic deletion 4p in CVS short term and long term culture

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Chorionic villus sampling (CVS) for prenatal diagnosis detects in about 1-2% of pregnancies a mosaic or non-mosaic chromosome complement which differs from that of the fetus. False positive non-mosaic chromosome aberrations that cannot from the outset be suspected of being confined to the placenta are very rare and were observed in only 0.07% of CVS in the EUCROMIC study (Hahnemann and Vejerslev, 1997). These false positive non-mosaic results mostly concern numerical chromosome aberrations and aberrations of ploidy level. However, non-mosaic structural aberrations have rarely been reported.
We report on the second pregnancy of healthy non-consanguineous parents (mother 35 y, father 44 y). CVS was performed in the 11th week of gestation because the first child was born with salt-wasting congenital adrenal hyperplasia and both parents are mutation carriers. On CVS, both, short term and long term culture showed a female karyotype with a large non-mosaic deletion 4p. As no fetal abnormalities could be detected on ultrasound, amniocentesis was performed in the 16th week of gestation. In amniocytes the deletion could not be detected by FISH with a Wolf-Hirschhorn region probe (Vysis) in more than 100 interphase nuclei and a total of 20 metaphases. Therefore, confined placental mosaicism (CPM) was the most probable explanation for these findings. In the 36th week of gestation a girl without dysmorphic features was born (measurements were within the normal range). Chromosome analysis from peripheral blood confirmed a normal female karyotype.
This exceptional case of CPM underlines how important it is to prompt further analysis in cases with discordance between CVS-karyotype and ultrasound before making a decision.

P087

Quantitative and qualitative analyses of the chromosomal instability in cells of patients with chromosome instability disorders by conventional and molecular cytogenetic techniques

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Fanconi anemia (FA), Nijmegen breakage syndrome (NBS), and ataxia telangiectasia (AT) are rare recessive disorders with an increased chromosomal instability. The patients' cells are characterized by spontaneous chromosomal breakage and an increased sensitivity to either DNA cross-linking agents (FA) or ionizing irradiation (AT and NBS). Patients of all three chromosome instability disorders have an increased tumor risk, especially for childhood leukemias. Genetic testing for FA, NBS, and AT is routinely based on conventional chromosome breakage analyses. Mainly chromatid breaks and radial figures are counted and compared to those of normal control cells. Subtle translocations are mostly not detectable by this test.
We established a semi-automated, molecular cytogenetic assay ("wcp-assay") as a methodical approach for the quantitative and qualitative analysis of chromosomal aberrations in FA patients. This

assay is based on the use of whole chromosome painting probes for single chromosomes together with an automated scanning microscope and the appropriate software. Here, we report the results of the "wcp-assay" in comparison to the normal conventional breakage test in cells of patients with different chromosome instability disorders (FA, NBS and AT). Our data show a high correlation between the data generated by the conventional breakage test and the "wcp-assay" indicating that the later can be used as a valid diagnostic approach. Additionally, the "wcp-assay" provides us with more detailed insights into the involvement of specific chromosomes in breakage events and with further information about the mechanisms of DNA repair. Furthermore, we expect a gain of information concerning the instability or genetic reversion of individual patients with chromosomal instability disorders over time.

P088

Four cases with subtelomeric deletions of 14q - clinical and genetic characterization results in genotype-phenotype correlations

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Molecular karyotyping in combination with a thorough clinical characterization holds the promise of establishing genotype-phenotype correlations for chromosome deletion syndromes e. g. concerning the subtelomeric regions. Although many cases with subtelomeric chromosome aberrations have been reported, these phenotype maps are emerging only slowly for many subtelomeric deletion syndromes including the terminal deletion 14q syndrome. Here, we report four cases with terminal partial monosomies of 14q32.3. Three of the patients carried de novo terminal deletions of 14q which have not been reported before. The fourth patient carried an unbalanced translocation der(14)t(9;14)(q34.3;q32.3) inherited from a mother carrying the balanced aberration [Leube et al., Clin Dysmorphol. (2003) 12:261-265]. The clinical phenotype of the patients was analyzed thoroughly. It included several characteristic symptoms of terminal deletion 14q syndrome such as mental retardation, pre- and postnatal growth retardation, microcephaly, muscular hypotonia, congenital heart disease as well as typical craniofacial and somatic dysmorphisms. Exact characterization of the deleted regions was performed using BAC FISH and the Illumina HumanHap550 SNP array. Deletion sizes were determined to be 3.25 (±0.013) Mb, 4.32 (± 0.168) Mb, 5.64 (± 0.353) Mb and 5.73 (± 0.080) Mb.
Based on these clinical and genetic analyses, a phenotype map for typical clinical findings of the terminal deletion 14q syndrome such as muscular hypotonia, growth retardation, congenital heart disease and several craniofacial and somatic dysmorphisms will be presented.

P089

Non-mosaic trisomy 7 in chorionic villi and trisomy 18 in the same fetus: Extreme form of mosaic variegated aneuploidy

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Mosaic variegated aneuploidy (MVA) describes cases with multiple trisomies. Meanwhile, 29 cases have been reported where one single patient carried a mosaicism for different trisomies. The parallel existence of two non-mosaic trisomies of two different chromosomes in the fetus and the placenta has not yet been published. We here report on an extreme form of MVA in a pregnancy with a non-mosaic trisomy 7 in CVS and a non-mosaic trisomy 18 in amniotic fluid. The 30-year-old mother underwent chorionic villous biopsy because of pathologic ultrasound findings for chromosome examination at 11+1 weeks of her first pregnancy. Cytogenetic analysis of uncultured CVS cells and long term culture revealed a non-mosaic trisomy 7. The trisomy 7 was not detectable in amniocytes, but a non-mosaic trisomy 18 was diagnosed. By STR typing, each aneuploidy could be confirmed in the respective tissue. In all informative markers the maternally inherited allele was stronger than the paternally

inherited one. Based on the observed reduction of maternal heterozygosity to homozygosity in all markers we conclude a postzygotic mitotic origin of both aneuplodies. It is well conceivable that mitotic errors in the early embryo briefly after differentiation into trophoblast and epiblast occur, resulting in a complete fetal-placental discordance as observed in our case. The observation of trisomies 7 and 18 as preponderant aberrations in MVA supports our assumption that the entire chromosomal complement discordance in our case represents the extreme end of MVA.

P090

The reverse painting of micro dissected chromosomes on DNA chips (Array painting) reveals cryptic chromosome aberrations

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Chromosomal rearrangements are a valuable resource for the identification of disease associated genes. Over 20 Years ago, the technology of micro dissection of aberrant chromosomes has been established to identify the chromosomal break points and to delineate the chromosome origin. During that time, the hybridization of these generated FISH probes were done on normal metaphase chromosomes with the known limitations of resolution. Recently, array painting, a technique that combines chromosome isolation and DNA array hybridization, has been introduced as a rapid method to fine map chromosomal breakpoints.
The individual spots on a DNA BAC chips representing parts of a contiguous human genome extend of 170 kb in average. Already this resolution allows the identification of genes of a break point region. On the example of a pericentric inversion of chromosome 1 we demonstrate the power of this approach. One copy of each chromosome arm was micro dissected and amplified by DOP-PCR. DNA was labelled differentially by random priming and hybridized onto a submegabase resolution tiling path BAC array. The aim of this study was to see whether micro dissected and DOP-PCR amplified FISH probes gave sufficient amount of probes to perform hybridization on this kind of platform. Using this strategy we were able to narrow down the breakpoints at 1p32 and 1q43 to about 150kb within one round of experiments. Surprisingly, our analysis revealed that the rearrangement was much more complex than initially suspected, involving a further paracentric inversion of 1q42. Both additional aberrations have been missed by conventional karyotyping as well as reverse chromosome painting.

P091

Partial trisomy of distal 22q and partial monosomy of distal 10q in two siblings with multiple congenital anomalies due to a maternal balanced translocation t(10;22)(q26.1;q13.3)

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The index patient, first child of healthy and non-consanguineous parents, is a 4-year old girl with motor and mental retardation. Additional clinical findings include hypotonia, cerebral palsy, defective position of the feet, microcephaly, and strabismus. Distinctive facial features are small upper lip and dysmorphic ears. Cytogenetic analysis revealed a terminal deletion on the q-arm of one chromosome 10 with the putative breakpoint in 10q26.1. FISH analysis with probes for 41 different chromosome telomers confirmed the terminal deletion on 10q and showed, in addition to regular signals on both chromosomes 22, 22qter-specific signal on the rearranged chromosome 10q. The patient is therefore trisomic for distal 22q and monosomic for distal 10q. The karyotype is 46,XX,der(10)t(10;22)(q26.1;q13)mat.ish der(10)(10pter+,10qter-, 22qter+). FISH analysis of the maternal chromosomes 10 and 22 showed an apparently balanced 10qter;22qter translocation while the father´s chromosomes revealed regular signals. The younger son of the couple was delivered after an uncomplicated pregnancy in 39th week of gestation by cesarian section. At the age of 8 weeks he presented with cryptorchism, ASD type II, single transverse palmar crease, failure to thrive and pes equinovarus configuration as well as some distinctive facial features. Cytogenetic and FISH analysis revealed the same unbalanced karyotype as found in his 4-year old sister. The 2-year old daughter

(not yet karyotyped) was reported of having speech delay, a small ASD and defective position of the feet. The clinical findings of the patients will be discussed and compared with those of patients described in the literature having either of the two unbalanced chromosomal rearrangements.

P092

Genome-wide genetic characterization of an atypical meningioma by single-nucleotide polymorphism array (SNP-A)-based mapping and classical cytogenetics

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In most meningiomas, accounting for about 20% of intracranial tumors, curative treatment can be achieved by surgical removal. Yet, 8-22% of these tumors are classified as atypical or anaplastic meningiomas (WHO grade II or III, respectively) presenting with a more aggressive behavior. We analyzed genomic alterations of an atypical meningioma using high-density single nucleotide polymorphism arrays (SNP-A) combined with GTG-banding, multicolor fluorescence in situ hybridization (M-FISH), and locus-specific FISH. In accordance to recent studies applying SNP-A in different malignancies we found that genomic lesions are present at a higher incidence (frequency) than predicted by traditional cytogenetics. Most of these aberrations have not been described by previous studies. Additionally, we unveiled loss of heterozygosity (LOH) without copy number changes on chromosome regions 1p31.1, 2p16.1, 2q23.3, 6q14.1, 6q21, 9p21.1, 10q21.1, and 14q23.3, suggesting partial uniparental disomy (UPD). UPD´s are currently considered to play an important role in the initiation and progression of different malignancies.
Furthermore, we detected two de novo reciprocal translocations, t(8;19)(q24;q13) and t(10;16)(q22;q12.1). While GTG-banding and M-FISH data suggested balanced translocations SNP-A analysis clearly demonstrated their unbalanced character.
The significance of the novel alterations in atypical meningioma presented here needs to be confirmed in a larger series of WHO grade I - III meningiomas.

P093

Banding and molecular cytogenetic studies detected a CBFß-MYH11 fusion gene in a baby with acute myeloid leukemia FAB M4-Eo

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The acute myeloid leukemia (AML) subtype M4Eo occurs in 5% of all AML cases and is usually associated with either an inv(16)(p13.1q22) or a t(16;16)(p13.1;q22). At the molecular level, these abnormalities generate a CBFß-MYH11 fusion gene. Patients with this genetic alteration are usually assigned to a low-risk group and thus receive standard chemotherapy. AML-M4Eo is rarely found in infants. In the present study, we described clinical, banding, and molecular cytogenetic data for a 12-month-old baby with AML-M4Eo and a chimeric CBFß-MYH11 fusion gene hidden by a yet unreported novel rearrangement between chromosomes 1 and 16. The karyotype was characterized as 46,XY,der(1)t(1;16)(1qter->1p22::16q22->16qter),t(3;10)(p24;q22),der(16)t(1;16)(1pter->1p22::16p13.13->16q22::16p13.13-16pter). This rearrangement characterizes a new case of inv(16)(p13.1q22) masked by a chromosome translocation. In summary, the description of the inv(16) masked by a novel translocation reinforces the utility of molecular assays to detect a submicroscopic abnormality even when a good banding cytogenetic study is performed.

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P094

Evaluation of copy number alteration by means of array CGH in childhood MDS

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Background and objectives: Myelodysplastic syndromes (MDS) account for less than 10% of hematopoietic malignancies in childhood. Monosomy 7 (-7) is the most common cytogenetic abnormality. However, there is evidence that deletion of chromosome 7 is not the initiating event and that additional alterations cooperate in the outgrowth of a malignant clone.
Design and methods: We therefore aimed to systematically evaluate copy number alterations (CNA) by means of array-CGH in 25 cases of MDS with monosomy 7 and in a further 20 cases of JMML (10 cases), advanced MDS (5 cases) and refractory cytopenia (RC, 5 cases). Microarrays containing around 8000 BAC/PAC clones leading to a genome-wide resolution of at least 1 Mb and oligo-CGH-arrays (Agilent) with a resolution of 7 kb were used. Selected CNA were validated by fluorescence in situ hybridization or quantitative PCR.
Results: With BAC arrays, CNA of 1 MB to 100 MB in size were found in individual cases like loss of 3p12-3p14 containing the TSG FHIT (1 advanced MDS), a microdeletion in 17p11.2 containing GRAP, a novel SH3-SH2-SH3 adaptor protein that couples tyrosine kinases to the Ras pathway (1 RC), a loss of 3q22-3q29 (1 JMML), a loss of 6q27 (2 JMML), a loss of 9q34 (1 JMML, 1 MDS, -7), loss of 12p12-13 (1 MDS, -7), a gain of 12q13 (1 JMML), a loss of 17p13 (1 JMML, 2 MDS, -7), loss of 21q21 with coexistent gain of 21q22 (1 JMML) and other CNA. Alterations were most frequently observed in the group of JMML and MDS with -7. In order to prove whether recurrent alterations in the sub-megabase range exist, 9 cases with monosomy 7 were analysed with oligo-arrays. Subtle shared deletions targeting NF1 (17q11.2), NOTCH1 (9q34) and NRAS (1p13) were identified and could be confirmed by qPCR.
Conclusions: The application of high resolution array-CGH to study karyotypic abnormalities in MDS leads to the identification of minimal shared regions of loss that may result in deregulation of important pathways like Ras signaling.

P095

Appendiceal GIST: Clinicopathologic, immunohistochemical and molecular study of two cases

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Gastrointestinal stromal tumors (GIST) constitute the most common mesenchymal group of tumours of the gastrointestinal tract. But, they are rare in the vermiform appendix. Only five cases have been reported so far, all without any molecular investigations. Here, we report two GISTs of the appendix. The first patient (case 1), a 78-old woman, had a history of endometrial adenocarcinoma and pelvic irradiation. Our second patient (case 2), a 72-year-old man, had a history of urinary bladder carcinoma. Both GISTs were incidental findings at surgery for appendicitis-like symptoms and on follow-up for bladder carcinoma, respectively. The GIST of case 1 was 5mm in size and located in the mid-portion. Case 2 had a pedunculated 25mm sized GIST in the tip. Both revealed a spindle cell histology with variable stromal hyalinisation. CD117 and CD34 were immunohistochemically positive and p16 immunostaining showed a loss in both cases. Furthermore, case 2 over-expressed the catalytic subunit of the human telomerase reverse transcriptase (hTERT) immunohistochemically. Molecular analysis of KIT revealed two types of mutations: case 1 showed a missense mutation K558R, case 2 had an in-frame deletion I571-_R588. Both mutations were in the juxtamembrane

domain (exon 11) of KIT. Comparative genomic hybridisation, successfully in case 2 (larger lesion), revealed no chromosomal imbalance. We suggest that the molecular pathogenesis of GISTs of the appendix might be different from gastric and intestinal GISTs. The coincidence of loss of p16 and over-expression of hTERT seems to contradict the small size, the benign nature and the limited growth potential of appendiceal GISTs.

P096

Systematic expression analysis of DNA repair genes in children with malignancies

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Because childhood cancer is unlikely to be due to unhealthy lifestyle or environmental hazards, it has been proposed that genetic factors are the primary cause for most malignancies in children. Our working hypothesis is that children with two malignancies are carriers of genetic information which makes them particularly susceptible for the development of different tumors. Because cells suffer from continuous DNA damage from exogenous and endogenous sources, highly efficient DNA repair systems are required for maintaining genome integrity and preventing malignant transformation of cells. Mutations in a number of DNA repair genes representing different pathways are responsible for many hereditary forms of cancer. The aim of our study is to identify DNA repair- and cell cycle-associated genes that are constitutively misregulated (without induction of DNA damage) in children with malignancies and, therefore, may contribute to cancer predisposition. With the help of the German Childhood Cancer Registry, we have recruited 20 persons who survived a childhood malignancy and then developed a second cancer as well as 20 carefully matched persons with a childhood cancer who did not develop a second malignancy. Using a customized cDNA microarray with essentially all important (>100) DNA repair genes and a selection of (approximately 150) DNA repair-associated genes, we have compared the baseline expression patterns in primary fibroblasts of persons with one or two childhood cancers with a pool of fibroblasts from 20 healthy controls (without cancer). Bioinformatic analysis of the expression profiles is underway. In addition, we will quantify the induced DNA damage (gamma-H2AX foci) and analyze the expression profiles after challenging different DNA repair pathways with gamma-irradiation, UVC, and mitomycin C treatment. Our results may prove useful for improving diagnostic classification of tumors, treatment selection and therapeutics development.

P097

Mutations in the IRF association domain of the transcription factor gene ICSBP may be associated with leukemogenesis in Shwachman-Diamond syndrome

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Patients with severe congenital bone marrow failure syndromes (CMBF) including Shwachman-Diamond syndrome (SDS), Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), and congenital neutropenia (CN) have a high risk for developing MDS or AML. It is not yet known which gene defects may induce the malignant transformation in these patients. ICSBP (interferon-consensus site binding protein), coding for a transcription factor of the IRF family controlling myeloid cell differentiation, is one putative candidate gene, since ICSBP has been identified as one of the genes most dramatically down-regulated in G-CSF-treated patients diagnosed as CN compared to G-CSF-treated healthy controls.
The aim of our study was to evaluate the occurrence of ICSBP mutations or methylation in patients diagnosed as CBMF leading to its down-regulation.
DNA of peripheral blood or bone marrow of 26 CBMF patients was screened for mutations in the DNA binding domain (DBD) and in the IRF association domain (IAD) of ICSBP by direct sequencing. Furthermore, promoter region of ICSBP was analyzed for de novo methylation.
We found base pair substitutions in the IAD: The substitution c:827 G>A leading to Arg276His was found in 3 of 15 SDS patients. Although this mutation occurred in two siblings and seems to be constitutional, this has to be proven by analyzing the parents or other tissues. Another substitution

c:602 C>T; p:Ala201Val was observed in the only DBA patient investigated. No alterations were found in CN (5), or FA (5) patients. By means of MS-PCR we found no evidence for promoter methylation in the patients. Loss of this transcription factor may disturb normal hematopoietic differentiation and may also induce leukemogenesis in humans. Larger patient groups are necessary to support the hypothesis of the relevance of ICSBP mutations for leukemogenesis in CBMF patients. Interestingly, one SDS patient carrying ICSBP mutation developed leukemia with a complex karyotype supporting our hypothesis.

P098

Establishment of an interlaboratory test for the quality assessment in leukemia cytogenetics

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Objectives: Numerous laboratories all over Europe offer chromosome banding analyses for leukemia diagnostics. The quality of the results depends on factors like cultivation conditions, chromosome preparation, banding technique and the experience of the investigator. To determine the quality of the cytogenetic investigations, we established an interlaboratory test (IT) of the complete procedure of chromosome banding analysis.
Methods: A sample containing a mixture of cell lines with normal and abnormal chromosomes as a surrogate leukemic blood sample was sent out to the participating laboratories. The cytogenetic results and reports of each laboratory were evaluated by a review committee according to a catalogue for minimum standards contained in the ISO15189 and for the formal correctness of the karyotypes. To compare cytogenetic findings target aberrations were defined for each cell line. Up to now, three interlaboratory tests (IT1-3) have been performed using cell lines with 3, 10, and 17 target aberrations.
Results: 20 (IT1), 25 (IT2) and 37 (IT3) laboratories took part in the ITs. The minimal contents of the reports were accomplished by 5 %, 56 % and 41 % of the participants. Most frequently informations of the date of sampling (44%) were missing. Inaccuracies within the karyotype formula were found in 65 %, 64 % and 73 % of the reports. All target aberrations were found by 70%, 20% and 8% of the laboratories.
Conclusions: The minimum standards of reports were fulfilled by the majority of the laboratories. The high degree of inaccuracies in the karyotypes points to the need of an internal or external review system. With increasing complexity of the karyotypes the detection rate of target aberrations decreased. This demonstrates that the newly developed IT system is a useful instrument to compare the results between laboratories. To identify the causes of the variation of the test results further investigations are needed.

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P099

Convergence of the proteomic pattern in cancer

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On the histological level the differentiation of normal epithelial tissues is well known and also the phenomenon of dedifferentiation which occurs the more the cells develop towards malignancy. To identify an epithelial tumor-specific proteomic profile as well as to measure the proximities between we used data from tumor tissue and adjacent normal tissue microdissected from 3 different epithelial tumor entities which were analyzed using ProteinChip technology and performed a bioinformatic meta-analysis on the resulting six complex data sets.
As a result all groups could be identified based on their proteomic signatures and the tumor tissues were found to be more similar to one another than to the normal epithelial tissue from which they progressed. This study shows for the first time at the proteomic level that changes in the histological features of tumors as compared to the tissues from which they arise are reflected in the convergence

of proteomic pattern during the development to cancer. Therewith the histopathological grading which forms the basis for the classification of tumors (grading), could be proven on the proteomic level.

P100

Gene expression profiling: A comparison of epithelial liver tumours

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Background and objective: Differential diagnosis of epithelial liver tumors using small biopsies is a difficult diagnostic field. More information on different gene expression profiles of epithelial liver tumors would help to delimit histopathologically malignant hepatocellular carcinoma (HCC) from benign hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH), respectively. Therefore gene expression profiling was performed on tumours cytogenetically well characterized by array based comparative genomic hybridization.
Design and methods: Global expression profiles of 24 HCC, 8 HCA and 7 FNH were measured by microarray analysis using a genome-wide microarray containing 43000 spots. Taqman assays were applied to validate the expression pattern of significant genes.
Results: Based on these microarray results, hierarchical cluster analysis branched all HCC from HCA and FNH. The most differentially expressed genes represent the family of metallothioneins, which are drastically decreased in HCC compared to FNH and HCA. The most significant gene is MT1F, expressed at high levels in HCA and FNH in comparison to HCC, followed by MT1G, MT1X and MT2A, showing the same pattern. Taqman Assays of MT1F and MT1G validated these significant microarray results.
Conclusion: We have shown that the expression of metallothionein is significantly decreased during development of malignant epithelial liver tumors. These data suggest that the different expression of methallothionein is a putative biomarker, differentiating between malignant HCC on the one hand and benign HCA and FNH on the other and therefore should be a focus of further research.

P101

Further evidence for heritability of an epimutation in one of twelve cases with MLH1 promoter methylation in blood cells clinically displaying HNPCC

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Germline mutations in mismatch repair (MMR) genes, tumours with high microsatellite instability (MSI-H) and loss of MMR protein expression are the hallmarks of HNPCC (Lynch-syndrome). While somatic MLH1 promoter hypermethylation is generally accepted in the tumorigenesis of sporadic tumours, abnormal MLH1 promoter methylation in normal body cells is controversially discussed as a mechanism predisposing patients to HNPCC.
94 patients suspected of HNPCC-syndrome with a mean age of onset of 45.5 years, MLH1-deficiency in their tumours but no germline mutation underwent methylation-specific PCR-screening for MLH1 promoter methylation.
In peripheral blood cells of twelve patients an MLH1 promoter methylation, in seven informative cases allele-specific, was found. Normal colonic tissue, buccal mucosa, and tumour tissue available from three patients also presented abnormal hemiallelic methylation in the MLH1 promoter. The heredity of aberrant methylation is questionable. Pro: MLH1 promoter methylation was found in a patient and his mother giving evidence for a familial predisposition for an epimutation in MLH1.
Contra: a de novo set-up of methylation in one patient, a mosaic or incomplete methylation pattern in six patients, and no evidence for inheritance of MLH1 promoter methylation in the remaining families. Our findings provide strong evidence that MLH1 promoter methylation in normal body cells mimics

HNPCC and constitutes a pathogenic pre-lesion in MLH1. The identification of hypermethylation as an epigenetic defect has important implications for surveillance recommendations, as these patients should be treated like Lynch-Syndrome patients, whereas the heritability of methylation is still under investigation.

P102

Somatic FGFR3 and PIK3CA mutations in a familial case of seborrhoeic keratoses

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Seborrhoeic keratoses (SK), also referred to as seborrhoeic warts, senile keratoses or basal cell papillomas, represent one of the most common benign skin tumours. Their incidence significantly increases with age. Most cases appear sporadic without familial aggregation. Rare cases of familial SK have been reported in the literature so far, but the genetic basis has remained unclear. We investigated a family with at least seven affected members in two generations who developed high numbers of SK at an unusual young age, suggesting a hereditary background with an autosomal dominant inherited predisposition. Because FGFR3 and PIK3CA mutations have been reported to be involved in the pathogenesis of sporadic SK, we analysed five SK of one affected family member for known hotspot mutations of these genes. FGFR3 mutations were present in three of five SK. These and other FGFR3 mutations recently identified in sporadic SK cause in case of a germline mutation skeletal dysplasia syndromes such as thanatophoric dysplasia or severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN syndrome). Besides an FGFR3 mutation, one SK of our patient additionally showed a PIK3CA mutation also known from sporadic SK. Germline mutations of both genes could be ruled out in the patient. Other causes have to be responsible for familial occurrence of early development of multiple SK such as inherited susceptibility factors predisposing to somatic FGFR3 and PIK3CA mutations in the skin.

P103

Multi-chromosomal aberrations in Wilms tumor detected by MLPA-analysis and Array-CGH

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Wilms tumor (WT) is an embryonal tumor of the kidney, which arises from pluripotent embryonic kidney precursor cells. WT affects 1 of 10.000 newborn children a year, making it the most frequent pediatric kidney cancer. The majorities of WTs are sporadic and usually arise in a single kidney before the age of 5. WT1 on chromosome 11, CTNNB1 on chromosome 3 and WTX on the X-chromosome are the major genes involved in WT development. WT1 point mutations are more frequent in a histological subgroup of WTs with stromal histology accompanied by rhabdomyoblastic differentiation, whereas intragenic deletions in WT1 are relatively rare in WTs. Mutations within the WT1 gene lead to a functional loss of its activity as a transcription factor and contribute to tumor formation.
In our studies, we previously analyzed three unilateral and stromal predominant Wilms tumors for mutations in the WT1 gene, using the PCR/SSCP-method and sequencing, but none were found. In order to search for WT1 aberrations not detectable with the previous methods we study these samples with two different MLPA kits. First we used the WT1 MLPA-kit, which focuses on aberrations in the chromosomal region 11p13, but also contains different control-probes to study other chromosomes. As aberrations were detected on several other chromosomes we used in addition the subtelomer MLPA-kit to verify these, in all cases the aberrations could be reconfirmed in this analyze. One tumor (MAM), with a complete deletion of 11p13, was additionally investigated with array-CGH to identify all aberrations on different chromosomes.
MLPA-analysis successfully detected an entire chromosome 11 deletion and other chromosomal aberrations in these tumor samples. Additionally we found a gain of chromosome 7 in all three WT (MAM, DE and AW) probes and several other chromosomal aberrations, which may contribute to tumor formation.

P104

Novel germline mutations of the RET protooncogene in patients with medullary thyroid carcinoma

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Introduction: Multiple endocrine neoplasia type 2 (MEN2) and medullary thyroid carcinoma (MTC) are autosomal-dominant inherited diseases caused by germline mutations within the RET protooncogene. Up to now genetic testing for mutations in exon 10, 11, 13, 14, 15 and 16 was recommended for these patients (familial and sporadic cases) to identify disease-causing mutations within known “hot-spots”. Extended genetic testing for the remaining exons of the RET protooncogene is recommended for patients with clinical manifest disease who do not have mutations in these “hot-spots” (Brandi, M. et al. 2001). Recent data suggests another “hot-spot” of mutations in exon 8 for MTC. Therefore we tested 100 patients clinically apparent MEN2 or MTC without disease-causing mutations in exon 10, 11 and 13 to 16 of the RET protooncogene for mutation within exon 8.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of exon 8 of the RET protooncogene and direct sequencing including corresponding exon-intron boundaries.
Results: Extended genetic testing for mutations within exon 8 revealed two novel heterozygous germline mutations: p.Ala513Gly and p.Arg552Gln.
Conclusion: In 100 consecutive patients with MEN2 or MTC, but without mutations in the known “hot-spots” in exon 10, 11 and 13 to 16, we have found two novel germline mutations in two patients with MTC. These data are in concordance with a new mutation “hot-spot” in exon 8 of the RET protooncogene. Genetic counseling and genetic testing for RET mutations should include rare mutations and be done in familial and apparently sporadic MTC/Men 2 index cases to confirm the diagnosis and define asymptomatic gene carriers for early therapy.

P105

Revaluation of 161 patients with leukemia investigated by conventional chromosome analysis and FISH with “BCR/ABL Dual Color, Dual Fusion Probe”: more variant translocations and partial deletions than expected?

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The BCR/ABL rearrangement, called Philadelphia chromosome, describes the balanced translocation t(9;22)(q34;q11). With conventional chromosome analysis the so called classical Philadelphia translocation can be detected for about 90% of patients with chronic myeloid leukemia (CML). This translocation also affects about 2% of patients with acute myeloid leukemia (AML) in addition to 25% of adult patients with acute lymphoblastic leukemia (ALL) respectively 5% of children affected of this disease.
Approximately 5-10% of the patients either do not present any translocation or show translocations with involvement of other chromosomes than 9 and chromosome 22. These translocations are called “variant“ Philadelphia chromosomes.
Fluorescence *in-situ* hybridisation (FISH) on metaphase chromosomes or interphase nuclei can be performed to detect the BCR-ABL translocation specifically and quantitatively.
Variant or masked translocations as well as partial losses of BCR and ABL, such as deletions on the der(9), are well known and described. Anyway, such cases still present specific challenges for the cytogeneticists.
Here, we report the results of the investigation of 161 patients by conventional chromosome analysis and FISH with special respect to variant translocations as well as partial deletions of BCR and ABL.

P106

A second fusion partner of *MAML2* in mucoepidermoid carcinoma

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The translocation t(11;19)(q21;p13) first has been described in our laboratory and is known to occur in mucoepidermoid carcinomas, hidradenoma, and rarely in Warthin's tumours of the salivary glands. Recently, the breakpoint was cloned and a fusion gene comprised of exon 1 of the *CREB regulated transcriptional coactivator CRTC1* gene on chromosomal band 19p13 and exon 2-5 of the mastermind-like gene *MAML2* on chromosomal band 11q21 was identified. We screened a total of 67 formalin-fixed, paraffin-embedded mucoepidermoid carcinomas for the presence of *CRTC1-MAML2*. In one of these samples for the first time a *CRTC3-MAML2* fusion gene was detected, as determined by RT-PCR and sequencing. Thus, this study demonstrates the existence of a fusion of *MAML2* with *CRTC3* additional to the already known fusion of *MAML2* and *CRTC1*. Both gene fusions seem to result in an identical tumor phenotype, so they may play a similar role in the development of mucoepidermoid carcinomas. This aspect could be an interesting starting point for further studies.

P107

Analyses of the cellular localization of the thyroid adenoma associated gene (*THADA*) by the use of GFP-fusion constructs

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Rearrangements of the thyroid adenoma associated gene (*THADA*) due to translocations involving 2p21 account for 10-15% of benign thyroid lesions with clonal chromosomal deviations thus belonging to the most common chromosomal aberrations in these lesions. The translocations affecting the chromosomal region of *THADA* result in truncation of the 3'-part of *THADA*. Recently, we have analyzed the genetic structure of *THADA* homologous genes in different vertebrates. We could identify a most conserved part of the protein with a homology of ~70% between the most different organisms suggesting that this part could be an important functional domain. Interestingly, this part is disrupted by the above mentioned truncations indicating a loss of function of *THADA* in benign thyroid lesions with 2p21 rearrangements and *THADA* truncations, respectively. Nevertheless besides this finding nothing is known about the function of the *THADA* protein. To obtain more information about the *THADA* protein we have performed localization analyses. We have cloned GFP-fusion protein expression vectors using pEGFP-C1 and pEGFP-N1 plasmids. *THADA-A3*, an alternative splice form of THADA, and the corresponding fusion gene *THADA-A3-FUS3p* were cloned into the above mentioned GFP-plasmids resulting in *GFP/THADA-A3, GFP/THADA-A3-FUS3p, THADA-A3/GFP* and *THADA-A3-FUS3p/GFP* constructs. With these plasmid constructs transient transfection was performed on different human cell lines by either electroporation or lipid-based transfection methods. Localization of GFP fusion proteins was detected by fluorescence microscopy. GFP alone (GFP-C1 as well as GFP-N1) was localized throughout the cell whereas the GFP-fusion proteins were localized within the cytoplasma. These findings suggest that there is no relationship between the function of the truncated part of the *THADA* protein and the intracellular localization of *THADA*.

P108

New mutations causing juvenile polyposis syndrome in Polish JPS patients

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Juvenile Polyposis Syndrome (JPS, MIM # 174900) is an autosomal dominant disorder, characterized by predisposition to developing juvenile polyps. These polyps are hamartomatous lesions of gastrointestinal tract, especially colon and rectum. The juvenile polyps are typified by markedly expanded lamina propria containing dilated cystic glands, an inflammatory infiltrate and show a normal epithelium with dense stroma. Occurrence of the juvenile polyps is also observed in other syndromes like Cowden syndrome, Bannayan-Ruvalcaba-Riley syndrome, Peutz-Jeghers syndrome and

hereditary mixed polyposis syndrome. The frequency of Juvenile polyposis syndrome is estimate to occur once in every 100000 newborn. Risk of gastrointestinal malignant transformation in JPS patient is increased and ranges more then 60%. Occurrence of the colon cancer is two fold higher in the upper parts of the gastrointestinal tract. Development of the juvenile polyps is caused by mutation in one of two genes associated with JPS. First of them is bone morphogenetic protein receptor 1A gene (BMPR1A also known as ALK3), which encodes a type I cell surface receptor, a serine/threonine kinase receptor, involved in bone morphogenetic protein signaling pathway (BMP). Second one is SMAD4 gene (also known as MADH4 or DPC4). The protein product of this gene is intracellular mediator of TGFβ superfamily signaling pathway. In result of research we observed four mutations. Using screening methods were found two mutations in the BMPR1A gene and two in the SMAD4 gene. Presented results are preliminary stage study which main aim is to determine the mutation spectrum of the BMPR1A and SMAD4 genes in Polish JPS patients.

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P109

Chromosomal microdissection in leukemia: a powerful tool for characterization of chromosomal rearrangements

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Chromosome microdissection has become an increasingly important method for assessing chromosome rearrangements in human cancer. Thus, micro-FISH has been used successfully in the identification of ring chromosomes, homogeneously staining regions, and double minutes.
Using reverse chromosome painting, we were able to characterize precisely structural chromosomal aberrations identified by conventional cytogenetics in the two cases:
Case 1: Additional clonal anomalies were found in an adult patient with Ph+ ALL. Cytogenetic analyses revealed loss of the Y chromosome, an extension of the long arm of chromosome 1, and a double Ph+ chromosome. Microdissection of the aberrant chromosome 1 displayed that the extra material on the add(1q) exclusively originated from the Y chromosome. The acquired small supernumerary marker chromosome previous designated as +der(22) was determined to be the short arm segment of the Y chromosome by complementary FISH analyses. Thus microdissection disclosed a so far unrecorded balanced translocation t(Y;1)(q11.1;q44) as additional anomaly in Ph+ ALL.
Case 2: In a patient with a clinically diagnosed acute myelogenous leukemia (AML) GAG banding revealed a 46,XX,-5,+ mar karyotype. The marker chromosomes consisted exclusively of chromosome 5 derived material as proven by whole chromosome painting. To characterize the aberration in more detail chromosome microdissection and multicolour banding (MCB) technique using a chromosome 5 specific probe set was applied for comparison. Both methods gave nearly identical results. The karyotype could be described as 46,XX,r(5)(p13.2q21). In consequence, the aberration leads to a partial deletion of chromosome 5, which would not have been identified using conventional banding techniques or 24-color FISH.
This report demonstrates the advantages of combining multiple techniques in order to obtain a detailed description of cytogenetic changes.

P110

HDAC inhibitors induce cellular senescence in neuroblastoma and prostate cancer cells

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Cellular senescence is a phenomenon that leads to an irreversible block of cellular division capacity. It is an event that appears in cell culture and also in vivo. In general, it is thought that cellular senescence is, similar to apoptosis, a mechanism to prevent cancer growth. So this mechanism could be a new approach for cancer therapy. Histone deacetylases (HDACs) are considered as therapeutic targets to treat cancer patients. They are of therapeutic interest because they inhibit cancer growth and are used in various clinical trials.
HDACs are important epigenetic regulators that deacetylate histones and other regulatory factors.

There are three different classes of histone deacetylases. The classification of HDACs is based on their homology to yeast proteins. HDACs class I are homolog to the yeast protein RPD3, class II to HDA1 and class III to the sirtuins, which play an important role for senescence. HDACs class III are known as SIRT (silent information repressor of transcription).
We analyzed whether inhibitors of HDAC class III induce senescence in mammalian cells. Further we aimed to detect the effect of HDAC inhibitors of class I and II on cell proliferation and senescence in human cancer cell lines. The aim of our work is to induce senescence in cancer cells and thus inhibit cancer growth. As a model system we used neuroblastoma and prostate cancer cells. The cellular senescence was confirmed with the staining of the senescence-associated beta-galactosidase a well-known marker for senescence.
We find that HDAC class III inhibitors induce cellular senescence. Furthermore, HDAC inhibitors of class I and II also induced cellular senescence both in neuroblastoma and human prostate cancer cells. In line with the induction of senescence specific markers, we also show that the cell cycle arrest is not reversible.
These findings underline that neuroblastoma and prostate cancer cells undergo cellular senescence a new possibility for tumor suppression.

P111

Expression of miR-193a and miR-365 in tumours involved in Neurofibromatosis type 1

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Neurofibromatosis type 1 patients develop multiple benign dermal neurofibromas. Other tumours associated with this disease are benign plexiform neurofibromas and malignant peripheral nerve sheath tumours (MPNST). In the NF1 microdeletion syndrome the complete NF1 and several contiguous genes are deleted. These patients often have a severe clinical phenotype including an excessive number and early onset of dermal neurofibromas and a higher life-time risk for the development of MPNSTs. Two genes coding for the microRNAs miR-193a and miR-365-2 also lie within the two most prevalent types of deletion. MicroRNAs are small non-coding RNAs who negatively regulate many protein coding genes. We investigated the expression pattern of these two microRNAs in tumours and cell types associated with NF1. We found a reduced level of both microRNAs in plexiform neurofibromas compared to dermal neurofibromas. An MPNST cell line showed a reduced level of both microRNAs in comparison to primary control fibroblasts. Surprisingly, we found a higher expression of both microRNAs in meningiomas compared to dermal neurofibromas. The rate of meningiomas is not different between classical NF1 and NF1 microdeletion patients. In addition we look for genes regulated by these microRNAs in cultured cells and their function in differentiation of melanoblasts. We suggest that these miRNAs lying in the NF1 microdeletion are involved in the tumour formation in NF1.

P112

Preliminary study of the Polish patients with Peutz-Jeghers syndrome

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Peutz-Jeghersa syndrome (PJS; MIM 175200) is rare, autosomal dominant disease. The frequency of PJS is estimated from 1/29,000 1/120,000 people. Hamartomatous polyps manifest during second or third decade of life and are observed in 80 to 100 % patients. Polyps can be located throughout digestive tract. Risk of malignant transformation is lower then others hereditary neoplastic disease, but PJS can be reason of many gastrointestinal discomforts like obstruction or bleeding. Besides all of that, the extraintestinal malignant transformations also are present. In Peutz-Jeghers syndrome high risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. Second of characteristic manifestation is mucocutaneous hypermelanocytic lesions. The brown or dark blue spots developing on lips, hands and feet, in the mucosa of the nose, conjunctiva or rectum appear in about 90% cases. Molecular study of Peutz-Jeghersa polyposis is not

described widely in literature. Up to the present, more then 160 mutations in LKB1 (STK11) gene causing PJS were established in computer database. Mutations in LKB1 gene occur in 70 % patients with familial form of PJS and in 30 to 70% sporadic cases of disease. LKB1 (STK11) gene is located on chromosome 19p13.3. Protein coding by LKB1 gene is serine/threonine kinase. Loss of LKB1 function causes many defects, since LKB1 participates in very important cell signaling pathways. Here we present the preliminary study considering 9 patients with PJS. First 4 exons of LKB1 gene were examined in all patients and as result of this study 4 point mutations were identified.

P113

C17orf79 as as a putative NF1 modifying gene lying in the *NF1*-microdeletion region

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominantly inherited tumor diseases. Several symptoms of NF1 as the dermal neurofibromas show a high interfamilial variability. To explain this variability the existence of NF1 modifying genes has long been proposed. In a very rare variant of NF1, the familial spinal neurofibromatosis, patients have spinal neurofibromas but few or no dermal neurofibromas. On the other hand, patients with microdeletions spanning the *NF1* and several contiguous genes have an earlier onset and a higher number of dermal neurofibromas as classical NF1 patients. We suggested that one of the genes additionally deleted in these patients contributes to the increased number of neurofibromas and may be one of the NF1 modifying genes. Our expression studies in dermal neurofibromas revealed four putative NF1 modifying genes: *CENTA2, UTP6, C17orf79* and *RAB11FIP4*. We investigated the expression and single nucleotide polymorphisms (SNPs) in these four genes and found differences in the expression pattern of *C17orf79* in all patients with familial spinal neurofibromatosis. So we assume, that *C17orf79* is involved in the occurrence of NF1 symptoms.

P114

Transcriptional silencing of *EZH2* using an anti-gene peptide nucleic acid in prostate cancer cells

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Overexpression of *EZH2* plays a major role for tumour progression in prostate cancer. The *EZH2* gene is physiologically expressed during embryogenesis, but is downregulated in adult mammalian cells, predestining *EZH2* as an ideal candidate for gene silencing therapy in prostate cancer.
In contrast to most approaches towards gene silencing, we chose the anti-gene strategy in order to achieve an optimized drug/target ratio compared to antisense therapy options. We apply single-stranded oligomers of peptide nucleic acids (PNA), a synthetic DNA-mimetic molecule with strong resistance to enzymatic degradation and high affinity for DNA in a sequence specific manner, to obtain long-term silencing effects. We have established a pre-screening reporter gene assay for testing series of PNA oligomers with respect to their capacity of inhibiting *EZH2* expression. Five PNA were tested so far, targeting the transcription start and the first intron of the *EZH2* gene. The best result was achieved by an 18mer-PNA eleven base pairs downstream of the *EZH2* transcription start, reducing reporter gene activity to 30%. In contrast the PNAs against intron 1 showed minimal repressive effects, indicating that targeting the transcription start is crucial for efficient gene silencing by the anti-gene method.
Cellular Uptake is a major obstacle in PNA applications. In order to verify, that the PNAs are capable to inhibit endogenous *EZH2* expression, we used partially complementary DNA oligomers, which formed stable and transfectable PNA-DNA duplexes. However our data showed that cellular uptake of PNA-DNA duplexes is highly depended on the sequence of the DNA oligomer impeding comparison among different PNAs, in particular between *EZH2*- and control-PNAs. To ensure constant uptake conditions we are therefore trying to accomplish cellular uptake through cell penetrating protein (CPP)-linked PNAs. First experiments are under way to study silencing of endogenous *EZH2* in prostate cancer cells.

P115

The APC gene mutations in Polish patients with FAP syndrome

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Familial adenomatous polyposis (FAP) is a genetically conditioned predisposition to occurrence of numerous adenamatous polyps in colon and rectum. The polyps arises in second decade of life and left untreated develop to the tumor. Other extracolonic features may include polyps in the upper parts of the gastroenterological tract, desmoid tumours, ocular lesions, osteomas, dental abnormalities, and malignancies in other organs. FAP incidence is estimated at 1/10,000. FAP arises due to germ line mutations in the adenomatous polyposis coli (APC) gene, which was first described in 1991. The APC gene mutations are studied at the Institute of Human Genetics in Poznan for last 10 years. The Institute of Human Genetics in Poznań cooperates with medical centers from all country, what permitted us to create the DNA bank for Polish FAP patients. Till now samples from 340 families were collected. Seven hundred DNA samples from Polish FAP families were banked so far. The APC gene we screened for mutation in 300 probands. We identified 74 point mutations in 124 FAP families. The detected mutation can be considered characteristic for Polish population due to 34 types of them has not described in other population. Among detected mutations seven occurred in two or to the greater number of families. Occurrence of the APC gene large rearrangements was studied in 95 families. We identified rearrangements in 24 families in two cases it were deletion of whole APC gene.

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P116

Characterization of the leukemogenic fusion protein CALM/AF10 as an aberrant transcription factor

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The t(10;11)(p13;q14) is a recurring chromosomal translocation that is found in acute myeloid and acute lymphoblastic leukemia as well as in malignant lymphoma. This translocation results in the fusion of AF10 (a putative zinc finger transcription factor) with the CALM gene (Clathrin assembly protein lymphoid myeloid leukemia gene). The fusion gene was cloned into pRTS-1 vector where the expression of CALM/AF10 is achieved by inducing the vector with tetracycline or doxycycline (tet-on system). The construct was stably transfected into the cell line DG75. The expression of CALM/AF10 after induction was confirmed by RT-PCR and Western blot analyses. Expression profiling using the Affymetrix hg U133 Plus 2.0 Array was carried out with the aim of identifying direct target genes of CALM/AF10. Expression profiling was performed at three time points: Non-induced, 24 hours and 72 hours after induction. The results were analyzed using the dChip 2007 (DNA Chip Analyzer) software. 1237 genes showed 2 fold differential expression 24 hours after induction. 594 (48%) genes were downregulated, while 643 (52%) were upregulated. Downregulated genes included genes involved in DNA repair (DDB2, TOP2A, BRCA1), cell cycle checkpoint (CCNE2, CHEK1, CDC2) and chromosome maintenance (MCM3, MCM7, MCM10). This suggests that CALM/AF10 might compromise genome stability. Upregulated genes included signal transduction molecules like RAB8B and STAT family members (STAT1 and STAT2), chromatin remodelling factors like BAZ2A and a positive regulator of Notch signaling, MAML3. Interestingly, no significant upregulation of HOX genes was observed, as had previously been observed in CALM/AF10 positive patient samples (Dik et al., 2005). These discrepancies can be explained by the fact that expression profiling conducted on leukemic patient samples examines different cell types and predominantly indirect target genes.

P117

Can breast cancer morphology help to predict a *BRCA1* mutation?

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Introduction: Since tailored therapeutic strategies are currently being developed, it would be useful to know at the time of diagnosis whether a *BRCA1* or *BRCA2* mutation is causatively related to an individual breast cancer. The aim of this study was to determine in an unselected hospital-based series of breast cancer patients, whether morphological features can improve the prediction of a *BRCA1* mutation.
Methods: In a retrospective approach, histopathological results of tumours from 897 unselected women previously diagnosed with breast cancer were re-evaluated regarding the age at diagnosis, subtype of cancer, tumour grade, estrogen (ER), progesterone (PR) and the Her2/neu receptor status as well as the p53 and Ki67 status. 142 tumours fulfilled the morphologic criteria indicative of a *BRCA1* mutation. Out of 59 women who were willing to participate in this study, 26 concomitantly showed a positive family history. After genetic counselling, a mutation screening in *BRCA1* and *BRCA2* was performed in 18 women.
Results: Pathogenic *BRCA1* germline mutations were detected in seven out of the 18 women (39%). None of these women carried a *BRCA2* mutation. All *BRCA1*-associated tumours were of high grade, of invasive-ductal subtype, PR and Her2/neu-negative, 91% were negative for ER. 60% of the tumours showed a high expression of p53 and 60% showed a high expression of Ki67. In particular, there was a highly significant difference between *BRCA1*-associated tumours and other familial tumours with respect to grading (p=0.001 for G3), ER negativity (p=0.0075), Ki67 ≥65% (p=0.0039) and "triple (ER-, PR-, Her2/neu-) negativity" (p=0.0019).
Conclusions: This study more clearly defines the histopathological criteria, i.e. triple negative, high expression of Ki67 and p53, that may help to improve the prediction of a *BRCA1* mutation in breast cancer patients.

P118

Correlation of DNA repair capacities measured with micronucleus test and mitotic delay assay

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Breast cancer has been associated with a variety of mutations in susceptibility genes, such as BRCA1 and BRCA2. Breast cancer patients also exhibit impaired average DNA repair capacity when compared to healthy controls, which has been assessed by different tests that measure the cellular ability to repair DNA damage. Yet unanswered is the question if those tests produce correlated results on an individual basis. For this purpose, micronucleus test and mitotic delay assay were performed simultaneously on the same probands.
Peripheral blood samples were obtained from 42 probands (breast cancer patients and controls). Cytokinesis-blocked micronucleus test was performed with spontaneous or radiation-induced lymphocyte cultures (2Gy (200 rad) of ionizing radiation at culture setup). Samples for mitotic delay were irradiated after 54 hours, 18 hours prior to measurement. Mitotic delay index was calculated as (ratio [cells in G2-phase]/[cells in S-phase] of irradiated cultures divided by the G2/S ratio of control cultures). No correlation of the mitotic delay index to either spontaneous micronucleus frequencies ($p = 0.45$) or to radiation-induced MN frequencies (2 Gy (200 rad) of ionizing radiation; $p = 0.19$) was observed. There is also no correlation between spontaneous and induced MN frequencies ($p=0.40$), which has already been shown previously.
As both increased mitotic delay index and micronucleus frequencies are known to be associated with defects in DNA damage repair and with breast cancer, the absence of correlation between these two tests indicates that they produce independent results to characterize cellular DNA repair capacity. Thus they seem to measure different aspects of DNA damage response. This could prove to be a useful step towards increased statistical significance when screening patients on DNA repair capacity.

P119

SPOC1 is regulated by E2F-1, a downstream target of the retinoblastoma protein (RB)

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy among women in developed countries, yet little is known about molecular events that drive the initiation and progression of this disease. We have recently identified a novel human gene (*SPOC1*) which encodes a protein with a putative chromatin interacting PHD domain (plant homeo domain). We could show that expression of *SPOC1* was clearly associated with worse prognosis in tumour tissue of patients with epithelial ovarian cancer. The median survival time was 1596 days for patients with low *SPOC1* expression versus 347 days for patients with high expression.
Since the present data suggested, that *SPOC1* functions as an oncogene in epithelial ovarian carcinogenesis, we wanted to know whether *SPOC1* is involved in one of the known cancer gene networks. We therefore performed an analysis of *SPOC1* promoter region in different species using the Genomatix-Software and identified a possible promoter with a large number of common transcription factor binding sites. Interestingly, we found two binding sites for E2F-1, a transcription factor which is a target of the retinoblastoma tumor suppressor protein (Rb) and crucial for the initiation of DNA replication. Using luciferase reporter gene assays we could demonstrate that a region of 928bp increases the promoter activity 68-fold compared with wild pGL3-Basic. In order to examine the effect of E2F-1 on *SPOC1* promoter activity, we co-transfected the *SPOC1*-luciferase reporter constructs with an E2F1 expression vector. Co-transfection reduced *SPOC1* promoter activity by 60% suggesting a downregulating effect of E2F-1 on the *SPOC1* promoter. Moreover, immunocytochemical co-localization experiments in cell cultures revealed a partial co-localization of *SPOC1* and E2F-1 in the nucleus, suggesting a functional interaction between both proteins and a possible autoregulatory effect.

P120

Characterisation of six novel rare APC variants on mRNA level

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Background: Detection of pathogenic APC mutations in patients with familial adenomatous polyposis (FAP) allows predictive testing in persons at risk. Evaluation of rare unclassified variants by mRNA analysis is a first step towards their classification into pathogenic mutations and variants of yet unknown functional relevance.
Methods: We characterised six novel APC variants identified in patients with classical and attenuated FAP on mRNA level.
Results: The variant c.531+5G>C in intron 4 results in a deletion of exon 4. The variant c.532-8G>A in intron 4 introduces a new splice acceptor site resulting in the insertion of 6 intronic nucleotids in the mRNA. The mutation c.1409-2_1409delAGG in the splice acceptor site of exon 11 destroys the normal splice site and activates two cryptic splice acceptor sites. The apparently silent substitution c.1548G>A;p.Lys516 at the last position of exon 11 leads to a complete deletion of exon 11, and the presumed missense mutation c.1742A>G;p.Lys581Arg localised at the second last position of exon 13 results in an almost complete deletion of exon 13. A large duplication of exons 10-11 was identified in two apparently unrelated FAP patients by MLPA. The mRNA analysis in one of the patients showed that exons 10 and 11 were correctly spliced and duplicated in the order of the exons: 9-10-11-10-11-12. Five of the variants result in a premature stop codon while the in-frame deletion of exon 13 leads to the removal of a complete functional motif from the N-terminal region of APC. The mutation c.1548G>A was detected in the index patient with classical FAP but not in his father affected with an attenuated FAP suggesting mutational mosaicism.
Conclusions: Based on mRNA analysis all six rare variants can be classified as pathogenic

mutations. The characterisation of rare unclassified variants on mRNA level is important for the evaluation of their pathogenicity and may elucidate mutational mechanisms in FAP.

P121

TLE1 is a new candidate tumor suppressor gene inactivated by DNA hypermethylation in lymphomas

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A recent study in Arabidopsis thaliana has found that the TTG1 gene is regulated by promoter DNA methylation and plays a key role in cell differentiation and cell-growth inhibition (Berdasco et al., submitted). In line with these functions, the human TTG1-homologue, TLE1 (Transducin-Like Enhancer of Split 1), displays tumor suppressor activity and has been shown to be hypermethylated in a cancer-specific manner. In particular, promotor methylation of TLE1 was detected in lymphomas but not in normal hematopoietic cells (Fraga et al., submitted). In order to further evaluate the importance of TLE1 hypermethylation in lymphomas, we analysed 13 B-cell lymphoma cell lines by methylation specific PCR (MSP) and pyrosequencing. With the newly established pyrosequencing assay we were able to quantify the DNA methylation on 14 individual CpG sites in the differentially methylated region of the TLE1 promoter. Commercially available completely methylated DNA and DNA isolated from healthy peripheral blood lymphocytes served as methylated and unmethylated controls, respectively. A total of 10/13 cell lines from different lymphoma subtypes (4 Burkitt lymphoma, 4 Hodgkin lymphoma, one diffuse large B-cell lymphoma and 4 B-cell lymphoma not otherwise specified) showed methylation using MSP whereas 3/13 were negative. MSP results revealed that DNA isolated from blood samples of 10 healthy individuals were all unmethylated whereas 12/20 biopsy samples of mature aggressive B-cell lymphomas showed a complete or partial methylation. In full accordance with the MSP results, pyrosequencing showed that all 10 cell lines with positive M-MSP displayed mean methylation values across the 14 CpGs of greater than 60% whereas the 3/13 cell lines with negative M-MSP showed values less than 20%. Pyrosequencing studies on a large panel of different subtypes of primary B-cell malignancies are currently underway.

P122

Submicroscopic aberrations are rarely detectable in isolated 5q- MDS

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Conventional cytogenetic diagnostic of Myelodysplastic Syndromes (MDS) is an important factor in the International Prognostic Scoring System (IPSS). 50% of MDS patients show cytogenetically visible aberrations. Thereby the deletion of the long arm of chromosome 5 is the most frequent structural karyotypic abnormality occurring in 30% of de novo MDS. 10% of these patients carry an isolated 5q deletion leading to a more favourable prognosis compared to patients with additional chromosomal alterations. In the newer WHO-classification the 5q- syndrome is a separate category but not all patients with isolated del(5q) have a 5q- syndrome.
For detailed genomic characterisation of patients with isolated del(5q) we performed high resolution array-based comparative genomic hybridisations (aCGH).
Our previous studies with 44K oligonucleotide platforms and a resolution of 35 kb revealed a low percentage (23%, 3 of 13) of cytogenetic hidden imbalances. A precise molecular definition of 5q deletion end points could be achieved. Another nine additional cases were analysed, four with 244K arrays and the higher 6,4 kb resolution and five with 44K arrays. The 5q deletion end points fall in the same common deleted regions as detected before. Submicroscopic aberrations were found in one case.
These studies contributed to the refinement of 5q breakpoints by aCGH, which will be important for further genetic analyses. Finally, this study indicates that the genomes of patients with isolated 5q deletions are relatively stable.

P123

Co-existing somatic promoter hypermethylation and pathogenic MLH1 germline mutation in Lynch syndrome: Observation from a systematic study

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Somatic epimutations in the MLH1 promoter mimic the phenotype of Lynch syndrome. To date, no somatic hypermethylation of the MLH1 promoter in the carrier of a pathogenic MLH1 germline mutation has been identified, prompting the recommendation that a germline mutation in MLH1 should only be sought in the absence of tumour tissue methylation. We aimed to determine whether methylation of the MLH1 promoter may coexist in carriers of a pathogenic germline mutation in MLH1. We examined the methylation status of the MLH1 promoter in 123 tumour tissue samples demonstrating high microsatellite instability and loss of expression of a mismatch repair protein (60 cases with MLH1 germline mutation, 25 cases without mutation, 38 cases with MSH2 mutations) using combined bisulphite restriction analysis (COBRA) and SNaPshot analysis. Methylation of the MLH1 promoter was found in two patients with pathogenic germline mutations, one a carrier of a MLH1 and the other a carrier of a MSH2 mutation. Our results demonstrate that methylation of the MLH1 promoter region does not exclude the presence of a germline mutation in an MMR gene. Hypermethylation of the MLH1 promoter may be present in most cases of sporadic colorectal cancers, but this does not exclude a diagnosis of Lynch syndrome.

P124

Imatinib resistance in CML is caused by BCR-ABL-independent activation of RAF

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Although the BCR-ABL tyrosine kinase inhibitor Imatinib has undoubtly revolutionized the therapy of chronic myeloid leukaemia (CML), drug resistance is still a common problem in Imatinib treatment of CML. Many cases show a resistance due to mutations in the catalytic cleft of BCR-ABL or an overexpression of BCR-ABL, but in about 20 % of cases the resistance mechanism is unknown. Here, we report on a mechanistic study carried out on Imatinib-sensitive and -resistant clones derived from the CML cell line KCL22. The results of sequencing and BCR-ABL activity assays support the assumption that Imatinib-resistance in the resistant clone is not caused by alterations in BCR-ABL. The simultaneous treatment of resistant cells with Imatinib and the Mek inhibitor CI-1040 led to a block in proliferation. Therefore it could be stated that the MAPK signalling pathway is involved in the acquisition of Imatinib resistance. We showed that Erk activity in the resistant clone was not compromised by Imatinib doses that fully repressed Erk activation in the sensitive KCL22 cells. A systematic analysis of the upstream MAPK pathway demonstrated the inhibition of Ras-GTP accumulation by Imatinib in resistant KCL22 cells, while B-Raf and C-Raf activity was largely resistant to the drug's action. Sequencing of the kinase domains of both C-Raf and B-Raf showed no constitutive activating mutations in either kinases. In sum, our studies we could highlight a novel mechanism of acquired Imatinib resistance in CML cells based on the non-mutational aberrant activation of Raf. Our results may prove helpful for a better functional classification of Imatinib-resistance in CML.

P125

Comparison of detected chromosomal imbalances in adrenocortical cancer (ACC) in children using CGH and SNP array analysis

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Adrenocortical tumours (ACT) in children are rare and, if malignant, often associated with poor prognosis. Relevant cytogenetic factors for prognosis are hardly available. In a former study we

analyzed fourteen adrenocortical carcinomas (ACC) by comparative genomic hybridization (CGH). The number of CGH aberrations appeared to have a predictive value for overall survival in paediatric ACC. So all patients with less than 9 individual imbalances were in remission while all but one patient with 10 and more individual imbalances had died from the disease. The patients could be separated in the low risk group with good prognosis and the high risk group with bad prognosis. To confirm this hypothesis we analysed fifteen adrenocortical carcinomas by chip based copy number analysis with Affymetrix 100K microarrays.
The aberrations detected by CGH could be confirmed with microarray analysis. Moreover additional genomic imbalances were discovered. The number of aberrations ranged from 1 to 17 in individual tumour samples by using CGH and from 2 to 37 by using Array-analysis. In contrast to our hypothesis 3 patients with 18 to 22 individual imbalances were in remission while 1 patient with 8 individual imbalances had died after diagnosis.
In contrast to CGH it is possible to analyse simultaneously deletions and loss of heterozygosity (LOH). Surprisingly we found only a few deletions accompanied by LOH, but much more deletions without LOH. Deletions accompanied by LOH might have been emerged in an earlier state of tumorgenesis. Homozygous deletions might be a reason for detected deletions without LOH or this kind of aberration could have been developed in different cell clones. Maybe this kind of deletion and the involved chromosomes could be seen as a marker for malignancy and provide some evidence for an imprinting effect in carcinogenesis.

P126

Concomitant BRCA1 and BRCA2 gene mutations in a woman with primary breast cancer - a case report

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Mutations in two major cancer susceptibility genes, BRCA1 and BRCA2, predispose to hereditary breast and ovarian cancer. In recent years several families have been described in which more than one BRCA mutation segregated, predominantly involving Ashkenazi Jewish founder mutations. However, double heterozygosity (DH) has also rarely been reported without prior knowledge of Jewish ancestry. In this report we present a non-Ashkenazi family with multiple cases of breast cancer, with the index patient harbouring BRCA1 and BRCA2 mutations (BRCA2 K944X exon 11, BRCA1 1303delG and M1915T exon 11). The presence of a second mutation does not seem to lead to a more severe phenotype, but has important consequences for genetic counselling.

P127

Interphase FISH detects leukemic IGH-CCND1-fusion-positive lymphoma cells in the peripheral blood and bone marrow of the majority of patients with mantle cell lymphoma

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Mantle cell lymphoma (MCL) is a B-cell neoplasm characterized by the translocation t(11;14)(q13;q32) resulting in a juxtaposition of the cyclin D1 gene to the immunoglobulin heavy chain (IGH) regulatory elements and the subsequent overexpression of the cell cycle regulator protein cyclin D1. MCL occurs at a median age of about 60 years, shows a male predominance and has a median survival of about 3-5 years.
In the European MCL Network we analyzed 275 German MCL patients with diagnostic peripheral blood (PB) or bone marrow (BM) involvement (detectable by consensus IGH PCR) for translocation t(11;14) by fluorescence in situ hybridization (FISH) and 4-color flow cytometry (FC). The present interim evaluation includes 205 MCL analyzed by the LSI IGH-CCND1 XT dual color dual fusion probe from Abbott/Vysis. The cut off level for a positive FISH result with 2 fusion signals, one additional red and one additional green signal indicating a t(11;14) was 1% for this probe. Using these criteria, we detected a IGH-CCND1-positive clone in PB or BM in 122 of 205 (60%) cases. According to the FISH results, the percentage of IGH-CCND1 positive cells ranged from 1%-96% (mean 30,4%). In comparison, PB/BM involvement of MCL by flow cytometry was detected in 194 of 216 (90%) cases. The percentage of MCL cells in FC ranged from 0,07% to 83% (mean 18,4%). Interestingly, in many

FISH-negative but FC-positive samples we detected few scattered IGH-CCND1 positive cells by FISH, but the percentage of aberrant nuclei was below the diagnostic cut-off. The conclusions from these prospective randomised trials are: a) MCL can be diagnosed from PB or BM in the majority of patients without need for diagnostic lymph node biopsies, b) the sensitivity of conventional double-color FISH assays might not be sufficient for leukemic involvement of MCL. The latter might be overcome by 4- and 5-color FISH assays which we have recently developed for the detection of IGH-CCND1 fusion in MCL.

P128

Histological dedifferentiation in human hepatocellular carcinoma correlates with specific genomic alterations accompanied by changes in gene expression

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Background/aims: Dedifferentiation of hepatocellular carcinoma (HCC) implies an aggressive clinical behaviour and is associated with genomic alterations like deletion of 13q. However, little is known what genes are directly or indirectly deregulated due to these genomic alterations.
Material and methods: Gene expression profiling was carried out on 23 HCC diagnosed as G1= well differentiated, G2= moderately differentiated or G2-3/3= dedifferentiated using the 43k cDNA chip covering the entire human genome provided by the Stanford Functional Facility. All cases were characterized by array-CGH (Steinmann et al. 2006). Bioinformatic analyses included unsupervised hierarchical clustering and SAM analyses.
Results: Dedifferentiated HCC branched from well and moderately differentiated HCC ($p<0.001$ Chi-Square-Test). Within the dedifferentiated subgroup, 887 genes were up- and 33 genes were down-regulated. SAM of HCC with and without 13q- did not display a deregulation of any gene located in the deleted region. However, 531 significantly up-regulated genes were identified. 6 genes (BIC, CPNE1, RBPMS, RFC4, RPSA, TOP2A) were among the 20 most significantly up-regulated genes both in dedifferentiated HCC and in HCC with 13q-. All these genes are involved in cell cycle control and proliferation.
Discussion: Dedifferentiation of HCC is associated with up-regulation of genes involved in cell cycle control and proliferation. Notably, a significant portion of these genes is also up-regulated in HCC with deletion of 13q. It remains unclear, how these genes located in chromosome regions other than 13q are up-regulated, since no down-regulated genes were identified within the deleted region of 13q. Three known miRNA (mir-621, mir-16-1, mir-15a) are located in the deleted chromosomal region of 13q and thus should be lost. We therefore speculate that these miRNAs induce the up-regulation of critical cell cycle control genes.

P129

Mutations in RET protooncogene in Polish patient with medullary thyroid cancer analysed by pyroseqencing

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Medullary thyroid carcinoma (MTC) is uncommon tumor of thyroid gland, descended from C type follicular cells and constituting for 10% of all types of thyroid cancer. Considerate malignancy and course of a disease takes place between high and low differentiated thyroid cancers. MTC may appear in incidental or familial form (FMTC) but also as a major feature of the multiple endocrine neoplasia type 2 syndromes (MEN2). Two different syndromes of MEN2 can be distinguished: more common, associated with phaeochromocytomas and hyperparathyroidism - MEN2A and second syndrome with mucosal neuromas, ganglioneuromatosis of the gastrointestinal tract - MEN2B. Familial form of medullary thyroid cancer is autosomal dominant hereditary neoplasm with very high and changeable expression. Genetic background of MTC is correlated with mutations in protooncogene

RET. For most of MEN2A affected persons mutation in one of the six cystein's codons located within exon 10 and 11 of RET protooncogene are observed. The most frequent mutation in patients affected with MEN2B syndrome is single nucleotide substitution at codon 918. In FMTC individuals specific mutations in exons 10, 11, 13, 14 and 15 are equally found.
Performed analysis covered nine codons of protooncogene RET, where mutations appear most frequently. As the most appropriate assay of point mutation identification leading to single nucleotide substitution, pyrosequencing was implemented. We examined group of 304 individuals including affected with diagnosed medullary thyroid cancer, MEN2A and MEN2B syndromes and also relatives with first or second-degree consanguinity.

P130

Overexpression of *HMGA2* in thyroid carcinomas as a novel molecular marker for preoperative discrimination of follicular neoplasias

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Fine needle aspiration biopsy (FNAB) with subsequent cytological examination is performed routinely in patients with thyroid nodules, providing reliable results in the majority of cases. However, routine cytology cannot distinguish follicular patterned lesions due to identical cell morphology. Thus, there is a high demand for supplementary molecular markers. We investigated the expression of the high mobility group A2 protein gene (*HMGA2*) and its protein as a possible marker detecting malignant growth of thyroid tumors. While *HMGA2* is highly expressed in most embryonic tissues, its expression in adult tissues is very low. Recently, a post-transcriptional regulation mediated by binding of the miRNA let-7 to seven complementary sites in the 3´-UTR of *HMGA2* mRNA has been reported. Reactivation of *HMGA2* expression has been described for various malignant tumors and often correlates with the aggressiveness of the tumors. Consistently, low levels of let-7 also correlate with less differentiated tumors. This study was aimed at the use of *HMGA2* expression to detect malignant thyroid tumors. The *HMGA2* expression of 64 formalin-fixed paraffin-embedded thyroid tissues including normal tissue, follicular adenomas as well as follicular, papillary, and anaplastic carcinomas was analysed by real-time quantitative RT-PCR. Moreover, immunohistochemistry with an antibody raised against *HMGA2* was performed to verify these results on the protein level. We found a highly significant difference with histology of the tumors being the gold standard between the benign lesions and malignant tumors. Based on *HMGA2* expression alone, it was possible to distinguish between benign and malignant thyroid tissues with a sensitivity of 95.9% and a specificity of 93.9%. Our results show that even as a stand-alone marker *HMGA2* expression has a high potential to improve diagnoses of neoplasms of the thyroid.

P131

Incidence of the FIP1L1/PDGFRA rearrangement in 24 patients with eosinophilia sent for routine chromosome analysis

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Hypereosinophilic syndromes (HES) constitute a rare and heterogeneous group of disorders, defined as persistent eosinophilia associated with evidence of eosinophil-induced organ damage, where other causes of hypereosinophilia such as allergic, parasitic, and malignant disorders have been excluded. HES may be due either to involvement of myeloid cells, essentially due to occurrence of an interstitial chromosomal deletion on 4q12 leading to creation of the FIP1L1-PDGFRA fusion gene (F/P+ variant), or to increased interleukin (IL)-5 production by a clonally expanded T cell population (lymphocytic variant). For F/P+ patients, imatinib has undisputedly become first line therapy. Here we retrospectively tested 24 samples of patients with marked eosinophilia of unknown pathogenesis by FISH using a triple colour break apart probe set that allows detection of the FIP1L1-PDGFRA fusion as well as other PDGFRA rearrangements. The samples were sent for searching for clonal chromosome abnormalities in a routine setting. Only one patient (4%) had a cryptic 4q12 deletion associated with the FIP1L1-PDGFRA fusion. FISH was a reliable diagnostic test for differentiating the

F/P+ variant from other forms of HES or eosinophilia, thus allowing an early diagnosis of good responders to imatinib therapy.

P132

Sequence variations in glutamate transporter genes in patients with inherited ataxias

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Glutamate, the major excitatory neurotransmitter, is removed from the synaptic cleft by excitatory amino acid transporters (EAAT). Through their rapid and efficient function they play an essential role in maintaining extracellular glutamate levels below neurotoxic concentrations. Inherited ataxias correlated with cerebellar neurodegeneration could be a consequence of transmitter abundance. To answer the question whether patients with dominantly inherited ataxias have mutations within glutamate transporter genes, SSCP screening for EAAT1 and EAAT4 in nearly 100 unrelated samples was performed. Afterwards samples with conspicuous patterns were sequenced. Due to the fact that an EAAT1 missense mutation had been described previously in a patient with episodic ataxia we performed SSCP analysis of the nine coding exons and the flanking intronic regions of the EAAT1 gene. As a result we found a known polymorphism in exon 6 as well as five changes within the introns or the 3´-UTR. Three of these five variations represent single nucleotide exchanges, and the other two represent single nucleotide insertions. Overall three of the variations are not listed in public databases. Protein levels of EAAT4 - a Purkinje cell specific glutamate transporter - are reduced in patients with SCA5. To test the hypothesis whether EAAT4 mutations may cause another subtype of ataxia, we screened the nine exons of the gene. In one patient we actually found the mutation p.R25W. In addition, two frequent but silent variations (in exon 1 and exon 8) could be identified. Out of five variations within intronic parts flanking exons and the 3´-UTR, which are all frequent (5% to 25%), three are not described to date.
The results of our study suggest that mutations in EAAT1 or EAAT4 are not a frequent cause for dominant ataxias in our population.

P133

Screening of the KCNC3 gene in patients with autosomal dominant cerebellar ataxia (ADCA)

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The autosomal dominant cerebellar ataxias (ADCA) are a clinical and genetical heterogeneous group of neurodegenerative disorders. The main part of ADCA are spinocerebellar ataxias (SCAs). To date, 28 SCA loci have been described, and for at least fourteen the respective gene or mutation has been determined. In 2006, Waters and colleagues identified mutations in the KCNC3 gene that are associated with SCA13. The KCNC3 gene is localised on chromosome 19q13 and encodes a voltage-gated potassium channel, which is highly expressed in the cerebellum. They described two missense mutations in two unrelated families. The missense mutation R420H was found in a French family with childhood-onset ataxia. The missense mutation F448L segregated in a Filipino family with adult-onset ataxia. To determine the frequency of SCA13 among German SCA patients we screened 108 DNA samples from patients with dominant ataxia for mutations in the KCNC3 gene. Sequencing the four coding exons and flanking intronic sequences revealed the missense mutation R420H in two unrelated patients and the known polymorphism S546S in one patient. In addition, five novel DNA variations could be detected: the missense exchange D63G, a rare silent 1bp substitution and three intronic sequence variations. Three patients were heterozygous for the missense exchange D63G, whereas the rest of the patients showed homozygosity for the glycine isoform. Apart from the known mutation, no relevant sequence variation in the coding region of the KCNC3 gene was found. In conclusion, SCA13 represents a rare cause for SCA in patients of German origin.

P134

Familial Beckwith-Wiedemann Syndrome caused by novel mutations in the CDKN1C-gene

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Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder. Common cardinal features are exomphalos, macroglossia, and neonatal gigantism. Individuals with BWS have an increased risk of developing specific tumors during infancy.
Several complex genetic and epigenetic abnormalities, of which most occurred de novo, have been identified as the cause of BWS. In addition to epigenetic alterations of the KCNQ1OT1- and H19-regions, mutations in the CDKN1C-gene are assosiated with BWS. While rare in sporadic BWS cases CDKN1C- mutations were detected in about 40% of familial cases.
Here we report the results of molecular genetic analyses of individuals from two families with BWS. In family 1 the index case and his mother presented with clinical symptoms of BWS. Both carried a nucleotide change c.951A>G (p.X317Trp) in exon 3 of the CDKN1C-gene.
The index patient of family 2 had clinical symptoms of BWS and developed a tumor. For this patient the nucleotide change c.325G>T (p.Glu109X) in exon 2 of the CDKN1C-gene was detected. His mother, who was up to now not known to have signs and symptoms, is carrier of this mutation, too. Clinical features presented by the patients of the families investigated are discussed within the context of molecular results.

P135

Decreased expression of SRY and SOX9 and features of testicular dysgenesis syndrome in patients with frasier syndrome

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Frasier syndrome is characterized by chronic renal failure in early adulthood, varying degrees of gonadal dysgenesis, and a high risk for gonadal germ cell malignancies, particularly gonadoblastoma. Although it is known to arise from heterozygous splice mutations in intron 9 of the Wilms´ tumor gene 1 (WT1), the mechanisms by which these mutations result in gonadal dysgenesis in humans remain obscure. Here we show that a decrease in WT1+KTS isoforms due to disruption of alternative splicing of the WT1 gene in a Frasier syndrome patient is associated with diminished expression of the transcription factors SRY and SOX9 in Sertoli cells. These findings provide the first confirmation in humans of the results obtained by others in mice. Consequently, Sertoli cells fail to form the specialized environment within the seminiferous tubules that normally houses developing germ cells. Thus, germ cells are unable to fully mature and are blocked at the spermatogonial-spermatocyte stage. Concomitantly, subpopulations of the malignant counterpart of primordial germ cells/gonocytes, the intratubular germ cell neoplasia unclassified type (ITGCN), are identified. Furthermore, dysregulated Leydig cells produce insufficient levels of testosterone, resulting in hypospadias. Collectively, the impaired spermatogenesis, hypospadias and ITGCN comprise part of the developmental disorder known as ´testicular dysgenesis syndrome´ (TDS), which arises during early fetal life. The data presented here show that critical levels of WT1+KTS, SRY and SOX9 are required for normal Sertoli cell maturation, and subsequent normal spermatogenesis. To further study the function of human Sertoli cells in the future, we have established a human cell line.

P136

A novel family with Cooks syndrome, a rare inherited malformation of the limbs

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Cooks syndrome (OMIM %106995) is a rare autosomal dominantly inherited brachydactyly with hypo-/aplastic distal phalanges and nails. To date this condition has only been described in four pedigrees. Although the phenotypic spectrum of this entity shows partial overlap to brachydactyly type B (BDB), it seems to be a genetically distinct condition. Here we describe a novel family with Cooks syndrome from Egypt with three affected individuals in two consecutive generations. Affected individuals in this family are characterized by an absence of the nails of all digits including the thumbs as well as an absence of the toenails. All affected persons have prominent finger pads. In addition, X-ray examination showed an absence of the distal phalanges of the hands and feet. Direct sequencing of genomic DNA did not reveal mutations in exons 8/9 of the ROR2 as well as in the coding region of the NOGGIN gene causative for BDB. These findings suggest that Cooks syndrome is a genetic entity separate from brachydactyly type B. Further work is needed to identify the underlying molecular defect of Cooks syndrome.

P137

SOX9 micro- and macrodeletions are not uncommon in campomelic dysplasia

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Campomelic dysplasia (CD), an autosomal dominant skeletal malformation syndrome, results from mutations within SOX9, a 5.4 kb gene consisting of three exons, or from translocations interrupting the 1 Mb cis-regulatory domain upstream of SOX9. Only three cases with complete deletion of SOX9 have been reported, all several Mb in size, and one case with a 1.5 Mb deletion located 380 kb upstream of SOX9. We have screened 60 cases with clinically and radiologically confirmed (15 cases, group 1) or suspected CD (45 cases, group 2) for SOX9 deletions, using quantitative PCR. In these non-translocation CD cases, no SOX9 coding region mutation had been detected by sequencing. We found deletions in four cases of group 1. Two are microdeletions that removed exons 1 and 2 (2225 bp deletion) or exon 2 plus part of exon 3 (2177 bp deletion). Two are macrodeletions of 2.2 Mb and 4.4 Mb. Sequencing of breakpoint-spanning PCR products showed 3 bp homologies at the deletion junctions in three of these deletion cases. Short 2-6 bp homologies occur frequently at deletion junctions in the human genome. In group 2, three deletions of approximate sizes of 150 kb, 850 kb and 1.4 Mb all encompassing the SOX9 gene were detected, but due to sample limitation, their exact endpoints could no be delineated. Scanning the 1 Mb SOX9 upstream control region with amplicons spaced 100 kb apart in 11 group 1 and 23 group 2 cases did not uncover another upstream deletion case. In conclusion, SOX9 deletions are more frequent than previously supposed and can efficiently be detected by quantitative PCR, including deletions in the range of a few kb that go undetected by array CGH.

P138

Genotype-phenotype study in infantile spinal muscular atrophy type I - important information for future clinical trials

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Clinical trials in infantile spinal muscular atrophy type I (SMA I) are a matter of debate because of ethical issues and methodological problems. Current concepts include open trials with life span as the primary outcome measure compared with the survival statistics of a historical control group.
We reviewed the natural history of those SMA I patients born in Germany between 2000 and 2005

whose diagnosis was confirmed by the presence of a homozygous SMN1 deletion in the first 6 months of life in one of our laboratories (Aachen, Cologne, Würzburg). 174 patients fulfilled these criteria. The parents of 66 (38%) patients agreed to participate, completed questionnaires, gave consent to review medical reports and to DNA studies. Disease endpoint was defined by age at death or the age when either tracheostomy or permanent (>14 days) ventilation was performed. Survival statistics in the total cohort corresponded well with previous studies (median/mean age at death 6.7/9.0 months), the probability to survive the 2nd birthday was 13%. Prognosis of SMA I was largely dependent on SMN2 copy number. Four patients (6%) carried one SMN2 copy and had a neonatal/lethal SMA with congenital joint contractures and respiratory distress. The largest group (86%) with two SMN2 copies showed a variable disease course. Motor development was limited to head control in 7%. Median/mean age at onset was 1.2/1.3 months and median/mean age at disease endpoint was 6.5/7.7 months (range 0.5-30 months). Those 5 patients (8%) who retained three SMN2 copies of at least exon 7 had an onset at 3-4.5 months and a much better prognosis. One patient was ventilated from 19 months (current age 47 months), the others were alive at 10-55 months (median/mean age 30.1/31.4 months). Thus, the presence of three SMN2 copies was associated with a chronic or stabilizing disease course.
Our data are important for prognostication and genetic counselling in SMA I as well as for clinical trials.

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Activation of a cryptic splice site of the MTMR2 gene in Charcot Marie Tooth neuropathy type 4B1

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Charcot Marie Tooth neuropathies (CMT) comprise a group of clinically and genetically heterogeneous disorders. CMT4B1 is an autosomal recessive demyelinating type, that is characterized by focally folded myelin sheaths in the peripheral nerve. CMT4B1 is caused by mutations in the MTMR2 gene on chromosome 11q22. MTMR2 encodes the myotubularin-related protein-2, a phosphoinositide phosphate (PIP) phosphatase that dephosphorylates PI3P and PI3,5P2. Here we report a novel homozygous intron 4 acceptor splice site mutation (IVS4-2A>T) in the MTMR2 gene causing a severe form of CMT with irregular myelin outfoldings in a consanguineous Moroccan family. As exon skipping (the most frequently observed consequence of a splice site mutation in humans) would result in an in frame deletion of a repetitive protein sequence the pathogenic character of this mutation was not entirely convincing. Therefore, we explored the possibility that this mutation might have a less common but more detrimental effect. Sequence analysis of the exon 4/5 junction at the mRNA level showed a deletion of the first 19 nucleotides of exon 5. Inspection of the genomic sequence suggested that a cryptic splice site was activated in exon 5. On the protein level, this will result in a premature stop codon and a severely truncated, non-functional protein. Several distinct nonsense, frameshift and missense mutations have been reported in the MTMR2 gene. The MTMR2 mutation described here is the first splice site mutation identified in this gene. CMT4B1 is caused by loss of MTMR2 or its function and the consequences of the novel MTMR2 mutation are in line with this observation.

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Mutation analysis of SBMA using the HPLC

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Spinal and bulbar muscular atrophy (SBMA, Kennedy disease) is an autosomal recessively inherited neurodegenerative disorder. It is caused by a CAG-repeat expansion in the androgen receptor (AR) gene located on the X-chromosome (Xq12) that affects only man. The number of CAG repeats in normal individuals varies up to 36. In affected patients the expanded allele contains 38 or more CAG repeats and leads to an abnormal gene product. Thus, identification and exact determination of alleles on the molecular level is very important for diagnosis and prognosis of the disease. Most methods used for mutation analysis, are time consuming, laborious and expensive, such as fragment length

analysis by polyacrylamide slab gel electrophoresis (PAGE). The aim of our study was to develop a highly sensitive, automated and economical molecular method for characterization of the CAG repeat region based on high performance liquid chromatography (HPLC) using non-fluorescent PCR. We analyzed 25 patients - suspected of SBMA - from the DNA bank of the Institute of Psychiatry and Neurology in Warsaw (Poland) and the Institute of Human Genetics, University of Leipzig (Germany). As a control group were analyzed DNA from 11 patients with clinical diagnosis of Myotonic Dystrophy (MD) without mutation in DMPK gene. Following a novel PCR protocol, fragments enclosing the CAG repeat region were sized using HPLC in comparison to a 20 bp DNA standard. In order to evaluate the HPLC against one of the classical methods, the lengths of fragments were determined using PAGE and sequencing, too. Our results indicated highest accuracy and consistency of the data obtained with the HPLC method between 180 - 280 bp (± 2 CAG) and between 280 - 340 bp (± 3 CAG). We conclude that HPLC can be used as a highly sensitive and efficient alternative diagnostic method for fragment length measuring in spinal and bulbar muscular atrophy.

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Spectrum and frequency of SLC26A4 mutation among Czech patients with early nonsyndromic hearing loss

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Mutations in SLC26A4, the second most common cause of inherited hearing loss (after GJB2 mutations), causing Pendred syndrome (hearing loss with goiter) or DFNB4 (nonsyndromic hearing loss /NSHL/ with Enlarged Vestibular Aqueduct/EVA/or Mondini Dysplasia/MD/ on temporal bone CT). Aims of our study were to determine frequency and spectrum of SLC26A4 mutations in Czech patients with early NSHL. 238 unrelated patients /pts/ with early NSHL were stratified in 4 groups by expected probability of SLC26A4 mutations. We performed direct sequencing of all 21 exons in 174 pts from groups A-C.
In group A - 24 pts (EVA, MD and/or progressive HL) we detected 5 pts (21%) with biallelic SLC26A4 mutations and 6 pts (25%) with monoallelic mutation. In group B 18 pts (with affected siblings) we found 2 pts (11%) with 1 mutation and no biallelic patient. In group C - 132 pts (with indiferent clinical picture and no CT available) 4 different mutations in 4 pts (3%) were detected (no biallelic patient). In group D - 64 pts (with NSHL but normal CT) no sequencing performed.
The spectrum of the SLC26A4 mutations in Czech NSHL patients seems to be broad, conversely to few most prevalent mutations in GJB2 gene. The most frequent mutation was V138F (23% of all pathogenic alleles). The other mutations weren´t found more than twice (9%). Our clinical selection criteria for SLC26A4 analysis seem to be efficient and correct, as 100% of all biallelic and 55% of all monoallelic patients were from group A and the mutation detection rate in group C was very low. Among 174 pts tested we detected 14 different mutations. Biallelic mutations were found in 5 cases (3% of all pts) and at least one mutation was found in 17 pts (10%). Surprisingly high number (70%)of monoallelic mutations in typically featured pts is presumably due to second mutation undetectable by sequencing. These patients may carry a copy number abnormality detectable only by quantitative methods as MLPA.

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N-terminus of MCPH1 regulates chromosome condensation

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Primary microcephaly (MCPH) is a heterogeneous autosomal recessive disorder characterized by pronounced reduction of brain size and variable mental retardation without additional neurological deficits. Four genes have been identified to date whose biallelic mutations cause MCPH. Hallmark of patients with mutations in the MCPH1 gene (MIM# 606858; MIM# 607117) is a cellular phenotype of premature chromosome condensation, PCC, in the G2 phase of the cell cycle and delayed decondensation in G1. MCPH1 is located on human chromosome 8p23.1, consists of 14 exons, and encodes the protein microcephalin containing an N-terminal and two C-terminal BRCT (BRCA1 C-terminus)-domains as well as an NLS sequence. Retroviral transduction with full length cMCPH1 resulted in complementation of the PCC phenotype in MCPH1-deficient cells. The same result was obtained using MCPH1 variants lacking the C-terminal BRCT-domains (MCPH1-B) or a variant lacking exon 8 (MCPH1-S) and absent NLS. In contrast, a construct containing MCPH1 without the N-terminal BRCT-domain (MCPH1Δ1-7) was not able to complement the PCC phenotype.
Previously, a patient had been reported with a homozygous substitution (c.80C>G, Thr27Arg) localized in the N-terminal BRCT domain of MCPH1 with a low proportion of PCC (Trimborn et al., 2005). These data suggest a role of the N-terminal BRCT-domain in regulating chromosome condensation, possibly through interaction with condensin II. Further studies are underway to elucidate the exact mechanism of the regulation of chromosome condensation by microcephalin.

P143

TSPY expression is variably altered in transgenic mice with testicular feminization

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TSPY genes encode the testis-specific protein, Y encoded, and are expressed in premeiotic germ cells and round spermatids. The topology and timing of TSPY expression, and also its homology to members of the TTSN-family, suggest that TSPY is a proliferation factor for germ cells. There is also evidence for a role of TSPY in the aetiology of testis cancer. TSPY is a candidate for GBY, the elusive gonadoblastoma locus on the human Y chromosome, which is thought to predispose dysgenetic gonads of 46, XY sex-reversed females to develop gonadoblastoma. We have previously generated a TSPY transgenic mouse line that carries approximately 50 copies of the human TSPY gene on the mouse Y chromosome. In order to elucidate TSPY expression under complete androgen insensitivity and to investigate a possible role of TSPY in gonadal tumorigenesis, we have now generated sex-reversed TSPY transgenic Tfm mice hemizygous for the X-linked testicular feminization mutation. We can show that the TSPY transcript is aberrantly spliced in the testes of TSPY-Tfm mice, and that TSPY expression is upregulated by androgen insensitivity in some but not all animals. TSPY transgenic mice showed significantly increased testes weights. In one TSPY transgenic Tfm animal spermatogenesis was proceeding beyond meiotic prophase. No tumors of germ cell origin were found in the testes of TSPY-Tfm mice. Five out of 46 TSPY transgenic Tfm mice, and 3 out of 31 age-related NMRI-Tfm controls developed Leydig cell tumors, whereas none of the age-matched Tfm mice (n=44) on a wild type background were affected by Leydig cell tumorigenesis.

P144

Partial rescue of the Kit-deficient testicular phenotype in *TSPY-W^{v}/W^{v}* mice

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Human TSPY (**t**estis-**s**pecific **p**rotein, **Y**-encoded) is the product of a Y-chromosome-specific gene family located within the male specific region of the Y chromosome (MSY). *TSPY* expression is restricted to the testis where expression is limited to spermatogonia, spermatocytes and round spermatids. The *TSPY* expression pattern in germ cells and its homology to members of the TTSN-family indicate that TSPY functions as a spermatogonial proliferation factor.
In contrast to bovines and primates, where *TSPY* is organized as a repetitive gene family, the laboratory mouse harbours a single-copy pseudogene. We restored Tspy activity in a *TSPY* transgenic NMRI mouse line that carries a human *TSPY* transgene of approximately 50 copies on the

mouse Y chromosome. In this study, we generated *TSPY* transgenic W^{v}/W^{v} mice and analyzed the histology of their testes and epididymides in order to contribute to understanding TSPY function in spermatogenesis. The Kit receptor, and its ligand stem cell factor (SCF) play a fundamental role in hematopoiesis, melanogenesis and gametogenesis. Homozygous W^{v} mutant male mice on a C57BL/6 background with a mutation on *c-kit* are infertile because of the almost total loss of germ cells in testes. We examined the testes of 52 adult *TSPY*-W^{v}/W^{v} males and 37 age-matched controls (NMRI-W^{v}/W^{v}) histologically. In the testes of 29 out of 52 *TSPY*-W^{v}/W^{v} mice spermatocytes and spermatids were detected, while differentiated spermatids were observed in testes of 15 out of 37 NMRI-W^{v}/W^{v} mice. We identified sperms in the epididymides of 22 out of 50 *TSPY*-W^{v}/W^{v} mice and in 3 out of 34 investigated NMRI-W^{v}/W^{v} males ($P<0.001$). We observed fertility in 4 out of 28 *TSPY*-W^{v}/W^{v} male mice mating with wild-type females, whereas none of the controls (18 NMRI-W^{v}/W^{v} males) produced offspring. Taken together our findings show that TSPY is able to partially rescue spermatogenesis in W^{v}/W^{v} mutants, an effect that is influenced to some extent by the NMRI genetic background.

P145

Mutations in α-1 Tubulin (TUBA1A), a new gene for classical lissencephaly, refine the phenotype

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Classical or Type 1 lissencephaly is an autosomal dominantly inherited disorder of neuronal proliferation and migration which results in agyria or pachygria, a thickened cortex and may be accompanied by various other structural brain malformations. Patients with classical lissencephaly have severe psychomotor retardation and epilepsy. Mutations in the α-1a Tubulin (TUBA1A) gene have recently been found to cause classical (Type I) lissencephaly (Keays et al. 2007, Poirier et al. 2007). To date, TUBA1A mutations have been described in eight patients, including 3 foetuses, with relatively variable phenotypes. Our aim was to contribute towards defining the phenotype associated with TUBA1A mutation and to establish how common TUBA1A mutations are in patients with classical lissencephaly. We performed mutation analysis in the TUBA1A gene in 45 patients with a clinical diagnosis of classical lissencephaly and in whom no mutation was found in the LIS1, DCX or ARX genes. We have identified two new and one recurrent missense mutations in the TUBA1A gene in four patients with different forms of classical lissencephaly. All mutations had arisen de novo in the patients. All four patients had microcephaly, accompanied by variable cortical malformations. Two patients with a R422H mutation had had pachygyria, subcortical band heterotopia, and severe hypoplasia of the corpus callosum and cerebellum, whereas the other two patients with different mutations affecting the R402 codon had severe agyria-pachygyria, a thin corpus callosum and vermis hypoplasia. Although mutations in TUBA1A are not as common (approximately 4% in our cohort) as those in the LIS1 or DCX genes in patients with classical lissencephaly, mutation analysis in TUBA1A should be included in the molecular genetic diagnosis of type I lissencephaly, particularly in patients with a combination of microcephaly, hypoplasia of the corpus callosum and cerebellar vermis in addition to the agyria-pachygyria spectrum.

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Analysis for FGF14 mutations in german ataxia families

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Spinocerebellar ataxia type 27 (SCA27) is a rare autosomal dominant neurodegenerative disorder caused by mutations in the fibroblast growth factor-14 gene (FGF14). FGF14 is composed of 6 exons, two of which are alternatively spliced. To date, only two FGF14 mutations have been identified worldwide in single families, a missense mutation (F145S) and a 1-bp deletion. Clinical features of SCA27 are mild ataxia, dysarthria and nystagmus. Depending on the mutation, additional symptoms

such as tremor, cognitive impairment, depression, and mild sensory neuropathy can occur.
29 independent patients with a proven family history of ataxia and tremor were screened for mutations in the FGF14 gene. Other known SCA-loci (SCA1-3, 6-8, 10, 12-14, 17) had been excluded previously. No pathogenic exchange in the coding region of FGF14 was detected in any of the ataxia patients. In two patients, a novel G to A transition in intron 3 (IVS3+24G>A) of FGF14 creating a potential splice acceptor site was observed. Analysis of 100 healthy control samples revealed that the observed exchange is a neutral polymorphism.

P147

Detecting CNV in autosomal recessive limb girdle muscular dystrophies by a two-color MLPA assay

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Limb-girdle muscular dystrophies are a heterogenous group of autosomal recessively inherited disorders characterized by progressive weakness of the pelvic and shoulder girdle muscles. Apparently, early onset occurs combined with faster progression and more severe disabilities than in late onset cases. Mostly nucleotide substitutions, short deletions/insertions cause sarcoglycanopathy in the sarcoglycan alpha, beta, gamma and delta genes (SGCA, -B, -G, -D). Previously, deletions were reported in SGCG. In order to screen all 4 SGCA genes for duplications/deletions, we designed a two-color MLPA (multiplex ligation-dependent probe amplification) assay allowing fast and reliable quantification of up to 28 probes in a single reaction. Using synthetic probes, the method is suitable especially for cases where limited numbers of patients do not justify cloning procedures. We screened the sarcoglycan genes in 110 patients for the presence of pathogenic duplications or deletions. Exclusively those patients with confirmed clinical diagnosis were included, in which mutations had not been identified in both alleles. Several cases with one exclusive heterozygous point mutation showed deletion of the entire other allele for. One patient harbored a homozygous deletion of exons 2-5 in SGCG, and several patients exhibited homozygous deletions of single exons. Altogether, we identified a heterozygous and a homozygous deletion in SGCA as well as 6 heterozygous and a homozygous deletion in SGCG. Thus more deletions are evident in SGCG than in SGCA, reflecting different lengths (146.02 kb vs. 11.2 kb).
Large pathogenic deletions/duplications causing LGMD are comparatively rarely demonstrated. Nevertheless since the method is easily performed and reliable, screening for exonic deletions/duplications should be performed in patients where point mutations have not been identified in both alleles.

P148

Testis specific exon of human RNA helicase DBY (DDX3Y) is responsible for translational repression

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Human DBY (DDX3Y) gene belongs to the DEAD-box RNA helicase family and is localized to AZFa (AZoospermia Factor a) region of the Y chromosome (Yq 11.21). Deletion of this Y chromosome interval is found in men with severe testicular pathologies, including Sertoli-cell-only syndrome (SCO). We wanted to know how DBY is involved in spermatogenesis and whether its dysfunction can cause the observed SCO syndrome by analysing DBY function in human male germ cells. Although DBY is transcribed in a variety of tissues, translation is restricted to premeiotic male germ cells, namely spermatogonia and occasionally pre-leptotene/leptotene spermatocytes. We found, that this translational control is probably triggered by transcription from a second, testis specific promoter domain resulting in a testis specific long 5' UTR region. Interestingly, this long 5' UTR contains a novel exon (exon T) spliced in three different patterns with DBY exon 1. In order to elucidate how exon T is involved in the control of translation of the DBY protein, we developed a series of DBY reporter constructs (DBY minigenes) which include the N- terminal part of DBY (either with or without exon T) cloned upstream from EGFP/3xFLAG reporter tags. After transfection of the different DBY minigenes in mammalian 293-T cells, protein extracts were isolated and incubated with specific DBY and 3xFLAG antibodies on western blots. Repression of translation of the DBY-EGFP-3xFLAG fusion

protein was observed always and only when DBY exon T was part of the fused DBY transcripts. Interestingly, after mutation of the ATG initiation codons of four upstream open reading frames (uORFs) mapped in exon T, translational repression of the minigene was released and its efficiency became comparable to those DBY constructs which did not include exon T. DBY transcripts including exon T seems therefore to be inhibited to subsequent translation by the efficient translation of four small upstream peptides localized in exon T.

P149

Mouse Arfgef2 gene undergoes alternative splicing and is necessary in early embryonic development

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Mouse Arfgef2 gene encodes Big2 protein, which belongs to the guanine nucleotide exchange factor (GEF) family. This protein catalyzes the nucleotide exchange on ADP-ribozylation factors (ARF) switching ARF from inactive form (bond to GDP) to the active form (bond to GTP). All ARF-GEF family members contain a Sec7 domain build of about 200 amino acids which is a minimum unit for the catalysis. Proteins containing the Sec7 domain can be subdivided into two major classes, the BIG2 belongs to the class of high molecular mass (>100 kDa) proteins and is localized in the Golgi region. The protein is believed to be involved in membrane trafficking. In human mutations in the ARFGEF2 gen lead to the autosomal recessive periventricular heterotopia with microcephaly (ARPHM). Using the gene-trap strategy we could generate a mouse line with functional disruption of Arfgef2 gene. Heterozygous mutants did not demonstrate any phenotypic abnormalities thus we conclude an autosomal recessive inheritance. Using the LacZ reporter gene from the gene-trap construct we could demonstrate that this gene is expressed very early in mouse embryonic development. Using SNP markers we could demonstrate that Arfgef2 mRNA is stored in oocyte (maternal storage) and used during the first embryonic division. The embryonic Arfgef2 gene is activated first at the 4-cells stage. We also detected that Arfgef2 undergoes alternative splicing and the splicing pattern differs among tissues of adult animal. From the breeding of heterozygous animals we could detect homozygous syngamy, but not homozygous 2-cells stage embryos. Here we present the data demonstrating that BIG2 protein is necessary for the first embryonic division in mouse. Using the brefeldin A (BFA), a known inhibitor of BIG2, we could inhibit the first embryonic division in wild type mice what confirms our findings.

P150

FBN1, *TGFBR1*, *TGFBR2*, and *SLC2A10* mutation analyses in patients with suspected Marfan syndrome: A Swiss study

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Classical Marfan syndrome (MFS) is usually caused by *FBN1* mutations. Many of the features of MFS show overlap with related disorders such as MFS type 2 (MFS2), Loeys-Dietz aortic aneurysm syndrome (LDS), familial thoracic aortic aneurysms and dissections (TAAD), and arterial tortuosity syndrome (ATS). In classical MFS patients, *FBN1* genetic testing detects only ~80% of mutations. This may be due to technical limitations of currently used PCR-based screening methods and/or because the disease-causing mutation occurs in a different gene. Here, we investigated the clinical impact of these possibilities. In a cohort of unrelated individuals with MFS-like phenotypes in whom previous sequencing and/or DHPLC analysis of all 65 exons and flanking intronic regions of *FBN1* revealed no mutation, we sequenced the genes *TGFBR1*, *TGFBR2* (70 patients), and *SLC2A10* (50 patients). We also screened for large deletions/duplications (100 patients) by multiplex ligation-dependent probe amplification (MLPA). The pathogenic impact of novel sequence variants was assessed by in silico predictions and/or RT-PCR, and segregation analyses. The breakpoints of large deletions identified by MLPA were narrowed down by using the Affymetrix Human Mapping 500K arrays. In three MFS2, two LDS, and four TAAD patients, we identified heterozygous *TGFBR1* or

TGFBR2 nucleotide substitutions and in one patient with ATS a homozygous *SLC2A10* nonsense mutation. The deleterious alleles occurred de novo or segregated with the disease in the families, indicating a causative association between the sequence variants and clinical phenotypes. Neither a *TGFBR1*- nor a *TGFBR2*-specific phenotype could be detected. In two patients, MLPA revealed a large genomic rearrangement affecting *FBN1*. Our data demonstrate for the first time that *TGFBR1* mutations are associated not only with LDS but also with MFS2 and TAAD, and that true *FBN1* haploinsufficiency is sufficient to cause MFS.

P151

Identification of interaction partners of the peroxisomal male germ cell-specific gene Pxt1

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The presence of peroxisomes in male germ cells was negated for many years until 2006. Therefore, the role of peroxisomes in different steps of spermatogenesis is poorly understood. We have identified a male germ cell-specific expressed gene, named Pxt1, with expression starting at the spermatocyte stage during mouse spermatogenesis. The PXT1 protein sequence contains a conserved NHL motif, which is characteristic for peroxisomal proteins. The cellular localization of PXT1-GFP fusion proteins and co-localization experiment of the PXT1 protein with known peroxisomal markers confirmed the hypothesis that the Pxt1 gene encodes for a peroxisomal protein. Furthermore, we could demonstrate that point mutations introduced in the conserved NHL sequence results in diffused cellular localization of PXT1-GFP fusion protein. To further elucidate the role of the Pxt1 gene in spermatogenesis a yeast two-hybrid assay was performed. By using a testis cDNA library we could identify putative interaction partners for the mouse PXT1 protein. Here we present data about two of them, namely the kinesin family member 9 (KIF9) and the HLA-B associated transcript 3 (BAT3). Our data strongly indicate that both proteins interact with PXT1 in mouse testis.

P152

Autosomal recessive hereditary spastic paraplegia with thin corpus callosum and/or cognitive deficits: clinical characterization and linkage analysis

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Aims: Hereditary spastic paraplegias (HSPs) comprise a clinically and genetically heterogeneous group of neurodegenerative disorders with progressive spasticity of the lower limbs. Based on the mode of inheritance (autosomal recessive, autosomal dominant and X-linked) and the linkage data, to date, 36 distinct genetic loci have been described, 15 genes could be identified. The aim of this study is the further clinical and molecular genetic characterization of families with autosomal recessive complicated HSP (AR-HSP) with thin corpus callosum (TCC) and/or cognitive deficits.
Methods: Neurological examination, evaluation of a clinical questionnaire and the HSP rating scale (SPRS) for a total of 32 families with AR-HSP plus TCC and/or cognitive deficits. Linkage analysis to the loci SPG7, 11, 14, 15, 20, 21 and 26.
Results: 4 consanguineous families from Turkey were compatible with linkage to SPG11 and subjected to a candidate gene analysis to identify the SPG11 gene. In 3 families data were compatible with linkage to SPG7, SPG20 or SPG21, respectively. A subgroup of 10 families with AR-HSP and TCC and/or cognitive deficits, not linked to either SPG7, SPG11, SPG20 or SPG21, were further characterized. Clinical evaluation revealed mean age at the time of first signs of spasticity around 1-4 years of age. Linkage analysis was compatible with linkage of one large Turkish family to SPG14 with a LOD score of 2.2 at D3S1601. So far, only one family with linkage to SPG14 had been reported (Vaza et al., 2000). In the remaining 9 families no clear evidence for linkage to any of the analyzed 6 loci was observed.
Conclusion: The subgroup of AR-HSP with thin corpus callosum and/or cognitive deficits is genetically heterogeneous. Further neurological and genetic evaluation of these families might reveal, if certain loci are associated with distinct clinical features and contribute to our understanding of the molecular mechanisms underlying hereditary spastic paraplegia.

P153

Bardet-Biedl syndrome: Novel interaction partners of BBS proteins in centrosomes

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Bardet-Biedl syndrome (BBS) is a rare, developmental disorder characterized by postaxial polydactyly, rod-cone dystrophy, central obesity, renal dysplasia, learning difficulties, and hypogonadism. It is a striking example of locus heterogeneity with at least 12 genes (BBS1 - BBS12) linked to the disease. Following an autosomal recessive mode of inheritance in general, the phenotype seems to require three mutations in two genes in some families. The proposed model of “triallelic inheritance” suggests BBS as a link between Mendelian and complex phenotypes.
BBS shares clinical features with prevalent traits such as obesity, diabetes and coronary heart disease - thus possibly providing important clues to the understanding of those common diseases.
Current data suggest a functional disturbance in ciliary function and intraflagellar transport being associated with the phenotype. However, the precise functions of the BBS proteins have yet to be unravelled.
Here, we report on our efforts to identify new interaction partners of selected BBS proteins applying yeast-2-hybrid technology:
i) We present novel plausible binding partners including enzymes (ALDOB), transcription factors (PAX2), centrosomal proteins (EXOC7), and components of the cytoskeleton .
ii) We show by immunohistochemistry, which of the putative interacting factors co-localize with BBS proteins in centrosomes.
iii) We describe direct interactions between BBS proteins and corroborate these findings by chemiluminescent immunoprecipitation.
The biological relevance of our findings for the pathophysiology of BBS will be presented and discussed.

P154

Developmental analysis of a calvarial ossification defect associated with a midline brain malformation in the “short digits” (Dsh) mouse mutant

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The radiation induced mouse mutant short digits (Dsh) harbors an 11.7 Mb inversion of the Sonic hedgehog (Shh) locus, which dislocates cis-regulatory long-range enhancers from the Shh promoter. Dsh/Dsh mice are characterized by a holoprosencephaly and strongly resemble Shh-/- mice. Heterozygous Dsh/+ mice show a brachydactyly, a calvarial ossification defect and a midline brain malformation. Whereas the Dsh/+ limb phenotype is caused by late Shh overexpression in the digital anlagen, the developmental mechanism of the craniofacial and brain phenotype is so far unclear. We performed a detailed developmental analysis of the Dsh/+ skull and brain at adult and various embryonic stages. In adult Dsh/+ mice the posterior frontal, sagittal and coronal sutures did not fuse properly, leading to a large ossification defect. At E17.5, the expression of Collagen1 and the osteoblast markers Runx2 and Osterix was reduced in the calvarial osteogenic fronts. Beginning at E15.5 we observed a delay of mineralisation of calvarial neural crest derived skull bones. To trace back the origin of the skull defect we are currently analyzing the expression of neural crest markers at earlier stages. In addition to the skull defect, the mutant exhibits a brain malformation that includes a trifurcation of the telencephalic midline and a dysgenesis of the corpus callosum starting at E18.5. Using the markers Ror-beta, Cux2 and ER81 we show that the cortical layering outlines the midline defect, but it is not perturbed. In a comprehensive expression analysis of Shh and the axon guidance molecule Semaphorin 3C (Sema3C), residing at the centromeric site of the Dsh inversion, we were not able to identify any relevant expression differences between wt and Dsh/+. These findings suggest that the Dsh inversion may potentially lead to deregulation of additional genes in the inversion interval.

P155

A novel locus for bilateral microphthalmia, corneal opacity and blepharophimosis at Xq27.3-qter

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Severe malformations of the eye such as anophthalmia and microphthalmia are often apparent at birth and are caused by disturbances in the normal development. Several genes for the anophthalmia and/or microphthalmia phenotype have been identified, however, the cause of most human ocular malformations is unknown. Here we report a 4-year-old girl with severe bilateral microphthalmia, corneal opacity and blepharophimosis. Routine chromosome analysis revealed a de novo terminal Xq deletion, with the karyotype 46,X del(X)(q27.2). To delineate the deletion breakpoint, we performed haplotype analysis in the patient and her parents using various polymorphic markers located in Xq27-q28. The patient was heterozygous for marker DXS8043 (Xq27.3), however, she carried only maternal alleles for two more distally located markers, DXS8045 and DXS1200 (both in Xq27.3). These data suggest that (i) the terminal Xq deletion breakpoint is located between markers DXS8043 and DXS8045 and (ii) the chromosomal rearrangement occurred on the paternal X chromosome. The size of the deletion was estimated to be ~11 Mb. By X chromosome inactivation (XCI) studies we identified a random pattern of XCI in peripheral blood cells of the Xq deletion patient. Two X-linked loci have previously been established for anophthalmia, one in Xp21.2-p11.4 (ANOP2) and another in Xq27-q28 (ANOP1). For ANOP2, heterozygous loss-of-function mutations in BCOR, encoding a transcriptional repressor, were identified in females with the oculo-facio-cardio-dental syndrome and a BCOR missense mutation was detected in affected males of the family that mapped to ANOP2. The ANOP1 locus partially overlaps with the deleted interval of our patient, encompassing ZNF275 that is a candidate for this ocular malformation as it encodes a putative transcription factor. In the future, mutation analysis of this gene will unravel whether it is implicated in microphthalmia/anophthalmia.

P156

Cellular consequences of *NSDHL* or *EBP* missense mutations

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Mutations in *NSDHL* encoding a 3ß-hydroxy-steroid dehydrogenase enzyme functioning in the cholesterol biosynthetic pathway are associated with CHILD syndrome (MIM 308050), an X-linked dominant, male-lethal trait characterized by a strictly lateralized ichthyosiform nevus as well as ipsilateral hypoplasia of the body including limb defects. The CHILD phenotype associated with missense mutations appears to be caused by loss of function, possibly by a dominant negative effect. However, these missense mutations exchange highly conserved amino acids located along the protein, outside the predicted functional domains of the enzyme (co-factor binding site, catalytic site, transmembrane helix).
Chondrodysplasia punctata (CDPX2, MIM 302960), a phenotypically similar but distinct X-linked dominant, male lethal trait, involves mutations in a delta8-delta7 sterol isomerase (emopamil binding protein, EBP). Missense mutations in *EBP* are frequently found to exchange three specific amino acids.
To compare cellular consequences of missense mutations detected in CHILD patients with artificial mutations changing critical amino acids of predicted functional sites, we cloned human wild type or mutated *NSDHL* into expression vectors to generate fusion proteins with GFP and DsRED. In cells transfected with these constructs, wild type protein is localized at the surface of lipid droplets (LDs) and on the ER. CHILD-specific mutant NSDHL is either localized as the wild-type protein or distributed evenly in the cytoplasma. Mutations induced in enzymatically relevant domains do not alter the normal cellular localization of the protein. In contrast, constructs expressing either wild type or mutated EBP are not accumulated on lipid droplets.
These findings suggest that both a block in cholesterol biosynthesis and disturbed intracellular traffic or signal transduction might contribute to the phenotype of CHILD syndrome, whereas loss of the enzymatic role of EBP alone might be critical in CDPX2.

P157

Multiple enhancer elements within the introns control spatiotemporal expression of *GLI3*

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During development, the products of the *GLI* gene family translate signals of the hedgehog proteins (HH) into specific patterns of gene expression. Their co-ordinate function appears to determine a *GLI*-code, which, e.g. in the limb, governs pattern formation in anterior-posterior direction and which is similarly employed to pattern a great variety of other tissues. Malfunction of HH signaling is associated with tumorigenesis. Factors controlling the localized and timely expression of *GLI* genes and their targets are unknown.
We demonstrate that a sequence element encompassing the minimal promoter region can drive reporter gene expression in transgenic mice. Evolutionary comparison of genomic *GLI3* and flanking sequences had identified highly conserved sequences residing exclusively in introns. The majority of these non-coding elements activated expression of a luciferase transgene in cell culture, whereas the others served as repressors or showed no specific regulatory capacity. The elements activating the reporter gene in cell culture were also able to function as enhancers of a reporter gene in transgenic mouse embryos, whereas the elements which did not activate in transiently transfected cultured cells, likewise, were unable to enhance reporter gene expression in transgenic mice. Each of the conserved sequence elements determined localization, quantity, and time course of a specific part of the established *GLI3* expression pattern.
The identification of sequence elements controlling *in cis* the expression of *GLI3* contributes to the understanding of pattern formation via hedgehog signaling, and confirms that evolutionary sequence comparison can detect successfully *cis* acting genomic elements.

P158

Uniparental isodisomy as a cause of Miyoshi myopathy

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Autosomal recessive limb girdle muscular dystrophy 2B and Miyoshi myopathy - a rare distal form of muscular dystrophy - are caused by diallelic mutations in the dysferlin gene on chromosome 2p. Until now no exceptions from this rule are proven, although a certain percentage of patients with a confirmed dysferlinopathy in a muscle biopsy has only one mutation. This could be due to a hidden second mutation in the non-coding regions of this huge gene (genomic size about 250kb). No recurrent mutations exist, and individual mutations are distributed over all the 55 coding exons. The large number of polymorphisms is posing an additional difficulty for diagnosis rendering the gene unsuitable for high throughput mutation screening methods and a candidate for direct sequencing.
We report on a male patient, aged 27 years, with a severe Miyoshi myopathy phenotype and small stature. Immunohisto- chemically dysferlin was absent. Mutation analysis detected a homozygous deletion of exons 37-40 in the dysferlin gene. Both parents were tested: Only the mother carried the mutation in a heterozygous state. Haplotype analysis in the family with polymorphic DNA markers flanking the dysferlin gene revealed a maternal haplotype only and a loss of paternal alleles in the patient. More comprehensive SNP genotyping in the patient using an Illumina 300k bead chip showed homozygous signals only for all SNPs on chromosome 2, pointing to a uniparental isodisomy of the entire chromosome 2. Two neighbouring SNPs within the dysferlin gene came out with null signals revealing the homozygous deletion. Using this information, the size of the deletion was estimated to between 3.9 and 14.8kb. Obviously the observed genotype causes the severe phenotype.
Our case demonstrates the importance of controlling for diallelic inheritance in autosomal recessive diseases, especially if rare homozygous mutations in non- consanguineous families are involved.

P159

Systematic evaluation of genetic defects in 220 patients with Tetralogy of Fallot and identification of a novel mechanism of TBX1 mutations

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Tetralogy of Fallot (TOF) is the most common complex congenital heart defect accounting for about 5-6% of patients. Various chromosomal anomalies, microdeletion 22q11.2 and mutations in NKX2.5 have been described as recurrent causes. In order to address the question about the incidence of these known causes in a larger cohort and to obtain further prove for the role of JAG1 and TBX1 mutations as causative for isolated TOF and the 22q11.2 microdeletion phenotype as reported in single families respectively, we performed chromosomal analysis, 22q11.2 microdeletion testing and sequencing of NKX2.5, JAG1 and TBX1 in a cohort of 220 unselected patients with TOF. 17 patients (7,7%) showed the common 3 Mb microdeletion 22q11.2, 8 patients (3,6%) had trisomy 21, 3 (1,2%) had other chromosomal aneusomies. Two patients (1%) each had known mutations in NKX2.3 and JAG1. Two patients showed rare variants in TBX1, one of which did not show any functional alteration in a transcriptional reporter assay. The second leads to elongation of a c-terminal polyalanine stretch and results in reduced transcriptional activity. We could also show an increased aggregation tendency for the mutant protein. This observation delineates a novel pathomechanism of TBX1 mutations.
Our results confirm that microdeletion 22q11.2 and trisomy 21 are the most common causes of TOF, while mutations in NKX2.5, JAG1 and TBX1 are only rare causes. In addition to TBX1 haploinsufficiency and point mutations resulting in gain of function or loss of nuclear localization signal, we could identify an additional pathomechanism of TBX1 mutations resulting in protein aggregation due to polyalanine expansion.

P160

RNA and protein expression of *EFNB1* mutation c.614_615delCT in CFNS patient fibroblasts

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The *EFNB1* gene (OMIM 300035) encodes ephrin-B1 protein. Mutations in this gene cause craniofrontonasal syndrome (CFNS; OMIM 304110). The spectrum of mutations includes nonsense, missense, frameshift and splice-site mutations. *EFNB1* gene consists of five exons. Here we describe a c.614_615delCT mutation of *EFNB1* that was found in a CFNS female patient. This deletion leads to a frameshift, causing a premature termination codon (PTC) in exon 4. Subcultured patient fibroblasts were analysed by reverse transcription polymerase chain reaction (RT-PCR) and restrictional enzyme digestion analysis. RT-PCR showed that this PTC does not elicit nonsense-mediated mRNA decay (NMD) of the mutant transcript. This probably occurs because PTC appears closer than 25 nucleotides to 3‘- end of exon 4. In order to determine whether this mutant transcript will give rise to a truncated ephrin-B1 protein, Western blot analysis was performed. In contrast to presence of wild type and mutant type RNA in patient fibroblasts, only wild type but not a mutant type ephrin-B1 protein was detected using a polyclonal anti-human ephrin-B1 antibody. From this we conclude that truncated ephrin-B1 is rapidly degraded in the patient fibroblasts. Absence of mutant protein shows that mutation c.614_615delCT probably has neither dominant-negative nor gain-of-function effects but rather loss-of-function effects.

P161

Transcriptional profiling of embryonic and spermatogonial stem cells

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Stem cells have the capacity to self-renew and the ability to generate differentiated cells. Until recently Embryonic Stem Cells (ESCs) which are derived from the inner cell mass of blastocysts were the only known pluripotent cells. In 2006 we reported that also Spermatogonial Stem Cells (SSCs) isolated from adult mouse testis are pluripotent. SSCs retain their pluripotency in culture, are able to differentiate into derivatives of the three germ layers in vitro, generate teratomas in immunodeficient mice and when injected into blastocysts show germline transmission in chimaeras. To further define SSCs as pluripotent cells we compared ESCs and SSCs at the transcriptional level by peforming DNA-microarray experiments. For this we isolated RNA from ESC and SSC cell lines, amplified it by in vitro reverse transcription and hybridized the samples to microarrays containing 44000 genes (Agilent 44K Whole Genome Microarray). Scanned arrays were analyzed to identify fold changes in gene expression between the cell lines. The results of the microarray experiments concerning the expression of several randomly chosen genes were validated by quantitative Real Time PCR. We

found that about 43000 genes are equally expressed in ESCs and SSCs, only 938 genes have a more than fourfold difference in expression. As compared with ESCs, in SSCs 594 genes were upregulated and 344 genes were downregulated. Depending on the pathways these genes are included selected genes will be further characterized. The analysis of more than 50 pluripotency marker genes revealed no significant differences in expression between both types of cells. Our results demonstrate that the transcriptional profiles of ESCs and SSCs are very similar which supports that they both are pluripotent cells. We now promote differentiation of both cell types by retinoic acid and compare their RNA profiles. It can be assumed that the profiles obtained from this could provide indications for new pluripotency-related genes.

P162

MLPA analysis of patients with Marfan syndrome

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Marfan syndrome (MFS) is an autosomal dominant hereditary disorder of connective tissue. Cardinal manifestations include proximal aortic aneurysm, ectopia lentis, and involvement of the skeletal system. The clinical diagnosis is based on a set of well-defined clinical criteria, the Ghent nosology. Heterozygous mutations in FBN1 (15q21.1, 65 coding exons), which codes for the protein fibrillin 1, are the main cause of Marfan syndrome. Overlapping syndromes such as the Loeys-Dietz syndrome or Marfan syndrome type II (MFSII) are caused by mutations in TGFBR1 or TGFBR2, genes coding for the transforming growth factor beta receptor type I and II, respectively. More than 600 entries are listed in the FBN1 mutation database, about 66% of them are missense mutations, whereas large deletions only have a proportion of 0.8% (www.umd.be:2030).
We used MLPA (multiplex ligation-dependent probe amplification) to search for large deletions in the FBN1 gene in a number of 45 patients. The patients fulfilled the diagnostic criteria (Ghent nosology) or had one major criterion and involvement of a second organ system at least. All were tested negative for a mutation in the genes FBN1, TGFBR1 and TGFBR2. We identified 3 large deletions in the 3' region of the FBN1 gene; ranging from exon 50 to 54, exon 55 to 58 and exon 58 to 63 (the last one was previously described by Singh et al., J. Mol. Cell. Cardiol. 2007). A fourth deletion comprised the complete FBN1 gene. Breakpoints of the deletions were determined by long-range PCR techniques. Implications for the resulting protein product, as well as the phenotypes of the patients will be discussed.
We estimated a MLPA detection rate of 9% in this pre-screened patient group, corresponding to an overall rate of 3% among all patients with MFS. Our data suggest that the fraction of large deletions in patients with MFS is higher than previously expected, indicating an inclusion of MLPA analysis in the routine diagnostics of Marfan syndrome.

P163

Identification and characterization of two different large deletions of the MLYCD gene in an affected girl

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Defects in the MLYCD gene are the cause of malonyl-CoA decarboxylase deficiency, a rare autosomal recessive disorder characterized by malonic aciduria, developmental delay, seizure disorder, hypoglycemia, short stature and cardiomyopathy. The gene comprises five exons and spans 17 kb on chromosome 16q23-24. Standard mutation analysis starting with amplification of the coding region prior to sequencing failed to amplify the two formats of exon 1 of the MLYCD gene in our patient, suggesting a homozygous deletion of this region. Using TaqMan real time-PCR it was shown that both parents - as expected - were heterozygous for a deletion of exon 1. Genomic walking via RT-PCR however revealed the differences in position and size of the deletions. On the maternal defect allele the deletion comprises the whole MLYCD gene with a 5` breakpoint about 3 kb upstream of exon 1 and a 3` breakpoint about 13 kb downstream of the transcript end, thus summarizing to a deletion of about 33 kb. On the paternal defect allele the deletion comprises 19 kb of the 5`UTR region plus exon 1 with a 3` breakpoint nearby exon 2, thus summarizing to a deletion of about 27 kb. The analysis of the breakpoint regions revealed that both deletions are probably the result of illegitimate crossing over between Alu sequences flanking the deleted regions.

Taken into account the recently published data of Salomons et al, who expanded the MLYCD analysis setup by MLPA and identified four large deletions in nine patients (J Inherit Metab Dis 30: 23-28), this type of mutation turns out to be a frequent cause of MLYCD deficiency.

P164

Is TIEG1 a maternal imprinting factor for human chromosome 15?

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Maternal imprinting of the PWS/AS region in 15q11-q13 is likely to involve *trans*-acting factors that bind to the AS-SRO element of the bipartite imprinting centre. In a yeast one-hybrid screen of a human ovary cDNA library we had identified three independent partial cDNA clones for the TGFβ-inducible early gene 1 (*TIEG1*). TGFß signalling plays an important role in oocyte maturation. To further analyse the role of TIEG1, we used two different approaches. With an electrophoretic mobility shift assay we confirmed the binding of TIEG1 to the AS-SRO and narrowed down the core-binding site of TIEG1 to six bp (GTGGGA). We also found that TIEG1 can bind to an Sp1 site in the AS-SRO. Furthermore, we carried out a methylation analysis in offspring of female *Tieg1*$^{-/-}$ mice. If TIEG1/Tieg1 played a role in maternal imprinting, female *Tieg1*$^{-/-}$ mice should be unable to establish a maternal imprint in the germ line. As a consequence the offspring should have an imprinting defect and therefore an unmethylated maternal copy of the *Snrpn* promoter/exon1 region. Methylation analysis at the *Snrpn* locus was performed by bisulfite cloning and sequencing. Thirteen CpGs in approximately 20 clones were analysed by sequencing. We found no evidence for abnormal methylation in any of the four pubs studied. However the control of maternal imprinting may not be conserved between mice and humans. In fact, several studies have failed to detect an AS-SRO in the mouse. Furthermore, it is possible that Tieg2 and Tieg3 can compensate for Tieg1 deficiency.

P165

CTFR mutational analysis: comparison between InnoLipa 17-Tn/19 and a new Chip-based HairLoop CF test

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Cystic fibrosis is one of the most common hereditary diseases worldwide. In Germany, 1 in 2,500 newborn children is affected by the disorder that is caused by mutations within the cystic fibrosis conductance regulator gene. To date, more than 1,000 mutations are known, many of them are rare mutations, occurring as spontaneous alterations affecting only one family or individual.
Recently, we were able to show that the Innogenetics InnoLipa test does not allow discrimination of the mutations R117H and R117P. In order to evaluate a new Chip-based test system, the HairLoop CF test, we have compared both systems with a panel of patient samples of known mutational status. The Innogenetics test comprises 36 CFTR-related mutations, the HairLoop CF test includes 49 mutations. Here, we describe the results of this comparative approach.

P166

Proteasomal turnover of the human hahirless protein

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Mutations in the human hairless gene (HR) are responsible for complete congenital hair loss within the first weeks of life (atrichia congenita, MIM 209500 and MIM 203655) indicating an essential role of HR for the perpetuation of hair cycling. HR acts as a transcriptional corepressor for thyroid hormone receptors (TR), the orphan nuclear receptor α, and the vitamin D receptor. We have previously identified a direct interaction between HR and the 20S proteasome subunit α-7 suggesting a proteasome mediated degradation of HR. Our objective was to obtain further insights into the mechanisms of HR proteasome interaction as a step towards a better understanding of hair cycle regulation. Fusion of the interacting domain of HR to the C-terminus of EGFP showed a significantly

destabilization of the protein in cell culture. The modified EGFP could be stabilized in presence of proteasome inhibitors to confirm the proteasome specific effect. Further investigation concentrated on the ubiquitin dependency of HR degradation. Since we were not able to detect ubiquitin conjugates at immunoprecipitated HR and inhibition of multi-ubiquitin chain formation showed no stabilizing effects on HR, we postulate that direct interaction between HR and the 20S proteasome leads to an ubiquitin independent degradation of HR.

P167

The transcription factor TRPS1 interacts with the RINGfinger ubiquitin ligase ARKADIA

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Mutations or deletions of the *TRPS1* gene on human chromosome 8q24.1 cause the tricho-rhino-phalangeal syndromes (TRPS), which are characterized by craniofacial and skeletal abnormalities. The gene encodes a transcription factor that functions as a repressor for GATA-mediated transcription. The activity of transcription factors is often controlled by post-translational modifications. And in fact, we have recently found that SUMOylation of specific sites within the repression domain (RD) of TRPS1 regulates its function as repressor. In a yeast-two-hybrid screen we could identify two clones encoding amino acids (aa) 352-505 of the 986 aa protein ARKADIA. ARKADIA is a RINGfinger ubiquitin ligase. In mice, Arkadia is known as a key regulator in the TGF-beta pathway by inducing the ubiquitin-dependent degradation of Smad7, SnoN and c-Ski. By using a variety of truncated TRPS1 and ARKADIA constructs we could narrow down the ARKADIA-binding region within TRPS1 to the last 100 aa, which includes the RD. ARKADIA appears to interact with TRPS1 via two different regions, which were also described to enable the interaction of ARKADIA with SMAD7. Luciferase reporter gene assays were used to demonstrate that ARKADIA decreases the repressional activity of TRPS1, which may indicate an ARKADIA-induced degradation of TRPS1.

P168

Combined mRNA and miRNA expression profiling of the cpfl1 mutant - a mouse model of cone dystrophies

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The cpfl1 mutant (cone photoreceptor function loss 1) is a mouse model carrying mutations in the cone specific phosphodiesterase 6C (pde6c). The phenotype is characterized by a loss of cone photoreceptor function and a progressive degeneration of the cones. To elucidate the biological events leading to the loss of photoreceptors we combined mRNA expression experiments with whole genome miRNA expression profiling.
Expression analysis of cpfl1 and wildtype retinas in 2 age stages was performed using Affymetrix MOE 430 2.0 microarrays. Differential regulated transcripts with a minimum change in expression level of 1.5 fold (p-value ≤ 0.05) were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. To verify the data 11 transcripts were analyzed by qRT-PCR using the LC 480 system.
338 transcripts were differentially regulated in the retinas of 4 week old mice and 223 in those of 8 week old mice. There was an overlap of 30 % between both experiments. A large number of genes encoding proteins involved in phototransduction were down regulated. Gene regulation networks revealed misregulation of genes associated with cell death, proliferation and gene expression. All the transcripts chosen for Real-time validation could be verified. In the miRNA array analysis of 4 week old mice we found differently regulated miRNAs which have potential target genes included in the differential gene list of our previous transcriptional analysis. Among these is miR182 which has been shown to be expressed extensively in retinal tissue and has 8 potential target genes including immune responsive elements. The expression analysis of the cpfl1 mutant highlighted a clear misregulation of phototransduction in accordance with the loss of visual function that characterizes the phenotype. The combination of mRNA and miRNA expression profiling permits a closer monitoring of the neurodegenerative events in the retina occurring during the degeneration.

P169

New 2p21 deletion in hypotonia-cystinuria syndrome

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The hypotonia-cystinuria syndrome (HCS; OMIM 606407) is an autosomal recessive disease associated with deletions of SLC3A1 and PREPL on chromosome 2p21. The main clinical features of this contiguous gene syndrome include a generalised hypotonia at birth, failure to thrive, growth retardation and cystinuria. Meanwhile 13 patients with HCS have been reported, they all were homozygous for deletions in 2p21. The patients carried five different deletions, two of which (deletions “A” and “B”) are globally distributed. Sizes of deletions range from ~38 to ~127 kb and both SLC3A1 and PREPL are always homozygously deleted. We describe a boy with cystinuria and further clinical signs of HCS. Based on the biochemical diagnosis of cystinuria, we initially screened for SLC3A1 and SLC7A9 point mutations. However, we did not detect any sequence variation but PCR amplification of exons 2 to 10 of the SLC3A1 gene failed repeatedly. Taking the addititonal clinical findings of the patient into account, we then assumed that he was homozygous for a large deletion affecting exons 2 to 10 of the SLC3A1 gene and the closely linked PREPL gene. We therefore checked the patient´s DNA for genomic imbalances by a 500K SNP array (Affymetrix). Indeed, we confirmed a deletion in 2p21. We could show that one allele corresponds to the previously described 38 kb deletion (deletion B). The second allele carries a large deletion that has not been described before. Both alterations affect SLC3A1 and PREPL.Our case illustrates that large deletions in the SLC3A1 region contribute to the cystinuria phenotype and that they have to be considered in cystinuric patients with additional symptoms, among them hypotonia.

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***FOXG1B*, a candidate gene for microcephaly with simplified gyration**

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Disease-associated, balanced chromosomal aberrations are powerful tools for gene identification. We report two patients with an overlapping phenotype and chromosome translocations with a common breakpoint region in 14q12. Female patient 1 carries a *de novo* t(6;14) with breakpoints in 6q22.32 and 14q12. She presents with congenital microcephaly, mental retardation, and short stature. By array comparative genome hybridization a heterozygous *de novo* deletion of ~3.4 Mb in 3q23 has also been identified in this individual. Female patient 2 has postnatal microcephaly, frontal simplified gyration, seizures, mental retardation, and a gastroesophageal reflux. She carries a balanced *de novo* 2;14 translocation [46,XX,t(2;14)(q12.1;q12)]. Delineation of the breakpoint regions by fluorescence *in situ* hybridization revealed that the two breakpoints in 14q12 mapped within a gene-poor region, however, both breakpoints are located downstream of the *FOXG1B* gene. *FOXG1B* encodes a winged helix transcriptional repressor which is mainly expressed in brain and regulates early steps in cortical development. Recently, haploinsufficiency of *FOXG1B* has been proposed to be associated with microcephaly, mental retardation and other brain malformations. The 14q12 breakpoint of patient 1 was found to map ~1.26 Mb distal to *FOXG1B*, while that of patient 2 is located ~265 kb away from the 3 end of *FOXG1B*. These data may suggest that a position effect of these chromosomal rearrangements altered expression of *FOXG1B*. *In silico* analysis using the Regulatory Potential score identified several candidate *cis*-regulatory elements that are evolutionary conserved throughout the ~1.3 Mb region downstream of *FOXG1B*. We hypothesize that dissociation of one of the potential downstream *cis*-regulatory elements from *FOXG1B* by the translocation breakpoints might be associated with the disease phenotype of patient 1 and/or 2.

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Search for mutations in the PLAGL1/ZAC1 gene in Silver-Russell syndrome patients

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Silver-Russell syndrome (SRS) is a heterogeneous disease which is associated with pre- and postnatal growth retardation, asymmetry and craniofacial dysmorphisms. In 7-10% of SRS cases a maternal uniparental disomy of chromosome 7 can be detected, more than 38% of patients carry a hypomethylation of the imprinting region 1 (ICR 1) in 11p15. These chromosomes harbour the genes IGF2, H19, LIT1, GRB10 and MEST whose products interact with PLAGL1/ZAC1, an imprinted gene in 6q24 involved in the control of embryonic growth. Aim of this study was the identification of genomic variants in the PLAGL1/ZAC1 gene in SRS. We screened 30 SRS patients by SSCP and/or direct sequencing, among them 15 carriers of 11p15 hypomethylation, and additional 14 patients with isolated growth retardation. To identify genomic imbalances we carried out realtime-PCR approaches. Mutation analyses revealed 4 genomic variants. Whereas two of them have been reported in the databases (rs2076684 and rs9373409), two are novel: the variant g.52790C/T in exon 1 was detected in similar frequencies in the study cohort and in the control group (n=44). In one SRS patient, heterozygosity of the variant g.9567G/T in the non-coding exon 3 was detected but needs further characterisation. The realtime-PCR analysis does not reveal any evidence for a genomic imbalance in the PLAGL1/ZAC1 gene in SRS patients. Furthermore sequencing analysis of exon 6 of PLAGL1/ZAC1 is not yet finished. However, our preliminary results do not indicate a relevant role of mutations in PLAGL1/ZAC1 in the aetiology of Silver-Russell syndrome.

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Diagnostic algorithm in Silver-Russell syndrome: Update and „rare“ findings

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Silver-Russell syndrome (SRS) is a heterogeneous syndrome which is characterised by severe intrauterine and postnatal growth retardation and typical dysmorphisms. Until recently the clinical diagnosis could be confirmed only in ~10% of patients carrying a maternal uniparental disomy of chromosome 7 (UPD7). With the identification of ICR1 hypomethylation in 11p15 and the establishment of MLPA based approaches for its confirmation, the detection rate for (epi)genetic alterations increases to ~50%. Additionally single patients carry chromosomal aberrations. Based on our data from a large cohort of SRS patients we now propose the following diagnostic procedure: The first step consists of a 11p15 methylation-specific (MS) MLPA which allows the detection of the ICR1 hypomethylation as well as of genomic imbalances in this region, thus covering all types of SRS-specific (epi)mutations in 11p15. Subsequently a MS-PCR for the PEG1/MEST locus in 7q31 should follow. The finding of maternal UPD7 should be confirmed by microsatellite typing to exclude isolated imprinting defects. However, screening of our cohort of 54 SRS patients did not reveal any evidence for isolated imprinting defects. Interestingly, confirmation of UPD7 in additional 11 positively tested patients showed segmental maternal UPD7q31qter in two of them. We have to consider that the MS-PCR test is restricted to a narrow region in 7q but all (segmental) UPD7 cases identified so far would have been detected. After exclusion of 11p15 hypomethylation and UPD7 cytogenetic analyses should be considered. In conclusion the aforementioned diagnostic algorithm allows the confirmation of the clinical diagnosis in ~50% of SRS patients. Preliminary clinical characterisations indicate that 11p15 epimutation carriers show a more typical phenotype than the UPD7 patients. However, the spectrum of clinical features is variable and thus all patients with suspected SRS should be tested for 11p15 hypomethylation and UPD7.

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Protection of transgenic swine fibroblasts from human complement-mediated lysis by the expression of human CD46, CD55 and CD59

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The complement system is a part of the immune system engaged in non-self recognition and destruction. The rapidly growing shortage of organs for transplantation can be overcome 3using genetically modified animal organs. The expression of human complement regulators such as CD46, CD55 and CD59 has been proved to be an effective strategy to overcome hyperacute rejection of the discordant organs in xenogenic transplantations. To protect swine cells from human complement-mediated lysis three different genetic constructs have been prepared. These constructs contained EF-1α promoter and respectively the coding sequences of CD46, CD55 or CD59. Swine fetal fibroblasts were transfected using these constructs were grown under blasticidine selection conditions. Stable lines were molecularly characterized for an integration of transgenes by PCR. Lines with a stable integration of transgenes were subjected for further characterization of expression by RT-PCR. All cell lines expressing transgenes were used to test hypotheses that the expression of one or three human complement regulators (CD46, CD55 or/and CD59) would provide effective protection to the swine fibroblasts. Each transgene was expressed in single transgenic line and had protective effect on the tested cells in human serum cytotoxicity assay. Also in triple transgenic lines the expression of the transgenes had a wide positive impact on the protection of cells from human complement-mediated lysis, but this effect was not additive. Lines with high protective effect can be used to obtain transgenic animals by somatic cloning. Organs from transgenic animals expressing CD46, CD55 and CD59 human complement regulators will be protected from hyperacute rejection after grafting.

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Analysis of the physiological role of Insulin-like factor 5

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Insulin-like factor 5 (INSL5) belongs to the insulin superfamily. To elucidate the physiological role of the gene in vivo, gene targeting strategy was used to generate mice deficient in INSL5 protein. INSL5-deficient mice were born at expected ratio, were viable and fertile. Phenotypic analyses showed no major alteration in organ morphology, physiology, and in most clinical chemistry parameters. In nociceptive tests, INSL5-deficient mice showed a significant delay in response to hot plate and increased paw flick latency in Hargreaves test. However, nociceptive responses to noxious stimuli in the tail flick test and mechanical stimuli in the von Frey test were normal. These results suggest that INSL5 plays a role in pain perception by acting at the supraspinal level. These findings were supported by expression of INSL5 in central nervous system. INSL5 showed a particular dense expression in brain areas concerned with the central mediation of antinociception such as anterior hypothalamus/preoptic area, the ventral nuclei of the periaqueductal gray, and the pontine Kölliker-Fuss nucleus. Moreover, the INSL5 expression in pain processing regions of brain such as medullary raphe nuclei, lateral reticular nucleus and nucleus of the solitary tract further strengthen the idea that INSL5 might be an important neuropeptide of central pain pathway.

P175

Are H19 mutations involved in Silver-Russell syndrome?

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(Epi)mutations in 11p15 are associated with the primordial growth retardation disease Silver-Russell syndrome (SRS) and with the overgrowth disorder Beckwith-Wiedemann syndrome (BWS). In 11p15 two imprinting control regions (ICR1 and ICR2) regulate the expression of 14 imprinted genes. More than 35% of SRS patients show a hypomethylation at the ICR1. The ICR1 is paternally methylated and regulates the expression of the oppositely imprinted genes IGF2 and H19. The function of the untranslated RNA H19 is still unknown. However, the finding that H19 is a gene with a relative highly conserved sequence among mammals indicates a profound functional relevance. Due to the supposed function of the H19 sequence in the regulation of the imprinted region 11p15 we searched for mutations in this gene in 59 SRS patients.
We detected three SRS patients with so far unknown variants in the transcribed region of H19. In two cases (SR17; SR81) different 3 bp deletions in exon 1 could be identified (g.8616_8618delGGG;

g.8818_8820delAGG (AF087017)). Patient SR93 carried a 39 bp duplication affecting exon 2 and intron 2 (g.9867_9906dup39). All three variants were not detected in controls and are localised in evolutionary conserved regions. SR93 additionally showed a hypomethylation of the ICR1.
We performed splicing as well as expression analyses to figure out the functional consequences of these mutations. Splicing studies revealed a deviation from the normal H19 splicing behaviour in two cases (SR81; SR93). Expression analysis on blood lymphocytes carried out in SR93 did not verify an altered expression pattern of H19.
Nevertheless, our results indicate a relevant role of H19 mutations in the aetiology of SRS: functional effects of these variants, e.g. chromatin restructuring of the ICR1 or an altered function of the antisense RNA, are well conceivable and make further investigations of the biological role of H19 necessary.

P176

Spatacsin (SPG11): a new gene for complicated hereditary spastic paraplegia with thin corpus callosum

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Aims: Hereditary spastic paraplegias (HSPs) comprise a clinically and genetically heterogeneous group of neurodegenerative disorders with progressive spasticity of the lower limbs. We have recently characterized in detail the phenotype of two German pedigrees with AR-HSP with thin corpus callosum (TCC; Winner et al. 2004 and 2006) and could narrow down the SPG11 minimal critical region to a 2.93 cM interval with a maximum LOD score of 11.84 (Oelmez et al., 2006). Recently, KIAA1840 could be identified as the spatacsin gene, associated with SPG11 (Stevanin et al., 2007). We assessed the long-term course and mutational spectrum of SPG11.
Methods: Clinical examination, brain MR imaging and linkage analysis, sequence analysis of 30 candidate genes from the SPG11 locus.
Results: Spastic paraplegia in patients with spatacsin mutations (n=20) developed during the second decade of life. The spastic paraplegia rating scale (SPRS) revealed severely compromised walking between the second and third decade of life (mean SPRS score >30). Impaired cognitive function was associated with a severe atrophy of the frontoparietal cortex, TCC and bilateral periventricular white matter lesions. Progressive cortical and thalamic hypometabolism in the FDG-PET was observed. Sural nerve biopsy showed a loss of unmyelinated nerve fibers and accumulation of intraaxonal pleomorphic membranous material. Mutational analysis of spatacsin revealed 6 novel and one previously reported frameshift mutation, two novel nonsense mutations and the first two splice mutations to be associated with SPG11.
Conclusions: We demonstrate that not only frameshift and nonsense mutations, but also splice mutations result in SPG11. Mutations are distributed throughout the spatacsin gene and emerge as major cause for ARHSP with TCC associated with severe motor and cognitive impairment. The clinical phenotype and the ultrastructural analysis imply a disturbed axonal transport of long projecting neurons.

P177

Characterization of an 1.7 Mb X-chromosomal deletion in a patient with McLeod syndrome by array CGH

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Introduction: McLeod syndrome (MLS) results from mutations in the XK gene on Xp21.1 and affects haematology and the central and peripheral nervous systems in middle-aged men. 32 different mutations have been described to date. In addition, at least 8 large multi-gene deletions encompassing the XK locus have been described. The description of the phenotype of the carriers of

multi-gene deletions can give an insight into the function of the deleted genes.
Patients and methods: Index patient is a 58 year old man from Finland diagnosed with MLS, who is member of an extended family. Exon-specific PCR of the XK gene and sequence tagged site(STS)-PCR was used for initial identification and preliminary characterization of the deletion encompassing the XK gene. By array CGH using the X chromosome chip from NimbleGen® the size of the deletion was defined more precisely.
Results: PCR of the exons of the XK gene suggested a deletion spanning the whole XK gene. Testing of 20 sequence-tagged sites (STSs) on Xp21.1 gave evidence for a deletion including the region between stFSX915 and XK, excluding CYBB. The array CGH experiment allowed the definition of the breakpoints with an accuracy of ± 4 kilobases (kb).
Conclusion: At least 10 genes of unknown function are co-deleted in this patient, including LOC139604, FAM47C, PRRG1, and LANCL3.

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Novel mutations in the NHEJ1 gene in patients with a Nijmegen Breakage Syndrome-like phenotype

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Patients with clinical and cellular features of Nijmegen Breakage Syndrome but without mutations in the NBN gene are classified as NBS-like patients. Some of these patients have mutations in the DNA Ligase IV, a gene involved in the NHEJ DNA repair. Recently, a new member of the NHEJ DNA repair pathway, NHEJ1, was discovered and mutations in 6 patients with features resembling NBS or LIGIV syndromes were described. Here we report on 4 patients from 3 families of different ethnic origin with the NBS phenotype but no mutations in the NBN or LIG/V genes. Sequence analysis of the NHEJ1 gene in a patient of Spanish origin revealed the homozygous mutation R57G. Two novel, paternally inherited truncation mutations - 495insA and R176X, both in exon 4, were found in a heterozygous state in one German and in one Malaysian patient, respectively. Further analysis of the gene failed to uncover the second mutation in both patients. RNA analysis in the German patient indicated an aberrantly spliced in-frame transcript with skipped exons 2 and 3. Direct sequencing of introns 1, 2 and 3, detected only polymorphisms but no mutation which could have explained the aberrant splicing. Haplotype analysis showed that the aberrant transcript was a consequence of a large genomic deletion. Quantitative PCR revealed a large maternal genomic deletion of 1, 9 kb between introns 1 and 3. Analysis of the younger brother of the German patient, who presented with mild immunodeficiency and no apparent microcephaly, showed that he was also a compound heterozygote for both mutations. Similar to the German family, haplotype analysis suggested the second allele in the Malaysian patient to be also a genomic deletion. Quantitative PCR revealed a large deletion leading to a loss of 6, 9 kb of genomic sequence between 5'UTR and intron 3. Our findings confirm that patients with NBS- / LIGIV-like phenotypes may have mutations in the NHEJ1 gene including multi-exon deletions.

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Expression and functional analysis of a novel member of the immunoglobulin superfamily

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Members of the immunoglobulin (Ig) superfamily play important roles in intracellular adhesion, which is critical for many events including tissue patterning, morphogenesis and maintaining of normal tissue. We have isolated a novel member (SX) of immunoglobulin superfamily. Expression analysis revealed that the 3-kb of SX transcript is highly expressed in stomach. A putative splice variant of 1.5-kb was detected in testis. No SX transcript could be detected in other adult tissues. The cellular distribution of SX in embryonic stomach was determined by immunohistochemical analysis. At E13.5, SX expression

was detected in the epithelium of posterior region and was absent in the epithelium of anterior region of stomach. In E14.5 and E17.5 stomach, a much stronger expression of SX was observed in primordial buds of the glandular gastric epithelium located in posterior region. The temporal and spatial profile of SX expression suggests a potential role of the SX in regulation of cytodifferentation of stomach epithelium. Expression analysis of SX in testis revealed that the expression of 3-kb SX transcript is restricted to Sertoli cells, while the 1.5 kb SX transcript is restricted to haploid spermatids. Immunohistochemical analysis on sections of adult testis revealed that SX-protein is located in the Sertoli-Sertoli cell junctions (Ssj) as well as in sertoli-spermatid cell junction (Sspj). To clarify the in vivo function of SX, we have generated SX-null ES cells and chimeric mice and determined the role of SX in differentiation of gastric epithelium.

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The Parkinson's disease causing gene LRRK2 influences actin cytoskeleton signalling cascades

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The Parkinson's disease key player LRRK2 (leucine-riche repeat kinase 2) accounts for 10% of familiar and 1-4% of sporadic Parkinson's disease cases. Although a lot of research was done, the impact of this protein in biological signalling cascades is only rare known. It was shown that LRRK2 is able to reduce neurite process length when overexpressed with mutations or enforces the process length when it is reduces on cellular level. In our whole genome expression analysis of LRRK2 siRNA transfected human dopaminergic cells, we supported these results with the identification of differentially expressed transcripts in the canonical pathways: Axonal guidance signalling, actin cytoskeleton signalling and integrin signalling. The differentially upregulated genes of these pathways are CDC42 (cell division cycle 42), ARHGEF7, ARHGEF9, ARHGEF12 (rho guanine nucleotide exchange factor 7,9,12), BMP2 (bone morphogenetic protein 2), GNAO1 (G protein alpha activating polypeptide O), CHRM3 (cholinergic receptor muscarinic 3), FGF2 (fibroblast growth factor 2), ITGB5 (integrin beta 5) and the differentially downregulated genes CXCR4 (chemokine receptor 4) and SEMA4F (semaphorin 4F). Due to the fact that CDC42 is an actin polymerisation enforcing gene and ARHGEF7 is responsible for the activation of CDC42 with GTP we investigated the colocalisation of both proteins with LRRK2 on protein level under endogenous and overexpressed conditions in SH-SY5Y cells. The analysis of apoptosis following cytochalasin D (inhibitior of actin polymerisation) and nocodazole (disruption of microtubule) treatment in LRRK2 expressing cells pointed on LRRK2 influence on actin cytoskeleton regulation. We further analysed the influence of LRRK2 mutations on the polymerisation of actin by using a pyrene-actin fluorimetric assay. The results support the hypothesis that LRRK2 has great impact on actin cytoskeleton signalling - a major aspect of the regulation of neurite process length.

P181

High intra- and postsurgical opioid requirement in Morbus Crohn is not due to a general change in pain sensitivity or common variants in the ORPM1 and COMT gene

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Crohn's disease (CD) is a painful inflammatory bowl disease with a complex multigenic trait. CD patients require significant higher intra and postoperative morphine doses than patients undergoing a comparable severe abdominal surgery. Therefore, Crohn's disease is supposed as a suitable model for the identification of novel pain susceptibility genes. In order to elucidate the underlying molecular mechanisms and involved genes, we investigated whether the higher opioid uptake of CD patients is due to a general change in pain sensitivity and whether common, well known modulators of opioid efficacy in CD patients are involved in this phenomenon. We therefore investigated polymorphisms in the μ-opioid receptor (ORPM1) and catecholamine-O-methyltransferase (COMT) and applied quantitative sensory testing (QST) to a subgroup of patients. Our results demonstrate that CD patients

do not show an altered general pain sensitivity as detected by QST. In addition, we could exclude a common variant in OPRM1 and specific “high pain sensitivity” COMT haplotypes as the cause of the high opioid requirement in CD patients. These results point to a more complex pathway involved in the higher postoperative opioid demand in CD and the presence of other, yet unknown, genes modulating opioid uptake in CD patients.

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Glioma-amplified sequence 41: Target gene identification by siRNA-mediated knockdown and cDNA microarray analysis in a glioblastoma multiforme cell line

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Gliomas are characterized by karyotypic abnormalities like numeric chromosome aberrations or double minutes which may influence therapy or survival time. Gene amplification is a characteristic event in human gliomas and is associated with tumor stage and progression. The chromosomal region 12q13-15 is amplified in approximately 15% of astrocytoma and glioblastoma multiforme. In this amplification unit, we identified twelve amplified and expressed genes including Glioma amplified sequence 41 (GAS41). This highly conserved nuclear protein belongs to the YEATS family of transcription factors.
By yeast-two-hybrid screening we identified binding partners of GAS41. Two of them, NuMa (nuclear mitotic apparatus protein) and KIAA1009 are known to be involved in mitotic spindle assembly and chromosome segregation. We showed by immunofluorescence analysis that both proteins co-localize with GAS41 in a stable glioblastoma cell line containing the 12q13-15 amplification unit. To shed light on the molecular function of GAS41 in glioblastoma multiforme, we performed a GAS41 knockdown by small interfering RNA in this cell line. Human whole genome cDNA microarrays identified several target genes of GAS41 that are involved in cell cycle regulation and mitosis.

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Reduced level of Gal epitope on the surface of pig cells expressing human alpha1,2-fucosyltransferase

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Flow cytometry analysis was performed to measure the impact of CMVFUT transgene containing human gene encoding α1,2-fucosyltransferase enzyme (HT, H transferase) under the human cytomegalovirus (CMV-IE) immediate early promoter on reducing the level of Gal epitope on the surface of transgenic pig cells. Staining with the BS-IB4 lectin (specific for Gal antigen) and UEA-1 lectin (specific for H antigen) followed by flow cytometry analysis revealed a reduced level of Gal antigen and increased level of H antigen on the cell surface of transgenic pigs relative to control. The influence of human complement was measured by testing the sensitivity of nontransgenic and transgenic cells to complement-mediated cytotoxicity upon exposure to human serum. Human serum served as a source of complement. The control nontransgenic cells were effectively lysed by human complement. In contrast to the control, transgenic cells from homozygote, as well as from heterozygote were protected from lysis by human complement. Obtaining transgenic animals lacking on the cell surface Gal epitope molecules which are exposed for binding with xenoreactive anti-Gal antibodies will lead to the end of the first part of study of the transplantation of animal’s organs to the humans.

P184

The TRPS1 transcription factor regulates *HSPG2* expression

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The TRPS1 transcription factor, containing a unique combination of zinc finger (Znf) motifs including a GATA-type and IKAROS-like Znfs, can act as repressor of GATA-mediated gene transcription.

Mutations in the *TRPS1* gene cause the trichorhinophalangeal syndrome (TRPS), which is characterized by craniofacial and skeletal abnormalities. Expression profiling in limbs of *Trps1*$^{+/-}$ mouse embryos revealed an upregulation of the *HSPG2* gene. *HSPG2* codes for perlecan, a component of the extracellular matrix with an essential role in cartilage formation and development of joints and skeleton. Overexpression of *HSPG2* is associated with defects in the growth plates of developing long bones and the articular surface of joints. Thus, an increase of perlecan may be responsible for some of the clinical features such as cone-shaped epiphyses or early-onset osteoarthritis in TRPS.
To investigate whether *HSPG2* is a direct target of TRPS1, we performed reporter gene assays. Initially, the entire human 2.7 kb promoter of *HSPG2* was fused directly to the ATG of the firefly luciferase gene in pGL3 to activate luciferase expression. The *HSPG2* promoter contains nine putative GATA-1 binding sites. Five other potential protein binding sites are located in the 461 bp core promoter (Iozzo *et al*. 1997). Coexpression of the reporter gene construct with a wild-type *TRPS1* expression plasmid led to a significant reduction of the luciferase activity, suggesting a direct regulation of *HSPG2* by TRPS1. Even if all GATA-1 and four of the five other protein binding sites were deleted from the reporter construct the luciferase activity was repressed by TRPS1. These findings exclude the nine GATA-1 binding sites and half of the core promoter as targets for the repressional activity of TRPS1. On the other hand, we observed an increased luciferase expression upon coexpression of a *TRPS1* construct with a missense mutation in the GATA-type Znf, indicating an important role of this Znf for *HSPG2* regulation.

P185

Identification of the first duplication mutation in the human LIPH gene in a Turkish family with hypotrichosis simplex

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Hypotrichosis simplex (HS) is a rare form of hypotrichosis which has been reported to follow both an autosomal dominant as well as an autosomal recessive mode of inheritance. HS affects men and women equally with variable extent of hair loss. A small number of genes are known for HS, namely corneodesmosin, desmoglein 4, lipase H (LIPH) and P2RY5.
The LIPH gene was recently identified as being responsible for autosomal recessive hypotrichosis in 50 families from the Volga-Ural region of Russia. The hair growth in affected individuals is retarded or arrested, resulting in short hair lengths. Histopathological studies showed an abnormal morphology of hair follicles, dystrophic and fragile hair. Two deletion mutations have been identified so far in exon 4 and in exon 2.
In this report, we describe a familiy of Turkish origin comprising two individuals with clinical features suggestive of HS. We therefore hypothesized that the affected members may carry a mutation in the LIPH gene. After sequencing the complete coding region of the LIPH gene in the index patient, we identified a homozygous duplication mutation of 30 amino acids in exon 2 (c.280_369dup; p.Gly94_Lys123dup) that could not been find in 320 control chromosomes of Turkish origin. Here, we describe the first duplication mutation in the human LIPH gene. Our study emphasizes the variability of different types of mutations in the LIPH gene in the molecular diagnostics of patients with HS.

P186

Towards understanding the role of the polyglutamine binding protein 1 (PQBP1) in mental retardation

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We have found that mutations in the polyglutamine binding protein 1 (PQBP1) gene cause X-linked mental retardation (Kalscheuer et al., 2003; Cossee et al., 2006). Up to date, a total of 13 families with a mutation in this gene have been described. Identical and similar mutations resulted in high clinical variability, ranging from moderate mental retardation (MR) to much more severe forms, including microcephaly, short stature and spasticity.
The identification and characterisation of novel PQBP1 interaction partners is a logical starting point to

gain more insight into its cellular functions and to unravel the pathomechanism of the disease. As a prerequisite we have established a neuronal cell line, which stably expressed tagged human PQBP1 protein. We have used this cell line to pull-down the PQBP1 complex followed by ESI-TOF mass spectrometry. This endeavour has led to the identification of 15 candidate interaction partners. Interestingly, some of these have been shown to be part of neuronal RNA granules, which play a role in the localisation and translation of specific mRNAs in dendrites and synaptic plasticity. Interaction of PQBP1 with several of the newly found binding partners, including polypyrimidine tract-binding protein-associated splicing factor (PSF), DEAD box polypeptide 1 (DDX1) and KH-type splicing regulatory protein (KSRP) has been confirmed by co-immunoprecipitation experiments. In addition, we could show that in mouse embryonic cortical neurons endogenous and overexpressed PQBP1 localises to the nucleus and the cytoplasm and co-localises with its interactors in the same cytoplasmic granules. Currently, we are characterising the PQBP1 containing granules in more detail. Our findings suggest that PQBP1 might play a role in RNA localisation, transport and local protein translation in neurons.

P187

Mutations in the newly identified 13th FA gene, FANCI

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Fanconi anemia (FA) is a heterogenous disease with today 13 known underlying genes (FANCA, B, C, D1, D2, E, F, G, I, J, L, M and N). FA subgroup FA-I was first reported in 2004 by Levitus and colleagues, who delineated this group by somatic cell fusion. Recently, FANCI was identified by three groups independently (Dorsman et al., 2007; Sims et al., 2007; Smogorzewska et al, 2007). Our approach (Sims et al, 2007) recognized proteins with the highly conserved monoubiquitination site LVIRK, which might be substrates of the FA nuclear core complex. A back search for homology to FANCD2 extending beyond the LVIRK motif resulted in the identification of only one protein, KIAA1794. KIAA1794 was a previously uncharacterized protein with 1328 aminoacids and a molecular weight of 140 kDa. Monoubiquitination occurs at Lys523 and depends on the FA core complex and on the presence of functional FANCD2. KIAA1794 and FANCD2 show a weak but specific interaction. KIAA1794 also forms nuclear foci in response to DNA damage where it resides with FANCD2 and phosphorylated H2AX. In this respect, FANCI appears as a non-complementing ortholog of FANCD2 in the signaling of DNA-interstrand crosslinks. The identification of biallelic mutations in this gene led to the conclusion that KIAA1794 is FANCI. Here we describe the mutations in KIAA1794/FANCI of the four patients assigned to FA-I by Sims et al (2007) and of two patients identified thereafter. The mutation spectrum includes nonsense mutations, frameshifts, splice and missense mutations, an in-frame deletion and a start codon mutation. Given the key role of FANCD2 in the FA/BRCA pathway and the close connection between FANCD2 and FANCI, careful mutation analyses in FANCI will be very important. Main points of interest include whether residual protein levels are required and whether pseudogenes of FANCI exist that might be problematic for mutation analysis.

P188

Molecular genetic characterization of restrictive dermopathy

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Restrictive dermopathy (RD) is a lethal human genetic disorder characterized by very tight, thin, easily eroded skin, rocker bottom feet, and joint contractures. So far, mutations in ZMPSTE24 (FACE1) and LMNA have been associated to restrictive dermopathy. ZMPSTE24 encodes a zinc metalloprotease, which is involved in the post-translational processing of prelamin A - a gene product of LMNA - to mature lamin A, an intermediate filament component of the nuclear lamina. So far we have investigated 14 unrelated families including 38 individuals with RD for mutations in ZMPSTE24 and LMNA by heteroduplex analysis and direct sequencing. The pathogenic effects of the mutations were

confirmed by segregation analysis, population study and comparison with locus specific databases (www.umd.be; www.dmd.nl). In our sample, no mutation in LMNA was found in a RD patient. Among the 38 individuals, 28 individuals have been found to carry mutations in ZMPSTE24. Three frame shift mutations, c.50delA, c.209_210delAT and c.1085 -1086insT, have been found resulting in non-functional truncated ZMPSTE24 peptides. The ZMPSTE24 mutation c.1085 -1086insT is most common with a frequency of 57%. ZMPSTE24 c.50delA and c.209_210delAT are novel mutations. Interestingly, the first missense mutation c.1385T>G resulting in an exchange of a highly conserved amino acid (p.L462R) was found in a clinically unequivocal case of restrictive dermopathy. The mutation was heterozygous in the patient and would not explain the disease by itself because the not-affected mother is heterozygous too. So, a second pathogenic mutation should be expected. Unfortunately, there was no more patient material available to look for a second mutation in the patient. Using the ZMPSTE24 mutation c.1085 -1086insT as a direct molecular marker, so far five prenatal diagnoses have been requested resulting in the diagnosis of three homozygous affected and two homozygous unaffected fetuses.

P189

Copy number screening in hereditary spastic paraplegia genes

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The hereditary spastic paraplegias (HSPs) are a group of disorders defined by spasticity and weakness of the legs as the major features. They are genetically heterogeneous: 36 loci have been described and 15 disease genes have already been identified. Until recently, the spectrum of known mutations in these spastic paraplegia genes (SPGs) largely consisted of “small” alterations, i.e. changes affecting one or a few basepairs. Using multiplex ligation-dependent probe amplification, we screened some of the more frequent genes for copy number alterations. For SPG4, the most commonly involved gene, we established partial deletions as a major mutational class accounting for 20-25% of cases. We also identified one instance of a partial duplication which, surprisingly, is associated with sex-dependent penetrance of the HSP phenotype. For SPG3A, the deletion of one copy had no phenotypic effect implying a pathogenic mechanism other than haploinsufficiency. The consequences of a recently identified SPG3A amplification are currently being investigated. For SPG6, both deletions and duplications were found. Segregation analysis, however, argue against these variants to be of relevance in HSP. In contrast, our cases with deletion of SPG7 or duplication of SPG31 are highly likely to be pathogenic. We conclude that copy number aberrations in SPGs are a hitherto underestimated cause of HSP. Screening for this kind of mutation may not only reveal diagnostic information but also help defining relevant pathomechanisms.

P190

Genotype/phenotype correlation in DFNA4: a new mutation in the MYH14 gene

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For hereditary hearing impairment, over 250 genes are expected to participate in the pathogenesis, however, only a small fractions of them were identified so far. Following linkage mapping in several DFNA4 pedigrees resulting in the candidate region 19q13.33 a causative gene was identified: MYH14. It codes for one of the heavy chains of the class II nonmuscle myosins. At first, three missense and one nonsense mutations as well as a de novo allele in a sporadic case were reported with absence of alterations in control individuals. In a new large consanguineous Turkish family a microsatellite segregation analysis was performed with a significant lod score (4.1) pointing to the DFNA4 locus. Subsequent mutation screening of the 42 exons containing MYH14 gene yielded a heterozygous mutation in exon 31 (c.4210C>A). This leads to a change in amino acid composition (p.1404R>S) within the myosin tail of MYH14 peptide and represents a de novo finding. In silico analysis of this mutation suggests a change from basic/positively charged to a polar amino acid containing two

hydroxy groups with a likely structural effect. Taken together, our four informative families display mutations occurring in a spread fashion at peptide positions 7, 120, 726, and 1404. In all cases a serine residue is involved. While the stop codon mutation (S7X) results in a severe-to-profound hearing loss a reported series of affected individuals and some small families with silent or intronic mutations display mild-to-moderate hearing impairment. At present, the clinical outcome and the precise nature of mutation are being correlated using our and other published data.

P191

Unexpected functional consequences of the novel *HRAS* p.E37dup mutation in a child with some features of Costello syndrome

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Costello syndrome (CS) is a rare congenital disorder characterized by failure to thrive, craniofacial dysmorphisms, cardiac and skin abnormalities, mental retardation, and tumor predisposition. Most of the CS-affected cases have a heterozygous mutation in *HRAS* leading to exchange of glycine at position 12 or 13. H-Ras is a small GTPase and cycles between an inactive, GDP-bound and an active, GTP-bound state. In the latter case, H-Ras binds to effector proteins that stimulate various downstream signaling pathways. The G12/G13 sequence alterations in H-Ras cause impaired GTP-hydrolysis. Recently, we identified a *de novo* 3-bp duplication in *HRAS* leading to a doubled glutamic acid (p.E37dup) in a patient with CS-overlapping features. E37 is involved in stabilization of the switch regions which mediate the conformational change depending on the bound nucleotide. Interestingly, H-Ras guanine nucleotide binding and kinetics are not affected by p.E37dup as we determined by molecular modeling and biochemical assays. E37 is also essential for H-Ras interaction with various binding partners, including effectors. In line with this, computational analysis indicates a defect of H-Ras E37dup in effector binding. We used the H-Ras effector proteins RAF1, PI3K, PLCε, and RALGEF to specifically pull-down active H-Ras from cells overexpressing various H-Ras protein variants and demonstrated markedly impaired interaction of H-Ras E37dup with any effector tested. Although this data suggest inhibition of H-Ras downstream signaling, we found evidence for increased phosphorylation of MEK, ERK, and AKT in serum-stimulated cells. Our data indicate that p.E37dup has different functional consequences on H-Ras compared with mutations affecting GTP hydrolysis. Moreover, compensatory effects may result in deregulated H-Ras downstream signaling. Based on our data we hypothesize that specific *HRAS* alterations may lead to different phenotypic outcomes that are even not reminiscent of CS.

P192

Non-syndromic SHFM3 in a four-generation family with a 420,5 kb duplicated region in 10q24

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Split hand/foot malformation (SHFM) is a developmental defect of the extremities presenting at birth either as an isolated manifestation or as part of a syndrome. At least five distinct autosomal and X-linked SHFM-loci have been identified. Here, we report on a family in which several members presented with non-syndromic SHFM including hypoplastic respectively triphalangyal thumbs in an autosomal-dominant inheritance pattern. STS analysis confirmed linkage to the SHFM3-locus on chromosome 10q24 and further suggested SHFM3 (MIM 600095) in the family. As previously shown for SHFM3 patients we found duplication of the SHFM3 critical region in 10q24 in all affected family members, whereas none of the unaffected relatives were carriers of the duplication indicating complete penetrance. Array-based comparative genomic hybridisation (Array-CGH) using the 105A-array (Agilent) revealed an extent of 420.5 Kb for the duplication on chromosome 10q24.32 in the index patient. This duplicated region comprises following genes: LBX1, BTRC, POLL, DPCD, as well as the 3' part of FBXW4 (exons 6 - 9). Which of these genes is causative for the SHFM3-phenotype still needs to be elucidated.

P193

Concordance of natural autoantibodies in monozygotic twin pairs

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Natural autoantibodies (NAAs) that are generated independently of exposure to foreign antigens remain still an unknown both in terms of genesis and function. By employing a combined genetic-immunological approach we analyzed the occurrence of NAAs in monozygotic twin (MT) pairs. A cDNA library derived from human fetal brain was screened by SEREX (serological analysis of recombinant cDNA expression libraries) that identified 49 cDNAs representing 30 distinct NAAs using sera of 7 MT pairs. Twelve NAAs were detected in at least two siblings corresponding to a total of 31 NAA responses. Out of the 31 NAA responses, 18 occurred concordantly within MT pairs and 13 as a singular finding in discordant pairs. Eight NAAs were associated with concordant NAA responses in MT pairs including one NAA being concordant in two MT pairs, corresponding to a total of nine concordant NAA responses in four of seven MT pairs. Statistical evaluation shows that concordance of natural autoantibodies in MT pairs is significant more frequent than expected under assumption of independence (p= 0.0033 exact permutation test). These data provide support for a pivotal role of genetic factors in the generation of NAAs in humans.

P194

Investigation into interactions between the Fanconi anemia proteins FANCJ, FANCD2 and FANCN

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Fanconi anemia (FA) is a rare recessive disorder associated with developmental anomalies, and malformations, progressive bone marrow failure, genomic instability and predisposition to cancer. The FA and related proteins interact in a common pathway, the FA/BRCA pathway for genomic stability. Increasing evidence shows that these proteins function as signal transducers and DNA-processing molecules in a DNA-damage responsive network. The thirteen currently known FA proteins are classified in three groups: Proteins that form the so-called nuclear core complex (FANCA, B, C, E, F, G, L and M), FANCD2 and FANCI that are designated as ID complex, and the downstream effectors FANCD1, FANCJ and FANCN which initiate translesion synthesis and homologous recombination. Interactions between the upstream FA proteins have long since been recognized and are prerequisite for their ability to form the FA core complex. Recently, weak, but significant interaction has been shown for FANCD2 and FANCI. The only established interaction among the downstream FA proteins is between FANCD1 (=BRCA2) and FANCN (=PALB2, partner and localizer of BRCA2). Their connection to FANCJ and the ID complex remains elusive. This led us to set up studies to detect potential interactions between FANCD2, FANCJ and FANCN using the mammalian two-hybrid assay system. The full-length FANCD2, FANCJ and FANCN genes were cloned separately into the pM vector with a GAL4-binding domain and into the pVP16 vector (both Clonetech) with an activation domain. Reporter gene in this assay is Firefly luciferase (pG5E1bluc). As a transfection control and co-reporter, we are using an expression vector for Renilla luciferase (pRL-null). Our findings rule out homo-dimerisation. Potential interaction between the FANCN and FANCD2 and the FANCN and FANCJ are currently being studied. Candidate gene and gene library searches may help to fill in current missing links in the downstraem FA pathway.

P195

Gene expression profiling of peripheral blood of patients with SCA1 and SCA3 identifies potential disease progression markers

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Spinocerebellar ataxias (SCAs) are dominant, late onset hereditary disorders characterized by a progressive ataxia that is variably associated with other neurological symptoms. The clinical hallmarks result from a progressive degenerative process that mostly affects the cerebellum, brainstem and spinal cord. To date at least 28 different loci are associated with SCAs and related diseases. We used

whole genome expression profiling of peripheral blood to search for easily accessible markers, which should
i) differentiate between patients with different SCA types and
ii) be able to monitor disease progression.
Whole blood of 12 patients with SCA1 and 15 patients with SCA3 were analyzed on Affymetrix U133plus 2.0 Gene Chips. Using Support Vector Machines (SVM) a predictor could be defined that has the potential to distinguish between the two disease types. To identify progression specific markers, the patient collective was subdivided in mild, intermediate and severe stage of disease according to their SARA value (Scale for the Assessment and Rating of Ataxias). Transcripts, that showed differential expression between mild and severe stage patients, were identified and subsequently used to define a gene predictor set of 18 genes, which is able to correctly predict the disease stage of the majority of the patients of all three disease stages. Neither of these transcripts showed significant changes in age matched healthy control samples. Real-time RT-PCR validation of the progression specific marker set will be presented as well as microarray hybridizations in an independent patient collective.

P196

Subcellular localisation of human and mouse SCYL1 provides evidence for different cellular function

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In mdf-mice, a 1bp-isertion in the Scyl1 gene is cause of an inheritable muscle-deficiency. Scyl1 is highly conserved among eukaryotes. The human orthologous gene SCYL1 is located on chromosome 11q13 and encodes a highly conserved protein with an inactive kinase domain. Up to now no human muscle disease is associated with mutations in SCYL1. Little is known about the cellular function of the protein. Splice-variants that show different expression profiles and localise in different cell compartments have been discovered in mice and human. One of these isoform, TEIF, is exclusively found in humans and apes. Due to out-of-frame alternative splicing its C-terminal amino-acid sequence differs from SCYL1. This alternative C-terminus contributes to its function as a transcriptional regulator of the telomerase-subunit hTERT. We analyzed the expression pattern of SCYL1 and TEIF by northern-blot and real-time PCR in different human tissues. We detected equal expression rates in all tissues analysed with highest in muscle-tissue, brain and testicle, which corresponds to the murine Scyl1 expression pattern. By cloning human SCYL1 and TEIF into GFP-derived vectors and subsequent subcellular localisation studies we could demonstrate that these GFP-fused proteins are located in different cell compartments. While TEIF is localised in the nucleus and in the cytoplasm, SCYL1 is exclusively found in the cytoplasm. Interestingly, we detected murine Scyl1 in the golgi apparatus. We verified these data by using newly created antibodies against SCYL1 and TEIF in immunofluorescence microscopy. Due to the different subcellular localisations of mouse and human Scyl1 further analysis are necessary to prove whether both proteins have the same cellular functions and mutations in human SCYl1 will also result in a muscle deficiency. Moreover, genomic mutations in human SCYL1 will alter TEIF function, which will result in a completely different phenotype compared to mice.

P197

Family with Langer mesomelic dysplasia and Léri-Weill Dyschondrosteosis caused by a novel mutation in the SHOX-gene

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Short stature homeobox (SHOX)-related haploinsufficiency disorders include a wide spectrum of short stature phenotypes, such as symptoms of Turner syndrome, Léri-Weill Dyschondreosteosis (LWD), Langer mesomelic dysplasia (LMD), and short stature without any specific features (idiopathic short stature, ISS).
For most of the patients with symptoms of this disease spectrum mutations affecting the SHOX-gene,

its regulatory regions, or the pseudoautosomal regions (PAR1) of the X and Y chromosomes have been described.
Here, we report the results of the molecular genetic analyses of three generations of a family. The index case and his brother presented with clinical symptoms of LMD. For both of them it was shown that they carry the nucleotide change c.478C>G (p.Arg160Gly) in exon 3 of the SHOX-gene in a homozygous state. For the consanguineous parents, a sister of his mother and maternal grandmother of the index case the mutation was detected in heterozygous state. Clinical features seen in the patients affected are discussed in the context of our molecular findings.

P198

The CYLD tumor suppressor sensitizes cells to microtubule destabilization

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Mutations in the CYLD tumor suppressor have been identified in patients with familial cylindromatosis and familial trichoepithelioma, which are both autosomal dominant genetic predispositions to multiple tumors of the skin appendages. CYLD has been shown to deubiquitinate TRAF proteins and Bcl-3, both leading to inhibition of NF-κB activation. We have now found that CYLD is a microtubule-associated protein that accelerates microtubule destruction in cells treated with the microtubule-depolymerizing agent nocodazole. CYLD protein carrying a point mutation that truncates the protein at a.a. 485 and thereby deletes the C-terminus, including the majority of the third predicted CAP-GLY domain, still associates to microtubules, but has no influence on microtubule stability. Accordingly, specific knockdown of CYLD results in an increase of microtubule stability and faster recovery after nocodazole withdrawal. Our data strongly suggest that, in addition to upregulation of NF-κB signalling, microtubule dynamics plays an important role in the development of skin cancer induced by mutations in the tumor suppressor CYLD.

P199

Functional studies of a coding short tandem repeat in the androgen receptor gene reveal differences in receptor function

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The androgen receptor (AR) is a member of the nuclear receptor superfamily and mediates the effects of androgens. Genetic studies have reported association of a polyglycine-encoding GGN repeat in exon 1 of the AR gene with common human traits. The polyglycine tract is located in the transactivating domain of the AR protein, suggesting an effect of repeat length on receptor function. Here, we compare the functional characteristics of the two most common alleles (23 and 24 repeats) and two extreme alleles (10 and 27 repeats) in a reporter gene assay in HeLa cells. A correlation between the repeat length and AR activity was observed. This is attributable to both a higher protein concentration, determined by ELISA, and a higher per-protein activity of long repeat alleles. Interestingly, the protein concentration does not correlate with the transcript quantity determined by real-time PCR assays in presence of active AR protein. In absence of active AR protein, the amount of transcripts showed the expected correlation. Software based analysis of the transcript stability revealed increasing thermo stability with prolonging repeat length. Translation inhibition assays could not detect an influence of the repeat length on protein stability. We suggest that the different amounts of AR protein may be due to either a negative feedback loop in AR regulation or different repeat length dependent translation efficiencies. In conclusion, our data provide evidence of functional differences between the examined alleles of the AR GGN repeat, supporting its potential role in the development of human traits.

P200

Nonsense-mediated messenger RNA decay of survival motor neuron 1 causes spinal muscular atrophy

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Autosomal recessive proximal spinal muscular atrophy (SMA) is a neurodegenerative disorder resulting from functional loss of survival motor neuron 1 (SMN1). Homozygous absence of SMN1 due to deletion or gene conversion accounts for about 96% of SMA cases. In the remaining 4%, subtle SMN1 mutations are commonly identified. Here, we describe two novel intragenic SMN1 mutations in three type I SMA indi- viduals: a point mutation in exon 3 (c.469C>T) and a substitution in intron 4 (c.628-140A>G). In-vivo splicing assays demonstrated that the intronic substitution creates a novel splice donor site, culminating in aberrant splicing and insertion of 65bp from intron 4 between exons 4 and 5 in SMN1 transcripts (c.627_628ins65). Both mutations render SMN1 transcripts susceptible to nonsense-mediated mRNA decay (NMD), resulting in mRNA degradation, insufficient SMN protein levels and development of an SMA phenotype. Treatment of patient cell lines with the translation inhibitors puromycin and emetine markedly increased the levels of mutant SMN1 transcripts. A similar effect was observed after siRNA-mediated knockdown of UPF1, a factor essential for NMD. This study provides first evidence that NMD of SMN1 transcripts is responsible for the molecular basis of disease in a subset of SMA patients.

P201

Promoter studies in the FA genes

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Fanconi anemia (FA) is a rare genome-instability disorder with the frequent presence of congenital malformations and bone marrow failure. Other characteristic features include predisposition to FA-typical malignancies and cellular hypersensivity to DNA-interstrand crosslinking agents such as mitomycin C. At least 13 genes and corresponding complementation groups are underlying the disease. Eight of the FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and other components assemble in a nuclear complex, the FA “core complex”.
Little if any is known about the promoter regions, in particular the core-promoters of the FA genes. However, identification and characterization of the promoters would be essential for understanding the regulation of transcription, including intergenic regulation, of the FA genes. Our aim is to provide an explanation for the equimolar composition of the core complex. We used a variety of in silico methods to predict potential promoter regions. To confirm these, we have set up dual luciferase reporter assays. We cloned the identified regions (~1kb) in the pGL3 basic vector that carries the reporter gene for firefly luciferase. As co-reporter we used the pRL null vector that contains the renilla luciferase gene. With these constructs, plus the pGBKT7 vector as “carrier-DNA”, we transfected HeLa and HEK293 cells to assay luciferase activity using a DLR-ready luminometer. In a second approach we delete large areas of the promoter region to detect any decrease of activity. This helps us to identify the core regions of the promoters. Further characterization includes transcription factor binding sites and conserved sequence elements. First results indicate that there is generally strong promoter activity. Short half-life of the gene products is consistent with high transcriptional activity of the FA genes, and rapid regulation in response to DNA damage.

P202

New mutations and genotype-phenotype correlations in Johanson-Blizzard syndrome

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Johanson-Blizzard syndrome (JBS, OMIM 243800) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency (PI), hypoplasia of alae nasi, dental anomalies, deafness, scalp defects, imperforate anus, hypothyroidism and frequent mental retardation. We have previously identified mutations in UBR1 as the underlying cause of this rare disorder. UBR1 encodes a ubiquitin ligase of the N-end rule pathway.
We have analyzed the UBR1 gene in 16 novel unrelated patients with a clinical diagnosis of JBS and in 33 patients with either isolated congenital PI or atypical phenotypes with some features suggestive of JBS. Mutations discovered by direct sequencing were further investigated on mRNA and protein level as possible. Likely causative UBR1 mutations were found in 14 of 16 JBS patients. However, in

two of them only one allele could be identified. Homozygosity for a probable UBR1 mutation was also found in two siblings with isolated PI. In contrast to the previously reported mutations, this study identified missense mutations on one or both UBR1 alleles in half of the patients. Comparison of clinical findings in the present and previously reported patients reveals clear genotype phenotype correlations. While patients with biallelic truncating mutations displayed more severe clinical symptoms including multiple congenital anomalies and mental retardation, those cases with less severe congenital anomalies and normal intelligence were exclusively found among the patients carrying missense mutations at least on one allele. In two patients with the diagnosis of JBS no defect in the UBR1 gene was found. In one family the UBR1 locus could be excluded suggesting locus heterogeneity of JBS.
We conclude that residual UBR1 function ameliorates the phenotype and may be able to rescue the neurodevelopmental deficits of JBS. In contrast, congenital pancreatic insufficiency and oligodontia are the most constant consequences of mutated UBR1.

P203

Regulatory functions of the embryonic limb gene HOXD13 on cellular differentiation - a principle for Hox-dependent modelling of skeletal elements?

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Hox genes are known to play a major role in patterning processes during embryonic development. 5´ HOXA and HOXD genes are essential for limb formation including limb bud initiation and limb bud segmentation. Currently little is known about their role in cellular differentiation. For this purpose, we investigated the functional role of Hoxd13 on chondrogenic and osteogenic processes. Besides the wildtype effect we analyzed the functional relevance of alanine repeat expansions (+7Ala and +14 Ala) in Hoxd13, mutations known to cause synpolydactyly.
We introduced almost physiological gene copy amounts into mesenchymal progenitor cells and measured their influence on differentiation and proliferation. These findings were correlated with gene expression of the Sox-gene familiy and of Runx2.
We found strong inhibitory effects of Hoxd13 on chondrogenesis and on osteoblast mineralization. Central chondrogenic factors were downregulated, while perichondrial markers were elevated. Furthermore Hoxd13 expressing cells maintained the proliferative capacity of undifferentiated mesenchymal progenitors, reflecting a regenerative function. Descending expression levels of Hoxd13 were measured during maturation steps, a mechanism which was enhanced by Runx2 in a negative feed back manner. In contrast, expansion mutations disrupted the Hoxd13 wildtype effect in correlation to the alanine length, even caused a prochondrogenic activity in later differentiation steps.
In conclusion, our results show that Hoxd13 has a cell fate determining function on mesenchymal progenitors, meaning that Hoxd13 positive cells carry perichondrial features, while mesenchymal progenitors with declining Hoxd13 expression levels enter into a chondrogenic differentiation process. We assume that the observed Hoxd13 role is part of a principle for Hox-dependent modelling of skeletal elements.

P204

Epimutation of the MEG3-DMR at chromosome 14q32 in a boy presenting with a upd(14)mat-like clinical phenotype

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Recent data suggest that the clinical phenotype of maternal uniparental disomy of chromosome 14 [upd(14)mat] is caused by abnormalities of imprinted genes at 14q32 under the control of a paternally methylated differentially methylated region (DMR). We studied a patient with upd(14)mat-like clinical manifestation including low birth weight, neonatal feeding problems, muscular hypotonia, motor and developmental delay, small hands and feet, and truncal obesity. Conventional cytogenetic analyses, subtelomere screening by FISH, MLPA analysis for microdeletion and microduplication syndromes, and methylation analysis for Prader-Willi-Syndrome gave normal results. Methylation-specific PCR and bisulphite pyrosequencing of the MEG3-DMR detected a complete absence of the methylated paternal allele and severe hypomethylation, respectively. Microsatellite analysis demonstrated

biparental inheritance of chromosome 14 and excluded the presence of upd(14)mat. Biparentally inherited microsatellite markers D14S1426 and D14S543 are located at a 4-Mb distance of each other proximal and distal of the DMR, respectively. Molecular studies to exclude a segmental UPD or microdeletion in this 4 Mb-region are in progress. In addition to a recently published case, this case most likely represents another case of upd(14)mat-like clinical phenotype due to a microdeletion or epimutation at the 14q32 DMR.

P205

Li-Fraumeni-plus - a variant of Li-Fraumeni-syndrome in a girl with a de novo 770 kb microdeletion of 17p

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Li-Fraumeni syndrome (LFS) is a rare genetically caused disorder usually characterized by familial occurrence of various malignancies of early onset. LFS is clinically and genetically heterogeneous. Based on criteria like disease onset and familial distribution of different tumor types it is possible to discern between classic LFS and Li-Fraumeni-like syndrome (LFL). LFS follows an autosomal dominant mode of inheritance with high penetrance and mutation rate in germ cells is very low. LFS in most cases is due to a constitutional mutation of one copy of the TP53 tumor suppressor gene in affected family members and somatic mutations in the second copy of that gene in the process of malignant transformation. This gene at chromosome 17p13.1 is the most frequent target for genetic alterations in human cancer. LFS has not been reported to be associated with non malignant disorders. Here we describe the first case of a constitutional chromosomal microdeletion of the entire TP53 gene and several closely linked genes that occurred de novo. The phenotype of the patient is striking and includes severe psychomotor retardation, Leber congenital amaurosis (LCA), a Dandy-Walker anomaly, seizures and dysmorphic features. Chromosomal breakpoints (BP) were mapped by high resolution array comparative genome hybridization (CGH) followed by quantitative real type PCR analysis. The distal BP disrupts the ACADVL gene and the proximal BP presumably alters upstream regulatory sequences of the GUCY2D gene which would explain LCA of the patient. More than 40 genes have been mapped to the 770 kb deletion interval including the FXR2 gene which is an autosomal homolog of the FMR1 gene. Sequencing of the two BP flanking motifs will allow to recognize if low copy repeats (LCRs) are involved in development of this microdeletion as it has been demonstrated for DiGeorge syndrome at 22q11.2 or two other 17p microdeletion syndromes like Miller-Dieker syndrome or Smith-Magenis syndrome.

P206

Novel mutations in *PORCN* in patients with focal dermal hypoplasia, and no mutation in patients with Aicardi syndrome and the microphthalmia with linear skin defects (MLS) syndrome

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Focal dermal hypoplasia (FDH) is an X-linked developmental disorder characterized by skeletal and dental malformations, microphthalmia or anophthalmia and patchy dermal hypoplasia/aplasia. Although the majority of cases are females, about 10% of FDH cases are male. FDH shows considerable clinical overlap with two other X-linked disorders, namely Aicardi syndrome and the microphthalmia with linear skin defects (MLS) syndrome. Recently, loss-of-function mutations in the *PORCN* gene have been described to cause FDH. *PORCN* is located in Xp11.23 and codes for the porcupine protein, a putative *O*-aceyltransferase involved in posttranslational modification of Wnt

signaling molecules. We performed mutation analysis of *PORCN* in 13 females and one male with the clinical diagnosis of FDH, two patients with Aicardi and nine patients with MLS syndrome. The latter had been tested negative for mutations of *HCCS*, the gene for MLS syndrome. In total, we identified 12 heterozygous mutations in patients with FDH, of which nine are novel. Of these sequence alterations seven are nonsense and two missense mutations, two are small rearrangements, and one is a putative splice mutation (c.374-46T>A). *In silico* analysis of this sequence change predicted that the novel ectopic acceptor site created by the mutation was recognized with much higher splicing efficiency than the wild-type site suggesting that the c.374-46T>A sequence change may represent the pathogenic mutation. No (mosaic) mutation was yet detected in the affected male; interestingly, two female patients have somatic mosaicism for the *PORCN* mutation. In neither of the patients with a clinical diagnosis of Aicardi or MLS syndrome a disease-associated sequence alteration of *PORCN* was identified. In summary, our data suggest that
(i) in patients with a clear-cut FDH phenotype a mutation of *PORCN* most likely will be identified,
(ii) FDH does not appear to be heterogeneous, and
(iii) Aicardi and MLS syndrome are not allelic to FDH.

P207

Clinical and molecular characterisation of the attenuated form of Sanfilippo type B in eight Dutch patients

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Sanfilippo type B (mucopolysaccharidosis IIIB, MPSIIIB) is an autosomal recessive lysosomal storage disorder caused by the deficiency of α-N-acetylglucosaminidase (NAGLU). Deficiency of this enzyme leads to accumulation of heparan sulphate in lysosomes and clinically in particular to neurological degeneration, behavioural problems and mental decline. An attenuated form of MPSIIIB is characterised by a protracted course and relatively mild somatic features. So far, mutation analysis in 15 patients with a documented attenuated form of MPSIIIB has been described in the literature. We present clinical and molecular data of eight Dutch patients with Sanfilippo type B. Natural history was characterised by onset in the first decade with non-specific developmental delay, challenging behavioral problems over a long time, slow decline, physical problems developing in the 4th-6th and loss of ambulation in the 5th decade, and survival into (late) adulthood. Biochemical analysis showed a (near) complete loss of NAGLU enzyme activity in leukocytes, indicating that enzyme studies in fibroblasts may be necessary to identify residual activity in MPSIIIB patients. Sequence analysis of all exons and adjacent intron regions of the NAGLU gene was performed and 15 of the 16 mutant alleles were characterized. There were eight different missense mutations, five of which were novel. Four patients showed homozygosity for mutation R643C (c.1927C>T) which is a common allele in Dutch Sanfilippo type B patients and is associated with an attenuated phenotype. In conclusion, MPSIIIB should be considered in patients of all ages with mental retardation and behavioural problems; molecular studies may be useful to confirm an attenuated phenotype in affected patients.

P208

Patient screening for new Fanconi Anemia genes

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Fanconi Anemia (FA) is a rare autosomal and x-chromosomal recessive disease with great genetic and phenotypic heterogenity. FA is characterized by bone marrow failure, high cancer risk and different developmental anomalies. FA cells show hypersensitivity to DNA crosslinking agents. To date, 13 FA genes underlying corresponding complementation groups have been reported. The FA gene products interact in the FA/BRCA DNA damage response network. Most of the FA patients have been assigned to complementation group A, followed by C, G and minor groups. However there are still FA patients who cannot be classified to any of the existing complementation groups. To understand the FA pathway completely, it would be essential to know all members. Here we present a candidate approach to identify elusive FA genes. A key step in the pathway is the modification of a

protein complex, addressed as FANCD2/I, by monoubiquitination. This step is used to classify any gene defect upstream or downstream of FANCD2/I monoubiqitination. Over years, we have accumulated more than 20 cell lines that failed to reveal defects of all known FA genes. They include both, lines proficient and deficient of the modification step. We therefore hypothesize that there exist at least two further FA genes, one located up- and the other downstream of FANCD2/I monoubiquitination. Our screen includes immunoblotting and DNA sequencing of candidate genes that are coding for FA-associated, BRCA1-associated and other DNA repair-associated proteins. Abraxas, one of the BRCA1-associated proteins, and HES1 were excluded as FA genes in our cell lines. Most likely, only recently recognized components of the FA core complex will contain new FA genes. Through our investigation we aim to close existing gaps in the FA/BRCA network.

P209

Identification of SMN1 point mutations by genomic DNA sequencing

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Infantile spinal muscular atrophy (SMA types I-III) is caused by homozygous deletions/mutations of the SMN1 gene in 5q13, while absence of the SMN2 gene is not associated with clinical signs if at least one SMN1 gene is present. More than 95% of 5q13-associated SMA patients show a homozygous SMN1 deletion, the remaining 1.8 to 4.8% are usually compound heterozygous for the deletion and a point mutation on the other allele. The high homology between the two SMN genes makes screening for point mutations in the SMN1 gene difficult and is therefore implemented in the Cologne laboratory on RNA basis including cloning and cDNA analysis. To complete the set-up for molecular SMA diagnosis the Aachen laboratory established a sequencing procedure based on a long-range PCR assay (Clermont et al. 2004). The test consists of an SMN1-specific PCR for exon 7, generation of a large SMN1-specific fragment for exons 2 to 6 followed by a nested PCR, and unspecific amplification of exon 1. All fragments are afterwards sequenced. Our study cohort consisted of 5 positive controls with known variants and 7 typical SMA patients heterozygous for the SMN1 deletion. We were able to confirm the mutations in all 5 control patients and detected 7 point mutations among them two novel variants in the compound heterozygous patients. The genomic sequencing approach based on long-range PCR has major advantages and is an efficient and reliable method for SMN1 mutation screening in routine diagnostics. It allows the analysis of stored DNA samples and avoids time-consuming and costly cDNA analysis. The test is hampered by the high homology of the two SMN genes, i.e. if more than two SMN2 copies are present, as seen in many SMA type II and III patients, the SMN2 sequence might mask a subtle SMN1 mutation. In these cases the laborious RNA-based testing procedure remains the most reliable technique, it furthermore allows the detection of subtle mutations associated with nonsense-mediated mRNA decay.

P210

Matriptase deficiency is causative for a syndrome of ichthyosis, follicular atrophoderma, and hypotrichosis

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Autosomal recessive congenital ichthyosis encompasses a large, heterogeneous group of disorders of cornification. Isolated forms and syndromic ichthyosis can be differentiated. We have recruited two consanguineous families with a similar phenoltype of ichthyosis, follicular atrophoderma, and hypotrichosis from the United Arab Emirates and Turkey. Histopathologically, the epidermis was of regular thickness, with the stratum granulosum thinned and the stratum corneum orthohyperkeratotic. Hair follicle epithelium was thinned, hair infundibulum showed hyperkeratosis and a very thin stratum granulosum. EM analysis showed deposits of lamellar bodies in the lower lamellae of the stratum corneum. After genome wide linkage analysis we identified mutations in the suppression of tumorigenicity 14 gene (*ST14*), which encodes matriptase, a type II transmembrane serine protease expressed in most epithelia. We found a homozygous splice site mutation (c.2269+1G>A) in the

Emirati patients and a 1-base deletion (c.2034delG) leading to a premature stop codon in the Turkish patient. Furthermore, Western blot analysis showed complete loss of matriptase in the patients, reduced proteolytic activation of prostasin, and loss of processing of profilaggrin. Since filaggrin monomers play a pivotal role in epidermal barrier formation, we suggest that matriptase acts upstream of prostasin in a zymogen activation cascade that regulates terminal epidermal differentiation. In order to investigate the role of these proteins in the pathophysiology of the syndrome, we have generated 3D skin models with primary keratinocytes and fibroblasts from patients and healthy donors. Primary keratinocytes from control persons were transfected with siRNA to knock down the expression of *ST14*, *PRSS8*, and *FLG*, respectively. Samples taken from transfected keratinocytes and 3D skin models are now compared to each other and untransfected controls and further analyzed using genomic and immunohistochemical methods.

P211

Functional characterization of murine *ZFYVE27*, the gene mutated in Hereditary spastic paraplegia

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Hereditary spastic paraplegias (HSPs) are a group of neurodegenerative diseases phenotypically characterized by spasticity of the lower limbs. Previously, we have identified ZFYVE27 as a novel spastin interacting protein. Furthermore, we reported a German family with autosomal dominant form of HSP, in which *ZFYVE27* was mutated. ZFYVE27 belongs to FYVE (Fab1P, YOTB, Vac1P and EEA1) domain family of proteins, which play a key role in membrane trafficking, signal transduction, cytoskeleton regulation and apoptosis. In particular, ZFYVE27 was shown to promote neurite formation by directional membrane trafficking. Tissue specific expression analysis of murine ZFYVE27 showed a higher expression mainly in the central nervous system. Immunohistochemical studies of brain sections revealed that ZFYVE27 primarily localize in the neuronal cell bodies as well as in the axons of hippocampus, cerebellum and corpus callosum. Minimal expression was detectable in oligodendroglial cell bodies as well as in white matter. A comprehensive immunohistochemical analysis of spinal cord revealed strong expression of ZFYVE27 in motor neurons and a weaker expression was detected in somatosensory neurons. To elucidate the molecular function of ZFYVE27, we are currently generating a loss of function mouse model system. Towards this end, we have generated a knock-out construct for ZFYVE27 using BAC mediated homologous recombination. Screening of embryonic stem (ES) cell clones for recombinant event led to the identification of four ES clones and generation of chimeric mouse is in progress.

P212

Mutation analysis in patients with familial pulmonary arterial hypertension (FPAH)

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Among all patients with pulmonary arterial hypertension (PAH), those with familial history of the disease are relatively rare. Recent findings have shown that heterozygous mutations in the *BMPR2* gene can be identified in the majority of familial cases. Beside the *BMPR2* gene, mutations in *ALK1* and *ENG* genes, were found in a subgroup of subjects with hereditary hemorrhagic telangiectasia (HHT) and PAH, and in some infantile PAH cases.
In order to characterise the genetic background of PAH in families, which we collected during our study, and to find out to what extend the *BMPR2* mutations are responsible for FPAH, the index patients from 14 families were subject to molecular analysis.
The analyses of the coding region and exon/intron boundaries with the use of DHPLC and direct sequencing yielded in identification of 10 point mutations: p.Q82X, p.R211X, p.C420R, p.C420Y, p.D487V, p.R491W, p.R899X (2x), c.418+1G>C and c.1128+1G>T (71.4%). One of the four remaining families showed a deletion of *BMPR2* exon 1. In another family, which was negative for both point mutations as well as for large rearrangements in the coding region, we found a novel mutation (c.1-211 insC) in 5´UTR of the *BMPR2* gene. In the next family, where no *BMPR2* mutation was detected until now, previous study showed linkage with the *BMPR2* region, indicating that the mutation must be located in uncoding sequence. Only in one of the analysed families, where in addition to PAH the

index patient showed symptoms of HHT, we found a mutation in *ALK1* gene (R484Q). The patient's nephew, carrying the same mutation, died of PAH in early childhood.
Our analyses clearly show that nearly all FPAH cases can be explained with mutations in the *BMPR2* gene. However, these findings do not suggest that FPAH is evidently a monogenic disorder. The fact that *BMPR2* mutations were often detected also in healthy relatives of the PAH patients imply that other factors, most likely also genetic are needed to develop the disease.

P213

Polyglutamine binding protein 1 (PQBP1) mutations within the alternative exon 4 affect pre-mRNA splicing in the patients

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Mutations in the polyglutamine binding protein 1 (PQBP1) gene cause X-linked mental retardation (Kalscheuer et al., 2003, Cossee et al., 2006). Most of the changes are frameshift mutations, which affect an AG hexamer located in exon 4 and result in premature stop codons. Strikingly, clinical phenotypes of the patients are quite different, ranging from moderate MR to much more severe syndromic forms. Understanding the primary effect of the PQBP1 mutations is crucial for elucidating the pathogenic mechanisms of the disease. Therefore we have investigated patient cell lines for the presence of mutant PQBP1 protein, which might have a dominant-negative effect. We have found that truncated PQBP1 protein of predicted size was present in most cell lines tested. Interestingly, in patients with an identical or similar mutation in exon 4, the diversity of PQBP1 isoforms and their expression level was altered. This result is further corroborated by the observed PQBP1 splicing pattern, which was also altered in a mutation-specific manner. To understand if the AG hexamer contains information in cis that is required for exon recognition by specific splicing factors, we have used exonic splicing enhancer (ESE) scoring matrices. The bioinformatic analysis showed that exon 4 AG mutations lie within three short overlapping predicted ESE motifs. To investigate this further, we have cloned wild type and different PQBP1 exon 4 mutations into an exon trap vector. Expression of these constructs in neuronal cells indicated that the mutations have an effect on exon 4 splicing. We are currently determining the splicing factors binding the predicted ESEs in wild-type PQBP1 and will assess the influence of the PQBP1 exon 4 AG mutations.

P214

Late onset hereditary sensory neuropathie type 1 (HSN1) caused by a p.C133R missense mutation in SPTLC1

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The hereditary sensory and autonomic neuropathies (HSAN), which are also referred to as hereditary sensory neuropathies (HSN) in the absence of significant autonomic features, are a genetically and clinically heterogeneous group of disorders associated with sensory dysfunction. HSN1 is a dominantly inherited sensorimotor axonal neuropathy usually with onset in the first or second decades of life. We investigated a female patient with age-of-onset at 50 years. Her mother was also affected but died. Her brother was diagnosed as chronic inflammatory demyelinating neuropathy (CIDP) but - typical for a inherited peripheral neuropathy - steroid treatment failed. Neurological examination of the index patient revealed a normal nerve conduction velocity (NCV) of N. fibularis at 46 m/s. No motor impairment was found in EMG and NCV studies, also no atrophies were present. Major symptom is a distal, symmetric hypesthesia. Sequence analysis of all coding exons of the gene for the Serine Palmitoyltransferase, Long-Chain subunit 1 (SPTLC1) revealed a heterozygous variation c.397T>C resulting in a missense mutation p.C133R. Serine palmitoyltransferase (SPT; EC 2.3.1.50) is the key enzyme in sphingolipid biosynthesis. It catalyzes the pyridoxal-5-prime-phosphate-dependent condensation of L-serine and palmitoyl-CoA to 3-oxosphinganine. The p.C133Y and p.C133W mutations were earlier described as cause of severe HSN1 accompanied by deafness and ulcerations. Bejaoui et al. (2002) found that the p.C133Y and p.C133W mutations do not alter the steady state levels of the SPTLC1 or the SPTLC2 subunit but result in reduced SPT activity and sphingolipid synthesis. These functional results indicated that both of these mutations have a dominant-negative effect on the SPT enzyme. The here reported p.C133R mutation may follow the same loss-of-function

mechanism, but the resulting phenotype is relatively mild indicating a reduced but not completely missing SPT activity.

P215

Denaturing Gradient Gel Electrophoresis (DGGE) for the detection of point mutations in Duchenne und Becker muscular dystrophies

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Duchenne (DMD) and Becker (BMD) muscular dystrophies are X-linked recessive diseases caused by mutations in the dystrophin gene. Approximately two-thirds of affected patients have large deletions and duplications. Point mutations are found in a considerable proportion of the remaining individuals although in some patients, no mutation is detected despite comprehensive deletion/duplication and sequence analysis. For the detection of point mutations in the 79 exons of the dystrophin gene in patients with negative results of MLPA analyses, we selected a mutational scanning approach with denaturing gradient gel electrophoresis (DGGE) as full sequence analysis of all exons is costly and time consuming. So far, we have investigated 7 males with DMD, 3 males with BMD, one obligatory, and 7 possible (non-obligatory) female carriers. To ensure formation of heteroduplices, DNA samples of the male patients were mixed with a control male sample. Point mutations were identified in all DMD patients, one BMD patient, and the obligatory carrier (altogether 9 out of 11). This is in line with the sensitivity reported in studies involving full sequence analysis of the dystrophin gene. Point mutation was also detected in one possible (non-obligatory) carrier. Mutations included 6 nonsense mutations, two frame-shift mutations, and one splice-site mutation. Interestingly, the BMD patient showed a missense mutation in a conserved helix of the actin binding domain of the dystrophin. Our results indicate that DGGE mutation scanning is a sensitive and cost effective alternative to whole gene sequencing for the detection of point mutations in DMD and BMD.

P216

The p97/VCP gene is highly conserved and not a frequent modifying factor in SCA3

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Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder which is also known as Machado-Joseph disease (MJD). SCA3 is caused by the expansion of a CAG stretch in the MJD1 gene encoding a polyglutamine repeat in the respective ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases.
Statistically, a correlation between the number of CAG repeats and the age-at-onset in SCA3 patients is obvious. Patients with a low number of about 60 CAG repeats develop symptoms in their 60s and 70s whereas the age-at-onset in patients with a high number of CAG repeats (e.g. higher than 75 repeats) might be around 25 years or earlier. However, this correlation is not perfect since it was show the some patients with 71 CAG repeats have an age at onset of 29 years whereas other patients develop symptoms not until the age of 50 years. For this reason it has been proposed that the number of CAG repeats contribute only 55 % to the age at onset and that the remaining 45 % is influence by other factors, which we try to identify in this study. Up to now the actual function of ataxin-3 is unknown. Recently, HHR23A and B, the human homologues of yeast RAD23 as well as p97/VCP were identified as interaction partners of ataxin-3. In this study we screened a cohort of 480 SCA3 patients for polymorphism within all 17 exons of p97/VCP as well as the 5' and 3' untranslated region and the putative promoter region using DHPLC. In this screening, p97/VCP turned out to be very stable so that only rare polymorphisms, mostly in intronic regions, and no amino acid change was identified. While patients carrying the identified polymorphism show a tendency of early or late ages at onset, a statistical correlation was not possible due to the limited number of observed carriers. We can therefore exclude p97/VCP as a frequent modifying factor in SCA3.

P217

Interleukin 4 and the interleukin 7 receptor α chain are associated with multiple sclerosis

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MS is a common neuro-inflammatory and neuro-degenerative disease which affects worldwide >1 million people. The ethiopathology of MS is characterized by inflammatory foci with various degrees of demyelination and axonal damage. Twin studies revealed that, both, genetic and environmental factors predispose for MS. Till now the only consistent association in European MS patients has been identified in the HLA-DRB1*1501-DQB1*0602 haplotype. Yet, it is unclear why females are usually affected more frequently than men. Here we present evidence for sex specific MS association as evidenced for the interleukin 4 (IL4) promoter polymorphism -589 C/T (rs2243250). Two flanking polymorphisms showed insignificant p values. In dual luciferase assays of cultured Jurkat cells the cloned promoter comprising the -589 T allele leads to higher expression as compared to the respective construct with the C allele.
Similarly the rs6897932 SNP located in the interleukin 7 receptor α chain has been shown to be associated with MS in a combined cohort of European and US multiple sclerosis (MS) patients with subsequent functional studies. We provide a replication study adding insights into the involvement of rs6897932 in MS chronicity.
Based on these results we generate new insights for two genes regulating immunoregulatory pathways which show to be associated with the complex multifuctorial disease MS.

P218

Identification of a new locus for Dyschromatosis universalis hereditaria on chromosome 12q21-q23 in a family with autosomal recessive inheritance

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Background: Dyschromatoses are a group of pigmentary dermatoses characterized by the presence of small and irregularly shaped maculae. There are two major forms of the disease: dyschromatosis symmetrica hereditaria (DSH) and dyschromatosis universalis hereditaria (DUH). DSH patients are affected by maculae on the dorsal aspects of their hands and feet, whereas DUH patients have a generalized distribution over most of their body. Both disorders usually show autosomal dominant inheritance, although cases with autosomal recessive inheritance were reported. The locus for autosomal dominant DSH was mapped to chromosome 1q21.3, and mutations in the gene ADAR (DSRAD) were identified. In two Chinese families, initially reported to be affected with DSH, but later suggested to have autosomal dominant DUH, a second locus for dyschromatosis was mapped on chromosome 6q24.2-q25.2.
Patients and methods: We investigated whether one of these two loci is linked to the development of DUH in a consanguineous Bedouin family from Saudi Arabia. Four siblings were affected, three siblings and the parents were unaffected.
Results: After mutations in ADAR and linkage to the two known candidate regions were excluded, we searched for the disease-locus by a SNP-based genome-wide linkage analysis. Under the assumption of autosomal recessive inheritance, we have identified a new locus for dyschromatosis on chromosome 12q21-q23 in this family. The candidate region (maximum LOD score of 3.4) is spanning a distance of 20.9 Mb (18.9 cM) and contains 125 known or predicted genes.
Conclusion: We identified the first locus for autosomal recessive DUH and support recent evidence that DSH and DUH are genetically distinct disorders.

P219

Up-regulation of the heat shock protein receptor CD91 on monocytes of HIV-1 infected long-term nonprogressors - search for association with CD91 gene polymorphisms

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CD91 expression has been shown to be increased on monocytes of HIV-1 infected long-term nonprogressors (LTNPs). The CD91 receptor on antigen presenting cells might participate in the uptake of antigens bound to heat shock proteins and allow for crosspriming of cytotoxic T cells. We aimed to determine whether polymorphisms of the CD91 gene are associated with increased CD91 expression in LTNPs. We quantified the CD91 expression by flow cytometry on CD14+ monocytes in LTNPs (n=8), who never received antiretroviral therapy, HIV-1 infected patients who received their first highly active antiretroviral therapy (HAART) (patient group 1, n=10), patients receiving HAART after at least 4 previous courses of therapy (patient group 2, n=11), and 102 uninfected healthy volunteers. All groups were genotyped for a CD91 promoter polymorphism (C-25G), a silent exon 3 polymorphism (C766T), and four additional SNPs (rs11172106, rs4759044, rs715948, and rs7398375) in the CD91 gene.
The mean fluorescence intensity (MFI) of CD91 was found to be increased in the LTNPs compared to the other patient groups (p=0.0022). We observed a significant increase of CD91 expression in patient group 1 in the presence of the T allele of exon 3 (MFI: 99.7→124.7, p=0.0242) and a similar but only borderline effect in LTNPs (MFI: 131.3→155.1, p=0.0403), if a robust threshold for significance of alpha'=0.033 (obtained from permutated data sets) was applied. This genetic effect was not observed in uninfected individuals. The other SNPs did not show effects on the expression level of CD91 in any group.
HIV-1 infected LTNPs expressed increased levels of CD91 on monocytes compared to patients on HAART. An increased CD91 expression was found to be associated with the presence of a T allele at position 776 of the CD91 gene in patient group 1 and likely in LTNPs. This exon 3 polymorphism appears to modulate the CD91 expression in response to HIV-1 infection.

P220

Follow-up analysis of association signals from two independent genome-wide association studies of bipolar disorder provides evidence for *JAM3* as a susceptibility gene

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Background: Bipolar disorder (BD) is a genetically complex neuropsychiatric disorder. Recently, the first two genome-wide association studies (GWAS) of BD were published applying different approaches: (A) Baum *et al* (2007) analysed DNA pools from a US-American and German sample of BD on Illumina HH550 arrays and reported consistent association findings between these two samples (B) The Wellcome Trust Case Control Consortium (WTCCC, 2007) employed individual genotyping in a large UK case-control sample on Affymetrix 500K arrays. In the present study, we aimed to (A) identify genes that showed evidence for association in both studies, (B) individually genotype Affymetrix SNPs from these genes in our German sample, which was part of the Baum *et al* study, to make the results of both GWAS comparable, and (C) follow-up promosing SNPs in an independent sample of BD originating from Russia. Such replication steps are essential in order to distinguish true disease-associated genetic variants from spurious findings.
Results: A gene-based comparison of association signals from both published GWAS resulted in 9 genes with overlapping signals. From these genes, we selected 21 Affymetrix SNPs from the WTCCC study and individually genotyped them in our German (691 cases, 944 controls, part of Baum *et al*) and in an independent Russian (212 cases, 179 controls) sample. We found that SNPs in *JAM3* (*junctional adhesion molecule 3*) were significantly associated with BD in the German and Russian population (best combined *P* value=1.95E-03).
Conclusions: Genetic variation in *JAM3* shows consistent association with BD in three independent samples (Baum et al, WTCCC, Russian). *JAM3* can therefore be regarded as a very promising susceptibility gene for BD. Further studies will aim at the identification of the functionally relevant variants and at the investigation of the pathophysiological role in the development of BD.

P221

Molecular detection of periodontopathic bacteria in synovial fluid

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Rheumatic diseases and periodontitis are sharing similar pathophysiological features. It is assumed that periodontopathogens could influence the aetiology of rheumatic diseases. However, the underlying pathomechanisms are still unknown. Therefore, we established a specific and sensitive method based on molecular techniques to detect 5 major periodontopathogens, Actinobacillus actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Prevotella intermedia, Treponema denticola, Tannerella forsythensis in synovial fluid.
Methods: Nine patients suffering from juvenile idiopathic arthritis or rheumatoid arthritis, respectively, were included in this study. DNA from synovial fluid of affected knee joints was isolated by QiaAmp-kit (Qiagen, Hilden, Germany) using an adapted protocol. PCRs specific for the 16S rRNA genes of these bacteria were developed. For positive control of DNA preparation the samples were spiked with E.coli strain XL2B. Subgingival bacterial colonization was analysed using micro-Ident® test (HAIN-Diagnostik, Nehren, Germany).
Results: The PCR was optimized in order to detect up to 10 DNA copies of each periodontopathogen. DNA of 2 periodontophatogens, A.a. and P.g., was detected in synovial fluids of two different patients suffering from both juvenile idiopathic arthritis and periodontitis. In the same patients a subgingival infection with P.g. but not A.a. could be detected.
Conclusions: For the first time a highly sensitive molecular based detection system was adapted for the identification of periodontopathogens in synovial fluid. The application of this method allows a better understanding of the interaction of periodontitis and rheumatic diseases. The revealed first results indicate that periodontopathogens may play a role in the pathway of rheumatic diseases.

P222

Evidence for interaction between DCDC2 and KIAA0319 in dyslexia

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Independent linkage studies for dyslexia have pointed towards a susceptibility locus on chromosome 6p21-p22 (DYX2) (1). This region harbours two candidate genes in close proximity to each other, namely DCDC2 and KIAA0319 (1). Only one single study has sufficiently covered both genes, which is necessary to understand the relative contribution of both genes and to identify possible interactions between them. Harold et al. (2) recently reported a combined analysis of both genes in two UK samples, supporting their previously observed findings and showing evidence for an interaction between the two genes. We have previously reported strong association of variants in the DCDC2 gene with dyslexia in German families, but did not obtain any evidence for a contribution of the KIAA0319 gene (3). In the present study, consisting of 244 German families with a severely affected child, we expanded our marker set in order to obtain a more comprehensive picture of the contribution of KIAA0319.
None of the additional six markers in KIAA0319 showed significant association with dyslexia, neither in the total sample nor with one of the subdimensions or under severity stratification. When testing for interaction between markers in KIAA0319 and our previously identified risk haplotype in DCDC2 (3), we obtained no evidence for interaction for dyslexia itself, but found a nominally significant association for the subdimension “word reading”, which was the core phenotype in the study of Harold et al. This may be seen as supportive evidence for an interaction between KIAA0319 and DCDC2. However, an effect of KIAA0319 alone, as reported for the UK samples, could not be demonstrated in our sample of German origin.

1. Schumacher J et al. J Med Genet 2007; 44: 289-297
2. Harold D et al. Mol Psychiatry 2006; 11: 1085-1091.
3. Schumacher J et al. Am J Hum Genet 2006; 78: 52-62.

P223

A family-based association study in Central Europeans does not confirm a contribution of IRF6 to non-syndromic cleft lip with or without cleft palate

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Orofacial clefts belong to the most common congenital disorders, with a worldwide prevalence of approximately 1/600. Orofacial clefts occur either as part of complex malformation syndromes or as an isolated anomaly. Non-syndromic cases represent about 60% of all orofacial clefts and have a multifactorial etiology encompassing both genetic and environmental components. IRF6, the causal gene for Van der Woude syndrome, an autosomal dominant clefting syndrome, is considered as a promising candidate gene in non-syndromic cleft lip with or without palate (CL/P). Several reports on positive association between single-nucleotide polymorphisms in the IRF6 gene and CL/P in different populations further support the role of IRF6 in the development of CL/P. We aimed to replicate these findings in our cohort of Central European families and performed a family-based transmission-disequilibrium test using three single-nucleotide polymorphisms, which were previously reported to be significantly associated with non-syndromic CL/P in European populations. Our sample comprised 261 non-syndromic CL/P triads of Central European origin. For statistical analysis we employed the TDT and the FAMHAP software for single and haplotype marker analysis, respectively. Neither single marker nor haplotype analysis revealed an association between the tested variants in the IRF6 gene and non-syndromic CL/P in our sample. The findings from our family-based association study do not support a potential involvement of the IRF6 gene in the etiology of non-syndromic CL/P in patients of Central European origin.

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P224

Genome wide linkage scan of nonsyndromic orofacial clefting in 89 families of Central European origin

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Orofacial clefts (OFC) belong to the most common congenital disorders. About 60% of the OFC cases are classified as non-syndromic orofacial clefts. Non-syndromic cases have a multifactorial etiology encompassing both genetic and environmental components. The two most common forms of OFC, cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) are generally considered to be distinct in etiology. We present results of a genome wide linkage scan in 89 families with non-

syndromic OFC of central European descent. The families consisted of 74 CL/P and 15 CPO families comprising a total of 438 genotyped individuals (213 affected and 225 unaffected individuals). We genotyped 542 microsatellite markers (average inter-marker distance = 7.25 cM). Multipoint non-parametric linkage analysis was performed with Allegro 2.0f. In CL/P families we observed the strongest evidence for linkage on chromosome 4q22.1-q24 (ZLR score = 2.82) and on two neighbouring regions on chromosome 1 (1p22.3, 1p13.1) (ZLR score = 2.29 and 2.13, respectively). Interestingly, the combined analysis of CL/P and CPO families resulted in increase of ZLR scores (3.05 on chromosome 4q22.1-4q24 and 2.92/2.57 on 1p22.3/1p13.1). Our results highlight promising chromosomal regions for the identification of OFC associated genes. The data might also suggest an overlap in risk-associated genes between CL/P and CPO.

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P225

Silent variants in CHRNA4 change functional properties of the α4β2 nicotinic receptor

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Single nucleotide polymorphism (SNP) and haplotype studies are essential for unravelling complex genetic traits. Recent association studies have identified various SNPs in neuronal nicotinic acetylcholine receptor (nAChR) genes associated with the development of nicotine dependence and other neurological disorders. Nevertheless, there is a lack of functional studies taking into account naturally occurring haplotypes, underestimating human genetic diversity. In particular, no systematic search for functional nAChR SNPs and haplotypes has been performed yet. Moreover, it is known that non-synonymous (changing amino acids) and synonymous SNPs can have an effect on protein function, but so far this has not been analyzed for nAChRs. Thus, we aim to systematically identify haplotypes in the coding region of nAChR subunits and then characterize their impact on nAChR expression and function. Based on human HapMap data as well as whole genome association studies, we generated up to date lists of the most represented haplotypes for the genes CHRNA2-CHRNA7, CHRNA9 and CHRNB2-CHRNB4. Additionally, haplotype data are also generated experimentally by genotyping controls. Of special interest are the CHRNA4 haplotypes identified using the HAP software with data from the Whitehead institute (WICVAR). The haplotype CCTCTA (SNPs rs1044393, rs1044394, rs2229959, rs2229960, rs1044396 and rs1044397) represents 52% of haplotypes while the opposite haplotype TTGTCG has a frequency of 9%. We used these data to generate expression plasmids containing both CHRNA4 haplotypes. After injection in X. laevis oocytes, we monitored their electrophysiological properties using a two electrode voltage-clamp automatic setup. The identified functional SNPs and/or haplotypes will afterwards be analyzed in association studies comparing non-smoking controls with different endophenotypes of smoking (dependent and non-dependent smokers), as well as with patients with schizophrenia or epilepsy.

P226

Genetic variants of the *DICE1/INTS6* gene appear to be rare in German prostate cancer families with linkage at 13q14

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A recent combined genome-wide linkage scan for prostate cancer-susceptibility genes identified linkage at 13q14 (The International Consortium for Prostate Cancer Genetics). The gene deleted in cancer cells 1 (*DICE1*; HGNC: integrator complex subunit 6 *INTS6*; MIM 604331) is located in this interval, that was also found to be deleted in prostate cancer specimens. A *DICE1* missense mutation has been detected in prostate cancer cell line LNCap and reduced *DICE1* expression appears to be associated with CpG promoter hypermethylation in prostate cancer cell lines. These results let us to consider the *DICE1* gene as a putative prostate cancer-susceptibility gene on 13q14. Patient DNA of thirteen prostate cancer families from Germany with linkage at 13q14 were analysed by genomic sequencing for nucleotide changes in the coding region of the 18 exons encoding the *DICE1* gene.

None of the patients harboured *DICE1* mutations. In one family including three affected brothers the variations c.1215A>C (p.T405T) in exon 10 and c.2568A>G (p.S856S) in exon 17 were detected in a heterozygous pattern. These variants have been previously reported in a hepatocellular carcinoma cell line. In sporadic prostate cancer patients, variant c.2568A>G (p.S856S) was detected in 10/325 (3.08%) compared to 5/207 (2.42%) control samples without statistical significance (95% confidence intervals with binominal distribution). We conclude that *DICE1* appears to be involved in prostate cancer progression but not in prostate cancer susceptibility.

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Spondyloepimetaphyseal dysplasia Omani type: report of a second family and a novel mutation in the CHST3 gene

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Spondyloepiphyseal dysplasia (SED) refers to a heterogenoueous group of disorders characterized by the involvement of vertebral bodies (platyspondyly) and the epiphyses. In some cases the metaphyses are affected as well (spondylo-epi(-meta)physeal dysplasias, SEMD). The changes usually result in shortening of the trunk, and to a lesser extend, of the extremities. Genetic analysis revealed the molecular basis of many of these conditions and demonstrated that in most cases the composition of the extracellular matrix is affected. Recently, a novel recessive form of SE(M)D with major involvement of the spine has been described in a large kindred from Omani (SED Omani type, MIM 608637). Thiele et al. (2004) demonstrated a missense mutation in the chondroitin 6-O-sulfotransferase (C6ST-1) gene (CHST3) in this family, which resulted in a defect of chondroitin sulfate chain sulfation. Here, we report a second family with SE(M)D Omani type and a novel mutation in the CHST3 gene.
We investigated the case of a 10 year old girl presenting with short stature, kyphoscoliosis, and multiple joint dislocations. Additional clinical findings included: supernumerary carpal bones, short metacarpalia, and short distal phalanges (especially of the thumb). The girl was born to first cousin parents of Kurdish origin. While a brother and a sister are also affected and show a similar phenotype, four additional brothers and a sister are unaffected. Using a genome-wide linkage approach we were able to map the underlying gene defect to an interval on chromosome 10q22.2 (rs2395053) to 10q23.1 (rs723195). We sequenced candidate genes from the region and identified a missense mutation in the CHST3 gene changing the glycine at position 259 of the chondroitin 6-O-sulfotransferase into valin (p.G259V). The amino acid is located in the evolutionary highly conserved 3´-phosphoadenosine 5´-phosphosulfate (PAPS) binding site.

P228

A promoter variant of the Annexin A5 (ANXA5) gene is associated with recurrent pregnancy loss

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Annexin A5 (placental anticoagulant protein) occurs on normal placental villi and it has been suggested that annexin A5 molecules form an antithrombotic shield on the apical surface of placental syncytiotrophoblasts. In this work we sought to verify whether variation in the promoter of (ANXA5) gene represents a risk factor for recurrent pregnancy loss (RPL). Sequence analysis of 70 German RPL patients, all known to carry neither factor V Leiden nor a prothrombin mutation, revealed four consecutive nucleotide substitutions in the ANXA5 promoter that were transmitted as a joint haplotype (M2). Reporter gene assays revealed that M2 reduces the in vitro activity of the ANXA5 promoter to 37-42% of the normal level. The possible relationship between M2 and RPL was evaluated by comparing RPL patients with two independent control groups recruited from the registry of the Institute of Human Genetics in Münster and the PopGen biobank in Kiel, respectively. Carriers of M2 were found to exhibit a more than two-fold higher RPL risk than non-carriers (odds ratio = 2.42, 95%

confidence interval: 1.27 - 4.58) when using unselected controls (PopGen), and an almost four-fold higher risk when using the Münster ´super-controls´, i.e. women with successful pregnancies and no previous history of pregnancy losses (odds ratio = 3.88, 95% confidence intervall: 1.98 - 7.54). This statistically significant association should facilitate the development of improved prognostic algorithms for RPL and provide the possibility for an early and preventive treatment of RPL.

P229

Association of functional CYP1B1 variants in German patients with primary open-angle glaucoma (POAG)

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Glaucoma, a major cause of blindness worldwide affecting mostly adults, is a complex and genetically heterogeneous disorder characterized by progressive death of retinal ganglion cells. CYP1B1 is the major gene responsible for primary congenital glaucoma (PCG), a rare recessive form already manifesting in newborns. Most glaucoma cases, though, occur in adulthood, presenting as primary open-angle glaucoma (POAG) with no associated ocular malformations and autosomal dominant inheritance in about 40% of cases.
To date, three causative genes have been described (MYOC, OPTN and WDR36) explaining only <10% of cases. Recently, PCG associated CYP1B1 mutations have also been observed in the heterozygous state in POAG patients in Spanish, French, Canadian and Indian populations.
We now investigate the reported association of CYP1B1 in German POAG patients. The entire coding region was sequenced in 399 unrelated patients and 376 controls. Five known PCG variants (P52L, W57X, Y81N, R368H, and A443G) and four novel ones (G168D, N203S, G329V, and V465A) were identified. To assess their role as causative variants, we are performing in vitro functional assays of all CYP1B1 variants embedded in their respective founder haplotypes, combining enzymatic activity and relative protein amount to determine relative enzymatic activity. Results for N203S show strong reduction of relative enzymatic activity, confirming its role as loss-of-function mutation as previously reported for P52L and W57X. Variant Y81N showed an intermediate reduction, compatible with a hypomorphic allele. Assessment of remaining alleles is ongoing. These 6 variants investigated so far, were present in 12 patients (3%) but only in 3 controls (0.8%; P=0.034).
In conclusion, our study suggests that heterozygous CYP1B1 mutations showing absent or reduced relative enzymatic activity, are more frequent in German POAG patients than in controls and thus may represent a risk factor for POAG.

P230

The incidence of the Abcc6-PXE gene mutation in patients with cardiovascular disorders, but no reported PXE disease

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Background: ATP-binding cassette, sub-family C 6 (Abcc6) is reported as determinant gene for both Dystrophic cardiac calcification (DCC) and Pseudoxanthoma elasticum (PXE) in mice and human, respectively. Both PXE and DCC share many features including calcium phosphate deposits. Previously, the frequent Abcc6-PXE gene mutation has shown to be associated with an increase in the prevalence of coronary artery diseases (CAD) in a Dutch population. Here we report data on the investigation of the frequency of the Abcc6-PXE gene mutation in two well defined case-control studies for CAD and coronary artery calcification (CAC) in a German population.
Results: We genotyped the c.3421C>T PXE mutation using allelic discrimination on genomic DNA in patients with CAD from the German Myocardial Infarction Family Study (n=1156 cases and n=983 controls). Using coronary angiography 877 CAD patients were further characterized for calcification and grouped into patients with CAC (n=511) and without CAC (n=304). Controls within each case-control study were well defined and selected to match for sex, age, BMI, blood pressure, and cholesterol level. Analysing the incidence of the c.3421C>T mutation, no significant difference in the

frequency of the heterozygous carriers could be found neither in the CAD study (0.60 % in cases and 0.71 % in controls, p=0.7936) nor in the CAC study (0.78 % in cases and 0.98 % in controls, p=0.7164). Few heterozygous carriers of the c.3421C>T PXE mutation were found in all both studies (14 carriers in total). Clinical data of the available files from 13 heterozygous carriers were examined for any reported PXE-abnormalities. No sign of PXE was reported in these heterozygous carrier patients.
Summary: Our data demonstrate no association of the Abcc6-PXE gene mutation either with CAD or with CAC. The use of a well defined case-control study is the optimal tool to carry out association analysis for SNPs with lower frequency.

P231

Genetic variation in the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) is associated with myocardial infarction in the German population

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Background: Genetic variation in the genes for ALOX5AP and LTA4H has previously been shown to contribute to the risk of myocardial infarction (MI) and stroke in the Icelandic and Scotish population. Both genes encode for proteins playing a role in the synthesis of the pro-inflammatory leukotriene B, possibly providing a link between MI and inflammation. The aim of the present study was to investigate whether these associations can be confirmed in a large study of German MI patients.
Methods: Two previously described four SNP haplotypes of the ALOX5AP gene (termed haplotype A and B) and one SNP (rs2660899) of the LTA4H gene conferring the greatest risk of MI in previous studies were genotyped in 1,211 unrelated MI cases from the German MI Family Study and in 1,015 healthy married-in spouses serving as controls.
Results: Haplotype B in the ALOX5AP gene was associated with an increased risk of MI in the German population, confirming previously reported associations of this haplotype with CAD in populations from Scotland and Italy. No association with risk of MI was detected for haplotype A of the ALOX5AP gene nor for SNP rs2660899 representing the LTA4H gene.
Conclusions: Haplotype B of the ALOX5AP gene is associated with an increased risk of MI in a large German study. This study is the third independent report from a European population describing increased risk of CAD for carriers of haplotype B of the ALOX5AP gene, which further substantiates a role of this gene in the pathogenesis of CAD in Europeans.

P232

Hair shaft abnormality Pili annulati - reduction of candidate region to 2.9 Mb and expression analysis of genes in the critical region

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Background: Pili annulati is an autosomal dominant transmitted hereditary hair disorder characterized by alternating light and dark bands in the hair fibre of affected individuals. Recently, a locus for pili annulati was mapped to chromosome 12q24.32-24.33 by linkage analysis in 5 families segregating this trait. Recombination events defined a critical region of 8 Mb.
Objectives: The aim of the current study was to reduce the size of the candidate region by analysis of further families and to investigate the expression of possible candidate genes in hair follicles and scalp tissue. Approach Genomic DNA was extracted from 96 individuals of 4 families, after examination to establish their phenotype. Finemapping was performed in all 96 individuals using 26 microsatellite markers spanning a 20 cM region at the telomeric end of chromosome 12. Candidate genes were analyzed for their expression in hair follicles, derived from plucked hair follicles, scalp and other tissues by RT-PCR.
Results: In family I, 7 individuals were affected, 5 unaffected. The largest family so far described for

pili annulati in the literature was family II, with 26 affected and 39 unaffected family members over 3 generations. Family III and IV were smaller families with 3 and 6 affected and 8 and 2 unaffected individuals, respectively. In family I and familiy II recombinations were identified which reduced the region by more than half from 8 Mb to 2.9 Mb containing 38 known and putative gene loci. We have analysed a majority of the genes in this region by RT-PCR and have found that 21 were expressed in plucked hair follicles.
Conclusion: In summary we confirmed the locus for pili annulati in 4 further families, reduced the critical interval to 2.9 Mb, and identified possible candidate genes expressed in the human hair follicle.

P233

Identification of a novel germline mutation p.Trp355Arg in the *HSD3B2* gene in a patient with hypospadias and micropenis

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Introduction: Classical deficiency of 3 beta-Hydroxysteroid-Dehydrogenase Type II, caused by mutations in the *HSD3B2* gene, is a rare form of congenital adrenal hyperplasia (CAH).
Patient: We report a 2-year-old patient of Turkish origin referred to our center for the evaluation of penoscrotal hypospadias and micropenis. Testicles were palpable bilaterally in the scrotum. Genetic testing of *CYP21A2* gene was already done due to initial biochemical and hormonal findings, and a compound heterozygous state of detected germline mutations (p.Val281Leu, p.Leu307fs, p.Gln318Stop, p.Arg356Trp) was already excluded by analysis of *CYP21A2* genes of both parents. His parents are first degree cousin.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding regions and corresponding exon-intron boundaries of the *HSD3B2* gene. PCR products were sequenced directly.
Results: Sequence analysis of the *HSD3B2* gene showed a novel homozygous germline mutation in exon 4, codon 355 (TGG>CGG), leading to the substitution of tryptophan (Trp) with arginine (Arg) and therefor to an alteration of the amino acid sequence within the enzyme. Both parents were heterozygous carriers for this mutation.
Conclusion: To find the underlying genetic cause and to judge a case of CAH in the right way it is important to look at all - clinical, biochemical and hormonal - aspects in a differentiated way. Comprehensive (clinical, biochemical, hormonal) analysis should be conducted and approved by genetic testing in line with a genetic counseling.

P234

New aspects of the molecular genetic diagnostics in Cleidocranial Dysplasia (CCD)

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Introduction: Cleidocranial Dysplasia (CCD) is an autosomal dominant skeletal disorder, characterized by hypoplastic or absent clavicles, large fontanelles, increased head circumference and wide sutures at birth that show a delayed ossification. In addition, short stature, dental anomalies and hand malformations are common. CCD is caused by mutations in the RUNX2/CBFA1 gene on chromosome 6 that encodes a transcription factor that promotes the differentiation of mesenchymal cells into osteoblasts in the cartilage anlagen. By direct sequencing point mutations in RUNX2/CBFA1 can be detected in about 70%. As mice lacking one allele of Runx2/Cbfa1 resemble closely the human phenotype, CCD is supposed to be caused by haploinsufficiency of RUNX2/CBFA1.
Methods: In 42 unrelated patients with CCD but without identified mutations quantitative PCR was performed to screen for copy number variations in the coding region of RUNX2/CBFA1. Furthermore, array CGH on BAC arrays was done to further characterize the size of deletions that exceeded the genomic region analysed by qPCR and in case where no aberration was detected by qPCR to screen for possible other genome wide copy number variations that might be related to the CCD phenotype.
Result: We found heterozygous deletions between 400bp and 2.3Mb in 10 of 42 investigated samples, i.e. about 25%. In one additional case we identified a 1.8Mb duplication 5' of another gene that had not been directly related to CCD so far.

Conclusion: As new aspect in the molecular genetic diagnostics of CCD copy number analysis in RUNX2/CBFA1 should be included in standard procedures. Additionally, we are going to screen for mutations in our new candidate gene, and 5' of it for smaller copy number variations that can not be detected by array CGH, as 23% of all cases still remained unresolved.

P235

In vitro characterization of the transcriptional effect of mutations in the retinoblastoma promotor-region

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Mutations that impair RB1 gene function can cause hereditary retinoblastoma (rb). In a few families, rb is linked to mutations in the promoter-region of the gene. These families can show incomplete penetrance and milder expressivity compared to families with RB1 null mutations. Several RB1 promoter mutations have been identified in isolated cases. In vitro assays of the transcriptional activity (TA) can help to decide if a variant allele is pathogenic. Moreover, it is interesting to test if any of the few promoter variants identified in *cis* to *bona fide* pathogenic mutations has distinct regulatory activity and thus may modify tumor predisposition.
We tested 8 promoter variants classified according to mutation site: Ia/Ib, in known Transcription Factor Binding Sites (TFBS); II, in predicted TFBS; III, non-coding but outside known or predicted TFBS; IV, genetic variation in exon 1. Reporter gene expression was determined by a Dual Luciferase Reporter Assay in CHO cells. Transcriptional activity (TA) of an Ia mutation (g.1862G>A, associated with rb in 10/11 heterozygotes) was markedly reduced (20% of normal) which is in line with reported data. No loss of TA was observed in an Ib mutation (g.1910G>T, reported in a family with rb in 3/9 heterozygotes). Putative non-pathogenic variations showed diverse activity: for g.1718G>A (type III) activity was moderately reduced (62%) whereas g.2101C>T (p.Ala14Ala in exon 1) showed an enhanced TA (165%). Another type IV alteration, g.2118C>T (p.Pro20Leu, reported in 3 patients with isolated rb and 2 unaffected heterozygotes), showed reduced TA (32%). Our results show that mutations in the coding part of exon 1 can influence promoter activity. Specifically, g.2118C>T is pathogenic but is not a missense but a regulatory mutation. Moreover, we found that putative normal variants can influence regulatory activity in vitro and thus are reasonable mediators of modifying effects.

P236

Refinement of the MYP3 locus on human chromosome 12 in a German family with Mendelian autosomal-dominant high-grade myopia by SNP array mapping

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Myopia, or short-sightedness, is the most common eye disorder worldwide. "Pathologic" high myopia, or myopia of $\leq$ -5.00 diopters, predisposes individuals to retinal detachment, macular degeneration, cataract, or glaucoma. Many forms of autosomal dominant non-syndromic high-grade myopia are known in humans. While the critical disease intervals have been identified and located to physical map positions, the gene defects and causative mutations responsible for autosomal dominant myopia has remained elusive to date. Examination of a German six-generation kindred with 46 individuals by 10K whole genome chips led to the identification of a 19 cM map segment as being the most likely familial myopia candidate region spanning from chromosome 12q14.3 to 12q21.31 (MYP3). The recombination breakpoints in this family and the interval of the originally reported German/Italian family defining the MYP3 locus within 12q21-2 (OMIM 603221) allowed us to drastically refine a minimum consensus region. This new composite region locates between the microsatellite marker D12S1684 and SNP_A-1509586, narrowing the original 30.1 cM of the MYP3 interval down to only 6.8 cM. Within the refined MYP3 interval the public databases list only 25 genes, from which several appear to be promising MYP3 candidates based on similarities with genes and proteins involved in vision physiology and eye disease. Future identification of this MYP3 gene may provide insights into the pathophysiology of myopia and eye development.

P237

PCView - finding positional candidate genes

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After linkage studies, researchers are usually confronted with many positional candidate genes. As it is still not feasible to sequence the whole region at once, the researchers usually prioritise genes, according to their own expectations of a good candidate. Depending on the disease under scrutiny, this can be based on OMIM reports, tissue specific expression, and the like. While much of the relevant data can be found on the Internet, collecting and assigning it to all the genes in the region can become a tedious effort usually involving a lot of clicking, and subsequent copy and paste work or stapling numerous print-outs. Although some applications offer an automatic prioritisation, they still require subsequent searches on the Internet because they do not display all the data that might be relevant to the researcher.
We have developed a web-based database application to simplify this. After the researcher enters a target interval, she will get a list with all the genes in the region plus gene-specific information. The output is presented as an HTML page that can be printed and studied at a cosier place than the computer monitor. On the results, projections and selections can be applied, i.e. the user can select the kind of information he needs and set criteria that must be fulfilled by a gene to be included. The results can be sorted according to the gene´s position or to user-defined parameters like tissue specific expression or interesting interactions.
PCview also offers a semi-automatic prioritisation. The researcher can decide by herself on which parameters this should be based. If, for instance, genes causing similar phenotypes are known, prioritisation can focus on genes interacting with them and showing similar expression patterns, yet considering other parameters as well. All this remains transparent to the researcher and can be fine-tuned to lay more attention on those parameters that appear important to her.

P238

Mutation analysis of DNA repair genes in patients with MHC-linked increased chromosome breakage rates in systemic sclerosis

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Systemic sclerosis (SSc) is a systemic connective tissue disorder characterized by proliferative vascular lesions, obliterative microvascular lesions, and residual atrophy with fibrosis of multiple organs, i.e. skin, gastrointestinal tract, heart, lung, and kidney. The etiology is still unknown. In 1988, the increased chromosomal breakage rates in cultured lymphocytes of SSc patients were linked to the Major Histocompatibility Complex (MHC) on the short arm of chromosome 6 (Rittner et al., Hum. Genet. 1988; 81:64-70). Our working hypothesis is that mutations of a gene(s) in the MHC region cause a DNA repair defect in lymphocytes of SSc patients and predispose to SSc. We therefore performed mutation analyses of DNA repair-associated genes in the MHC region in two SSc patients with increased chromosome breakage rates. So far, three candidate genes, FANCE, MAPK14, and FKBPL, have been excluded. In another DNA repair-associated gene we found a hitherto undescribed A-G nucleotide exchange causing a non-synonymous substitution of the neutral amino acid glutamine by the basic amino acid arginine in one SSc patient. This nucleotide exchange was not observed in 50 normal control individuals. Sequence analyses of more SSc patients and additional 50 controls are underway. In conclusion, systemic sclerosis may be a disorder with DNA repair deficiency in heterozygous state.

P239

Analysis of genetic polymorphisms in B-cell chronic lymphocytic leukemia and their putative association with cytogenetic subroups

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B-cell chronic lymphocytic leukemia (CLL) is a common type of leukemia that particularly affects elderly people. As the clinical course of CLL is highly variable, prognostic factors for disease progression such as particular cytogenetic abnormalities, the mutational status of the immunoglobulin heavy chain (IgH) genes, lipoprotein lipase (LPL), or CD38 expression, have been identified. However, a convincing prediction of disease progression cannot be made in early stages of the disease. Further prognostic factors would be helpful for meaningful prediction of disease progression. There is evidence that multiple low-penetrance genetic factors, including genetic polymorphisms, predispose to CLL or modify its clinical course.
The aim of this study was to identify such susceptibility alleles for CLL. We genotyped 32 polymorphisms in 20 candidate genes primarily involved in DNA repair and detoxification in over 400 patients and over 400 matched controls to determine whether they alter the risk for CLL in a case-control study. Additionally, we investigated 18 polymorphisms for a putative association with specific chromosomal aberrations that have been found to play an important role in the prognosis of CLL. Comparison between patients with 13q14 deletion that correlates with a favorable prognosis and controls showed differential distribution of alleles in three genes (CYP1A1, CYP2C9, mEH), whereas differences between patients with deletions in 11q22~23 and 17p13 that correlate with an extremely unfavorable prognosis and controls could be detected in four genes (CYP1B1, CYP2C19, GSTP1, XRCC1). Further investigations are now under way to prove the relevance of these polymorphisms in progression-free survival and overall survival within these cytogenetic subgroups. The results of this study may help to predict disease progression.

P240

DRD3 genotypes and nicotine dependence

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Nicotine dependence is assumed to have a strong genetic background. Several candidate genes have been shown to be associated with smoking. To further test possible associations between genetic factors and parameters of smoking behaviour (e.g. level of nicotine dependence, number of cigarettes per day, duration of smoking, age and gender) we recruited 219 smokers joined a smoking cessation program. Genetic analyses for several candidate genes (dopaminergic, acetylcholinergic, serotoninergic pathways) have revealed one significant association signal for the G9V polymorphism in the DRD3 gene, which encodes for the D3 subtype of the dopamine receptor.
In order to replicate these findings, a further 245 samples were added to this study group. The samples have been genotyped for 10 informative single nucleotide polymorphisms (6 HapMap tagSNPs, and 4 additional SNPs) in the DRD3 region. Genotyping was performed with the Roche LightCycler480 instrument using the 384well format and FRET HybProbes or high resolution melting analyses with unlabelled probes.
Data accuracy was very high with a drop-out rate below 1%. After reconstruction of DRD3 haplotypes, we conducted a test for linkage disequilibrium and calculated multivariate association analyses. At this point, there is however no significant correlation between the genotype data and the anamnestic data. As anamnestic items like smoking intensity are suitable for categorical analysis (CatReg), we are currently undertaking more multivariate association analysis under this paradigm. This will help us to conclude upon the genetic effect of DRD3 haplotypes on smoking behaviour in our cohort.

P241

Modulators of cystic fibrosis disease severity encoded on 19q13

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Two cystic fibrosis (CF) modifying genes have been described on 19q13 albeit the designation of the risk and the benign allele is contradictory for TGFB1 [Arkwright PD et al. 2000, Drumm ML et al. N Engl J Med 2005] and the genetic entity associated with CFM1 [Zielenski J et al Nat Genet 1999] is still unknown. We have investigated 37 nuclear families with F508del-CFTR homozygous siblings exhibiting extreme clinical phenotypes [TwinRes 2000;3:277-293] for modulators of cystic fibrosis disease severity typing 7 microsatellite markers and 17 SNPs selected for high polymorphism

information content within a 6 Mb region encompassing TGFB1 and CFM1. Association with sibling intrapair discordance, previously reported for marker D19S197 (P = 0.003; NEnglJMed 2006;354:88-90) could be confirmed at a genomic fragment 200.000 bp distant to the microsatellite marker. Asymmetric transmission of parental genotypes which is most likely mediated by a maternal non-genetic effect was observed at TGFB1 SNP Leu10Pro (P = 0.000132, EurJHumGenet. 2007;15:774-778). Evidence for modulation of disease severity was neither observed at TGFB1 nor at CFM1, but haplotype analysis of the genomic fragment encompassing the entire CEACAM- gene cluster revealed a modulator for CF disease severity (P = 0.0057, P-value corrected for haplotype block). Fine-mapping of causative variants on the corresponding haplotype block comparing concordant mildly and concordant severely affected sib pairs is currently underway to determine the underlying molecular mechanism. Our data demonstrate that 37 families with contrasting phenotypes allow the observation of parental non-genetic confounders and furthermore enable the identification of novel modulating genes by systematic fine-mapping comparing haplotype distributions derived from informative SNP markers.

P242

Homozygosity for a *MPL* mutation in an Arab family with autosomal recessive familial thrombocytosis

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Familial thrombocytosis (FT) is mostly caused by dominant mutations of the thrombopoietin (*TPO*) gene. We recently mapped a locus for autosomal recessive FT in an Arab family to the *JAK2* region on chromosome 9. This region was excluded as the candidate region in a second Arab family with autosomal-recessive FT. However, in this family co-segregation with thrombocytosis was observed for markers linked to the myeloproliferative leukemia virus oncogene (*MPL*), which codes for the thrombopoietin receptor. Additionally, positive linkage to the *MPL* region on chromosome 1 was obtained through a genome-wide linkage scan. Assuming presence of a germ-line mutation, we sequenced the entire coding region of *MPL* including flanking intronic sequences and identified homozygosity for the missense mutation p.Pro106Leu (c.317C<T) in the affected family members. p.Pro106Leu was absent among 193 healthy Germans. Of 108 healthy Arabs, 4 were heterozygous for p.Pro106Leu. Pro106 is conserved across species. While p.Pro106Leu is predicted to alter thrombopoietin receptor function by biometric analysis with the computer program SIFT, no pathogenic effect was predicted when using PANTHER PSEC.
Unexpectedly, TPO serum levels were elevated in the affected sibs. *MPL* missense mutations in conjunction with elevated TPO levels are commonly found in patients with congenital amegakaryocitic thrombocytopenia (CAMT). Further investigations (including functional tests, Western blot and RNA studies) are under way to define the pathogenesis of *MPL* mutations leading to thrombocytosis in some cases and thrombocytopenia in others.

P243

Role of mammalian heat shock proteins 110 (HSP110) in germ cell development

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The mammalian HSP110 gene family consists of three proteins: Hspa4l, Hspa4 and Hsp110. Constitutive expression of Hspa4l is high in testis and moderate in other tissues, while Hspa4 and Hsp110 are ubiquitously expressed in various tissues. Immunohistological analysis in testis sections from different stages of postnatal development showed that Hspa4l is highly expressed in spermatogenic cells from late pachytene spermatocytes to post-meiotic spermatids. To study the physiological role of Hspa4l in vivo, we generated Hspa4l-deficient mice. Hspa4l-deficient mice were born at expected ratios and appeared healthy. However, approximately 42% of Hspa4l-/- male mice suffer from fertility defects. To determine the expression pattern of Hspa4 and Hsp110 in germ cell development, we performed RNA and immunohistological analyses. Whereas the seminiferous

tubules of Hspa4l-/- in testes contain all stages of germ cells, the number of mature sperm in the epididymis and sperm motility is drastically reduced. Reduction of sperm count is due to elimination of significant number of developing germ cells via apoptosis. No defects in fertility were observed in female mutants. In contrast to meiotic and postmeiotic expression of Hspa4l, Hspa4 and Hsp110 are activated in premeiotic spermatogonial cells. To determine the role of Hspa4 in germ cell development, we have generated and characterized Hspa4-deficient mice.

P244

Genetic variation of the FAT gene at 4q35 is associated with bipolar affective disorder

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A recent study (Blair et al., Mol. Psychiatry 11:372-383, 2006) suggested that the cadherin gene FAT exerts an influence on susceptibility to bipolar affective disorder (BPAD). We aimed to replicate this finding in a German sample (425 BPAD I and 419 controls). In addition, we performed a comprehensive linkage disequilibrium mapping of the whole genomic region of FAT and the neighboring circadian gene MTNR1A (48 single nucleotide polymorphisms (SNPs) covering 191kb). No significant association was observed for SNPs located in the MTNR1A gene. In FAT, however, 9 SNPs showed association, 8 of them being located in the same haplotype block found to be associated with BPAD by Blair et al.. The smallest p-value of 0.00028 (OR 1.71) was seen for non-synonymous SNP rs2637777. A combination of 5 markers including this marker showed a haplotype distribution with a nominal p-value of 1.8 x 10-5 that withstands correction for multiple testing. While the control allele frequencies between our sample and the samples studied by Blair et al. are comparable, tendencies of risk allele frequencies are opposite. Possible explanations for this include potential differences in linkage disequilibrium structure between the German, Australian, UK, and Bulgarian populations sampling variation, multilocus effects, and/or the occurrence of independent mutational events. We conclude that our results support an involvement of variation at the FAT gene in the etiology of BPAD, but that further work is needed both to clarify possible reasons for the observed risk allele differences and to ultimately identify the functionally relevant variant(s).

P245

No association between *CALCA* polymorphisms and clinical outcome or serum procalcitonin levels in German trauma patients

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Severe trauma induces sustained inflammatory changes that are related with secondary organ dysfunction. Recently, we showed that (i) post traumatic systemic inflammatory response syndrome (SIRS) was associated with a SNP in the cytokine IL-6 gene, and (ii) adult respiratory distress syndrome (ARDS) was associated with an IL-8 SNP and IL-8 plasma concentrations in German trauma patients. Another clinically relevant marker in polytraumtized patients is the serum procalcitonin (PCT) level. Therefore, we conducted a prospective cohort study to investigate whether polymorphisms in the calcitonin (*CALCA*) gene are associated with PCT levels and posttraumatic complications like SIRS, multiple organ dysfunction syndrome (MODS) or sepsis.
137 patients fulfilling the inclusion criteria (i) Injury Severity Score (ISS) >16; (ii) age 18-60 years; (iii) survival >48 hours after injury and 104 healthy German controls were studied for a series of SNPs from within or flanking *CALCA*. No differences were observed for allelic and genotypic distributions between patients and controls. When trauma patients were grouped according different clinical outcome parameters, we did not observe an association between any of the studied parameters and *CALCA* SNPs. Additionally, no association was observed between *CALCA* SNPs and PCT levels. We conclude that *CALCA* polymorphisms are unlikely to influence PCT levels and clinical outcome in polytraumatized patients.

P246

TGFB3 displays parent-of-origin effects among central Europeans with nonsyndromic cleft lip and palate (CL/P)

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Knock-out mice and association studies among different ethnicities in humans support the involvement of TGFB3 in the formation of orofacial clefts. The most comprehensive association study to date tested 23 SNPs in a Japanese CL/P population (Ichikawa et al. 2006). The authors observed significant association for 9 of these SNPS. We wanted to investigate the relevance of their findings for CL/P patients of Central European origin and in addition searched for parent-of-origin effects. We applied a family-based association design and included 204 patients and their parents. We investigated three SNPs (IVS1+2118, IVS1+5321 and IVS1-1572) which were most significantly associated with CL/P in the previous study and which had minor allele frequencies >0.05 in Japanese and Central Europeans. For statistical analysis we employed the TDT and the FAMHAP software for single and haplotype marker analysis, respectively. The LEM program was applied to estimate the relative risks of Weinberg´s log-linear model. TDT analysis showed neither significant transmission distortion for single markers, nor for any of possible haplotypes. However, we found strong evidence for parent of origin effects with lower risk of maternal than of paternal transmission (IM = 0.38; CI: 0.17 - 0.86) of the risk allele T to the affected offspring at marker IVS1+5321. This is also expressed in an increased risk of heterozygous children having the T-allele inherited from the father (RP = 3.47; CI: 1.32 - 9.11). The present analysis suggests a role for TGFB3 in the Central European population if the risk allele is inherited from the paternal side. Although these data suggest an intriguing role of TGFB3 in the development of oral clefts, the hypothesis still requires a convincing degree of support from independent studies.

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P247

Analysis of contrasting haplotypes G10 and A13 [rs767455 D12S889] in intron 1 of *TNFRSF1A*

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Objectives: The haplotype G10 [rs767455 D12S889] and the contrasting haplotype A13 in intron 1 of *TNFRSF1A* have been identified to modulate disease severity in the monogenic hereditary disease cystic fibrosis. [HumGen2006;119:331-343] By sequencing, we identified seven SNPs, which seem to be linked to each of the haplotypes.
Since the function of the two contrasting haplotypes is unknown, *in silico* analysis of the intron 1 and analysis of samples from patients of a former study in patients with cystic fibrosis (CF) (BEAT- study) were done. [AmJRespirCritCareMed2004;169:719-25]
Methods: The samples were genotyped with regard to the haplotypes G10 and A13 as well as the seven SNPs in these haplotype blocks.
In silico the two haplotypes were compared if any differences in promoters, transcription factor binding sites, CpG-islands and transcription variants are predicted.
Results: Ten patients were identified to be homozygous for the haplotype G10 and 13 to be homozygous for the haplotype A13. Linkage analysis of the seven additional SNP will be done. *In silico* analysis of the intron 1 predicted that some of these SNPs introduce differences in transcription factor binding sites between the haplotypes G10 and A13. Promoters are probably similar for both

haplotypes. No CpG-islands could be found. Additionally four different splicing variants of TNFRSF1A were found *in silico*.
Conclusions: The *in silico* comparison of the two contrasting haplotypes predicted that the haplotypes differ in the binding sites of certain transcription factors. Whether the four alternative spliced transcripts exist *in vivo* is still unknown. Linkage analysis as well as functional analysis of protein using serum and BAL samples of patients homozygous for the haplotype G10 or A13 are underway.

P248

A locus for autosomal recessive trait of familial thrombocytosis within the region of Janus-Kinase (*JAK2*) gene on chromosome 9p

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Familial thrombocytosis (FT) is mostly inherited in an autosomal-dominant manner. Previously, we reported an Arab family in which FT was inherited as a recessive, possibly X-linked trait. To map and identify the disease gene in this family, we re-evaluated the clinical status of the family members and performed a genome-wide linkage scan. We excluded X-chromosomal inheritance and mapped a possible candidate locus to a 19.69 cM interval (corresponding to 11,811,633 bp) between markers D9S1792 and D9S285, maximum two-point/multipoint LOD=2.48/2.55 for D9S286. This region on 9p encompasses 56 genes, including *JAK2*. Assuming presence of a germ-line mutation, we sequenced the entire coding region of *JAK2* including flanking intronic sequences, as well as 10,000 bp of the 5´-end region. No *JAK2* mutation could be detected in the affected family members. Additionally, we excluded presence of the somatic *JAK2* mutation Val617Phe in granulocyte DNA from affected family members. Real-time quantitative RT-PCR analysis from granulocyte RNA revealed higher *JAK2* expression in the family members than in controls. We assume that FT in this family is caused by homozygosity for an as yet unidentified mutation in *JAK2* or in another gene in the same chromosomal region, leading to direct or indirect activation of *JAK2*.

P249

GPFrontend and GPGraphics - graphical front- and backends for TGen's GenePool software

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Performing whole genome association (WGA) studies on pooled DNA is potentially an alternative to genotyping of individual DNAs, as hybridization intensities at single array oligonucleotides of the respective alleles should reflect allelic frequencies in the DNA pool. This approach, though, has been hampered by the lack of user-friendly analysis software for such DNA pooling-based studies. Recently, Pearson et al. (Am J Hum Genet. 2007; 80:126-39.) have developed the GenePool suite of programs to extract and analyze data from pooled SNP array data - for both Affymetrix and Illumina platforms. The GenePool programs, however, were originally intended for GNU/Linux operating systems; hence, they are rather cumbersome to handle for the average PC user and completely lack any graphical output. After compiling the software for Microsoft Windows, we have created two novel programs to overcome this: GPFrontend enables the user to run all GenePool Programs with a graphical user interface, where all options can be manipulated and seen at once. Furthermore, it takes care of managing and generating metadata files which are necessary for GenePool to properly analyze the data. GPGraphics enables the user to graphically evaluate SNP scores or ranks calculated by GenePool, generating a set of image files. These images can in turn be viewed and analyzed with GPGraphics to determine the underlying data for particular SNPs or regions thereof. In addition, GPGraphics is able to apply several mathematical filters to the data points before generating images, thus further facilitating analysis.
The software was successfully used to conduct DNA pooling-based WGA studies for a mendelian trait (cystic fibrosis) and a quantitative trait (optical disc diameter). For details on these studies refer to the abstract by Krumbiegel et al. at this conference.

P250

Clinical and molecular genetic studies show no association of alopecia areata and inflammatory bowel disease

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Alopecia areata (AA) is one of the most common hair loss disorders which affects approximately 1-2 % of the general population, equally distributed in men and women. The etiopathogenesis of AA is incompletely understood, but it is thought to be a tissue-specific autoimmune disease directed against the hair follicle. Association of AA with a variety of autoimmune disorders has been repeatedly reported including association with inflammatory bowel disease (IBD). To further examine a possible association between AA and IBD we investigated our large sample of unrelated AA patients for 1) the prevalence of clinical IBD by using anamnestic questions and 2) the frequency of CARD15 variants. Only three of 750 patients had a personal history of IBD (two presenting with CD and one of them with UC) which was comparable to the frequency observed in western populations. Since mutations in the CARD15 (NOD2) gene have been observed to be a strong risk factor in IBD they may be considered promising candidates in the development of AA if an etiological overlap exists between the two disorders. Therefore the CARD15 variants R702W, G908R and 1007fs have been genotyped in a case-control-sample of 300 AA patients and 200 controls. No significant differences in genotype or allele frequencies between patients and controls have been observed for any of the three mutations. Furthermore, the analysis of subgroups of individuals with either severe AA, an early age of onset (onset age $\leq$ 20 years) or a positive family history did not reveal an association. Our clinical results and the genetic association study do not support the hypothesis that AA is associated with an increased risk for IBD.

P251

Candidate gene analysis for CAD using data from the 500k Affymetrix GeneChip® Array

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Introduction: Genome wide analysis offer the change to identify new gene regions associated with complex diseases. Recently, we identified several novel gene regions associated with CAD/ MI in two large GWA studies. Nonetheless, the 500k genotyping platform also offers an opportunity to examine genetic variants in genes with prior associations (candidate genes).
Method: We searched PubMed for original articles published before May, 2007 that reported statistically significant associations between specific genotypes and MI or CAD. Search terms included: myocardial infarction, coronary artery disease, association study, polymorphism, and genetic association. On the Affymetrix GeneChip® Array (500k) we distinguished between SNPs that are identical with those previously showing association and SNPs that are in complete or near-complete linkage disequilibrium (LD) with previously associated SNPs. We used two LD measures, r2 or D, based on data from the International HapMap Project.
Results and discussion: From a literature search we identified 142 SNPs in 91 candidate genes previously associated with CAD/MI. Only 13 of these SNPs are represented on the Affymetrix 500K array. For 36 genes there were no primary or tagging SNPs. For the other genes we identified 270 SNPs in complete or almost complete LD with the previously associated SNPs. While a number of SNPs displayed promising associations in either the WTCCC or the German studies only two linked SNPs (rs17489268, rs17411031) tagging the Ser447Ter variant in the lipoprotein lipase gene showed consistent association across both studies.
Conclusion: Our results are in good agreement with most recent systematic studies that were largely

unsuccessful in replicating initial findings on candidate genes. However, it should be borne in mind that many of the previously studied gene variants are poorly tagged on the Affymetrix 500k array and it clearly fails to cover the full extent of even common variation in these genes.

P252

High throughput mutation screening in patients with isolated respiratory chain complex I deficiency

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Almost 50 causal variants in nuclear genes associated with OXPHOS disorders are known. Despite this success, in the majority of cases the molecular defects remain unknown. Primary mitochondrial disorders are biochemically characterized by deficiency of respiratory chain complex (RCC) activities. They can be divided into three main categories: defect of respiratory chain complex I (RCC I), complex IV (RCC IV) and combined RCC deficiencies. We established a DNA collection of 200 samples each from patients with isolated RCC I and combined RCC deficiencies. In parallel we deployed high-throughput protocols for genetic screening using melting point analysis (Idaho-Light-Scan). In the current setting, 96 samples with 4 replicates each are analyzed in parallel followed by direct sequencing of those PCR products that display divergent melting curves. So far, we have screened 50 genes (250 amplicons) coding for the subunits and assembly factors of RCC I in the first set of 92 DNA samples from patients with isolated RCC I deficiency. Mutations have been identified in 14 patients. A single variant was identified in 12 additional samples and in 66 samples no mutations have been found.

P253

Association analysis of the polymorphisms of the VDR gene with bone mineral density and the occurrence of fractures

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Associations of the FokI, BsmI, ApaI, and TaqI polymorphisms of the vitamin D receptor (VDR) gene with the bone mineral density (BMD) of the lumbar part of the spinal column (BMD LS) and the neck of the femur (BMD FN), and with the occurrence of fractures, were studied using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis on DNA isolated from peripheral blood of 239 women and 40 men from the region of western Poland. Three polymorphisms of the 3′ end of the VDR gene (BsmI, ApaI, TaqI) indicated a strong linkage disequilibrium. Association analysis of the VDR gene FokI polymorphism with BMD LS showed a dose effect of allele f. The association of the bAT haplotype of the BsmI, ApaI, and TaqI polymorphisms of the VDR gene with BMD FN was statistically significant. The association of the ApaI polymorphism with the occurrence of fractures was observed. Associations were also observed between the occurrence of fractures and the baT haplotypes of the VDR gene.
J Bone Miner Metab (2007) 25:310-319.

P254

Results from a replication linkage scan with 50 German prostate cancer families: Some regions rediscovered

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Previously, we published results from a genome-wide linkage scan for prostate cancer, based on 139 German families (collected in Ulm, Germany) with 309 cases genotyped at 500 micro-satellite markers from the deCode panel (Maier et al., EJHG 2005). There, we focused on markers with nonparametric linkage Zlr-scores > 2, yielding regions of putative prostate cancer loci on chromosome #1 (1p31,

1q12), on #5 (5q14, 5q34, 5q35), on #6 (6q27), on #8 (8p22), and on #15 (15q13), if the sample was not stratified. Later, this scan was part of an international effort initiated by the International Consortium of Prostate Cancer Genetics (ICPCG).
In 2007, the ICPCG launched a collaborative scan, where Ulm contributed 50 new pedigrees (containing 114 genotyped cases). This time, the Illumina Linkage IVB SNP panel with about 5900 SNP markers was applied. We evaluated the Ulm families separately, this time using the software MERLIN. We found four notable signals, two of them, namely at 1p13 and 8p22, are close to regions that had already emerged in the first scan. The highest score (non-parametric LOD under the Kong and Cox linear model) in the first region was 1.87, in the second 1.05. A further region of interest was recognized at 22q13 (LOD=1.51), in close vicinity to one described by Camp et al. (Hum Mol Genet, 2007). Finally, we found a linkage signal on chromosome #14, at 14q12 with LOD=1.18.
Our study shows that also with comparatively small pedigree collections linkage regions can be (at least informally) re-identified. We think the main clue is the homogeneity of the underlying population.

P255

Epistatic interaction between the ACE D and MTHFR 677T alleles may increase the risk of progression of retinopathy of prematurity

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Retinopathy of prematurity (ROP) is characterized by neovascularization, occurs in infants with low gestation age and can lead to retinal detachment and blindness. We set up prospective study of 100 infants from Poland born at gestational age of 30 weeks or less for studying the role of angiotensin-converting enzyme (ACE) gene (I/D) polymorphism nad methyleneterahydrofolate (MTHFR) 677C>T SNP in the network of factors contributing to or concurring with the proliferative form of ROP and the specified diseases of preterm infants was assessed in terms of specificity of associations. The ACE I/D polymorphism was determined using PCR and MTHFR 677C>T SNP using PCR-RFLP methods. The genotypes were ascertained in the group of infants (N=99) and the random population sample (N=238). In infants with proliferative ROP there was a tendency to higher frequency of ACE D allele carriers, as compared to the group of no-ROP controls and spontaneously regressing ROP cases (91,7% vs. 75,0%; p=0,09). This association was statistically significant among newborns from single pregnancy (OR=4,9; p=0,03), but no significant association between the ACE genotypes and the susceptibility to other diseases was noted. MTHFR 677CT+TT genotypes were significantly associated only with patent arterial duct (PDA) (single pregnancies OR=3,5; p=0,047). However, in the group of newborns with proliferative ROP the frequency of subjects carying 3-4 MTHFR 677T and ACE D risk alleles (20,0%) was increased, as compared to the frequency of subjects with 0-1 risk alleles (10,0%; p=0,1). MTHFR 677CT+TT genotypes were associated with PDA (OR=3,5; p=0,047). In conclusion, the ACE D allele seems to be the causative variant involved in ROP progression and there may be the epistatic interaction between D and 677T alleles increasing the risk of ROP progression. No association has been found between the ACE and MTHFR genotypes and the preterm delivery.

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P256

Genome-wide association study of refractive error

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Non-syndromic refractive error (myopia, hyperopia) is a complex heterogenous disorders generated by genetic and environmental factors. The prevalence of pathologic myopia varies between populations. Depending on the definition used 25% (whites of European origin) to 50% (Chinese and Japanese populations) of all adults are affected. Heritability estimates for myopia as assessed in twin studies range from 0.5 to 0.96 (Young et al 2007). We collected refraction data from 3 x 10exp3

individuals aged older than 35 years by handheld autorefractometry (Retinomax), eye glass analysis and/or inspection of their optometric prescriptions. In half of these individuals genome-wide SNP data (500K Array Set, Affymetrix) were available. Along with other studies (Wang et al 1994, Gundmundsdottir et al 2005) we detected an average increase of refraction with age (= 0.07 x age - 4.0). We limited our analysis to the range of -15 D to 15 D and excluded individuals with congenital eye disorders or anisometropy higher than 5 D. Previous eye surgery (e.g. LASIK) also was an exclusion criterion. 1357 individuals and 272802 SNPs that passed all quality control filters were included in the genome-wide association analysis. After correction for age, we identified 8 gene loci that comprised at least 2 SNPs with a nominal significance level of at least 10exp-4 (max: 5.7 x 10exp-7) in a genotypic association model. A replication study is underway to test whether some of these loci may actually be involved in the genesis of refractive error.
(K.Oe. and T.Be. contributed equally to this study.)

P257

Array CGH analysis on 22 patients with mental retardation and dysmorphological signs

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Array CGH (comparative genome hybridization) is a powerful method to identify chromosomal imbalances of the genome. In comparison to classical cytogenetic analysis small deletions or duplications/amplifications can be detected. Recent studies on patients with idiopathic mental retardation showed in up to 10 % of the cases genomic imbalances identified by array CGH.
We analysed a set of 22 patients with mental retardation and dysmorphological signs with 244K Agilent oligonucleotide arrays. The 60mer oligonucleotides presented on these arrays cover the whole genome with a medium distance of 6,5 kb.
One patient showed a de novo 18,3 Mb duplication on the short arm of chromosome 1 (1p34.3-1p32.2). The patient has profound mental retardation, auto-aggressive behaviour, hypoplasia of corpus callosum, mitral valve prolaps, facial dysmorphisms and minor malformations (e.g. undescended testes, uvula bifida). The duplication is located in a tandem repeat as identified by FISH analysis. Despite its large size the duplication was not detected by conventional cytogenetics. In another patient a 9,3 Mb deletion at the end of the short arm of chromosome 4 was identfied followed by an adjacent 5,3 Mb duplication. The deletion encloses the Wolf-Hirschhorn region. Two further patients with imbalances bigger than 1 Mb showed a 1,6 Mb duplication on chromosome 7 and a 1,4 Mb duplication on chromosome 17, respectively.
Smaller imbalances between 100 kb-500 kb outside known regions of copy number variations were found in six further cases. Whether these alterations are responsible for the symptoms of the patients cannot be determined at present and further experiments are in progress to answer this question.

P258

Establishment of a core unit laboratory for microarray analysis

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With continued advances in microarray technologies, a variety of genome-wide analysis techniques like whole genome linkage and association analysis as well as expression profiling have become a powerful research tool for elucidating genetic variability in human as well as in animal models. Recently copy number variation analysis (CNV) has also become a widely used application. Especially the high density oligonucleotide based SNP microarray supports a much higher resolution of CNVs than BAC arrays. The newest generation of Affymetrix GeneChip arrays, for example, with 1.8M markers provides a median intermarker distance of 700bp. Chemically manufactured microarray platforms offer robust and reproducible results. The Affymetrix platform has an extremely wide acceptance supported by over 4,000 publications to date and a higher within-platform correlation of technical replicates than on other platforms. Our equipment enables the unattended barcode tracked scanning of up to 48 GeneChips.
We are using the Human GeneChip 500K and Human SNP5.0 arrays from Affymetrix for family based linkage analysis, whole genome association analysis in a case control setup and association analysis in setup with pooled DNA-samples. For the simplification of the analysis of allele frequencies we have developed software which enables the direct comparison of different pooling data. Up to now we also

used the Human GeneChip 500K-arrays from Affymetrix and developed software for copy number analysis. This setup has been successfully applied to the analysis of copy number variation in mental retardation (Hoyer et al., J Med Genet. 2007). In about 10% of cases a probably causal CNV is detected. Recently we established the protocol for Human SNP6.0 arrays and are in transition to this high resolution array type.

P259

HapMap-based association analysis between genetic variation at the positional and functional candidate gene SV2A and schizophrenia in the German population

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Schizophrenia is a genetically complex neuropsychiatric disorder with a life-time prevalence of 0.5-1.0 %. There is combined evidence from linkage, functional, animal, and clinical studies which makes the gene for the synaptic vesicle glycoprotein 2A (SV2A) a promising candidate gene for schizophrenia: SV2A is located in chromosomal region 1q21.2, where a genome scan found genome-wide significant evidence for linkage to schizophrenia (Brzustowicz et al, 2000). The gene has been shown to be part of the regulation process of synaptic vesicle exocystosis, and homozygous knockout mice show reduced hippocampal GABAergic neurotransmission. Further, SV2A is the primary binding site for the antiepileptic drug levetiracetam, and at least one clinical study revealed schizophrenia-related adverse reactions (auditory hallucinations and suicidal thoughts; worsening of pre-existent schizophrenia; depression). In the present study, we describe the results of a systematic association study between SV2A genetic variation and schizophrenia. In a large patient-control sample originating from the German population (796 Cases and 845 Controls), we genotyped 5 haplotype tagging SNPs selected from HapMap, covering the entire SV2A gene and flanking sequences that capture all haplotypes at a frequency>1% in the CEU population. In the German population the region under study is covered by a single haplotype block which is tagged by the 5 selected markers.
Single marker analysis yielded nominal Armitage's Trend Test P values of 0.0016 and 0.0061 for two SNPs. The results remain significant after permutational correction for multiple testing. Separate analyses of phenotypic subgroups are currently underway to refine the finding. In conclusion, our results suggest that SV2A may be involved in the development of schizophrenia. Independent replication studies are warranted to confirm our finding.

P260

Molecular genetics of alopecia areata: Different loci in humans and rats and association with HLA

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Alopecia areata (AA) is a chronic inflammatory disorder of the hair follicles in the actively growing anagen stage. It is characterised by one or several circular regions of hair loss on the head. Isolated hair loss on other parts of the body can occur, and hair loss can further extend, culminating in alopecia totalis or alopecia universalis. Alopecia areata has been suggested as a tissue-specific autoimmune disease, considering a potential role for loss of immune privilege. Frequent familial occurrence of the disease demonstrates a strong genetic susceptibility. We have been analysing the Dundee experimental bald (DEB) rat as a rodent model of AA. A whole-genome scan for linkage in an F2 population, obtained using an intercross approach with DEB and PVG rats, resulted in one highly significant and several suggestive loci. Fine mapping performed by haplotype analysis with newly developed markers identified a candidate interval of 3 Mb in length. Transcript and comparative maps were generated for rats, mice, and humans, and positional candidate genes were determined.
In parallel, more than 130 human affected sib-pair families were genotyped using 500,000 SNP

markers in a chip-based approach. Preliminary data indicated a small number of loci with highly suggestive results. Linkage analysis using NPL and HLOD scores for 20,000 selected markers revealed a major locus outside the HLA region and the region homologous to the candidate region in DEB rats. In contrast, HRR analysis in our human samples identified different alleles in the HLA region to be associated with AA. Analysis of additional samples suggested *NOTCH4* and *TAP2* as candidate genes, with a minimum p-value $<4x10^{-6}$. Interestingly, a recent report described an association with AA in an overlapping region in extended pedigrees. Furthermore, a significant increase of *Tap2* expression was observed in a mouse model for AA.

P261

Differentiated thyroid cancer: analysis of associations with CHEK2, P53 and NOD2/CARD15 gene polymorphisms

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Thyroid carcinomas amounts to 1% of general and are the most often carcinomas of endocrine system with still arising frequency. The most frequently occurring type is differentiated thyroid cancer (DTC), therein papillary and follicular thyroid cancer. DTC belongs to group of tumors well prognoses and slow progress and benignity. Major problem are recurrences and regional or remote metastasis. Numerous cases of osteolytic, cerebral and pulmonary metastasis were observed. Possible progression from well differentiated thyroid cancer to malignant anaplastic carcinoma is also observed. Considering this extended search for disease course, good or poor prognosis and response to medical treatment studied with molecular markers is substantial. It is expected that SNP studies of genes indicating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development.
The dependence of differential thyroid cancer occurrence upon DNA variation: I157T in CHEK2 gene, R72P in P53 gene and 1007fs in NOD2/CARD15 gene was examined. Group of 296 patients with differentiated thyroid cancer and 200 individuals from population group was examined. I157T and R72P variants were analyzed by pyrosequencing and 1007fs by PCR-SSCP and DNA sequencing. There were no significant differences in allele or genotype frequencies in analysis of R72P in P53 gene but mutated allele frequencies of 1007fs and I157T was 8.95% and 4.9% in patients with thyroid cancer, compared with 2.92% and 2.1% in control individuals respectively. Frequencies between patient and control groups were tested using Pearson's chi square statistics. This analysis shows that 1007fs and I157T mutations are associated with susceptibility to differential thyroid cancer.
Our findings indicate that particular characteristics of cancer risk genes is necessary. Additionally a summary effect of different SNP changes as a cancer predisposing factor is possible.

P262

A genome-wide association study in a patient-control sample of bipolar disorder originating from the German population

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Bipolar disorder (BD) is a common and disabling neuropsychiatric disorder that is considered to be a complex trait resulting from genetic and environmental determinants. Genome-wide association studies have recently become feasible and are expected to open new insights into the genetic factors involved. We examined a large sample of 700 DSMIV-diagnosed patients with BD, all of German descent, as well as 1,363 ethnically matched population-based controls. All samples were genotyped

using Illumina's HumanHap550 BeadArrays. For inclusion in the final data, individual DNA samples and SNPs met a variety of stringent quality criteria. Single-marker analysis of the quality-controlled data set resulted in a distribution of P-values matching the distribution expected, with some excess noted in the low p-value range consistent with the presence of multiple risk alleles of modest effect size. A total of 103 SNPs produced Armitage's Trend Test p-values smaller than 10E-04, the most significant SNP reached a p-value of 2.4 x 10E-07 and is located in chromosomal region 1q. The most significant findings of our study are currently compared to data available from the WTCCC genome-wide association study on BD (The Wellcome Trust Case Control Consortium, Nature 2007) and will supplement the selection process of SNPs for replication studies in independent cohorts of BD from the German population.

P263

Increased frequency of the IVS4+39C>T allele of PGHD-15 gene in group of patients with severe course of IBD

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Inflammatory bowel diseases (IBD) are autoimmunological disorders (MIM #266600) with genetic background, characterized by chronic inflammation of the wall of gastrointestinal tract. IBD include two clinical entities: Crohn's disease and ulcerative colitis (MIM#191390). The incidence of these diseases in Western population range from 100 to 300 per 100 000. The symptoms of Crohn's disease may arise in any part of gastrointestinal tract, however most often the distal portion of the ileum and caecum is affect. Inflammatory process penetrates through the whole thickness of the bowel wall and skip lesions are typical. Continuous inflammatory lesions confined only to colonic and rectal mucosa are characteristic for ulcerative colitis. Fistulas are absent and the inflammation never adopts the granulomatous form. In about 10% of the cases bowel inflammation may adopt the form hard to differentiate and the term indeterminate colitis is then used.
In our research we examined the frequency of alleles in the NOD2 gene and 15-@hydroxyprostaglandin dehydrogenase gene (PGDH-15) in 58 patients with severe postoperative relapses of inflammatory bowel diseases, 27 children with inflammatory bowel diseases and in control group (100 persons). The average age of onset was 31 years in the group of patients with severe disease course and 11 in the group of children. We observed the elevated frequency of the alleles 3019-3020insC and 802C>T of the NOD2 gene in comparison to the control group. The allele IVS4+39C>T of the PGDH-15 gene in the group of ill with the heavy course occurred two times more frequent than in the control group and three times more frequent in comparison with the group of affected children.

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P264

Genetic variations associated with diabetic nephropathy and type II diabetes in a European population

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Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD). Genetic susceptibility plays an important role in the development and progression of diabetic nephropathy. In this study, we examined whether the SLC12A3 (solute carrier family 12 member [sodium/chloride] 3) g.34372G>A [Arg913Gln] and CKFG (chronic kidney failure gene) D10S558 and D10S1435 are associated with ESRD resulting from diabetic nephropathy.
Methods: DNA polymorphisms were determined in 135 case (diabetic nephropathy) and 107 control (diabetes without nephropathy) subjects.
Results: In our European subjects, D10S558 (P = 0.042) and D10S1435 (P = 0.036), revealed

statistically significant associations with diabetic nephropathy. The SLC12A3 [Arg913Gln] showed no significant differences in genotype and allele frequencies between the cases and controls (p = 0.4562, p = 0.3755, respectively).
Conclusion: The microsatellite DNA polymorphisms of CKFG gene is associated with the genetic predisposition to develop nephropathy in European patients with type 2 diabetes mellitus.

P265

Position of chromosomes 18, 19, 21 and 22 in 3D-preserved B-lymphocytes interphase nuclei of human, gorilla and white hand gibbon compared to human sperm

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Comparative nuclear architecture studies in hominoids are sparse. Here for the first time a combination of multicolor banding (MCB) and three-dimensional analysis of interphase cells was used to characterize the position and orientation of human chromosomes #18, #19, #21 and #22 and their homologues in primate B-lymphocytes. In general, our data is in concordance with previous studies. The position of the four studied human chromosomes and their homologues human were conserved during primate evolution. However, comparison of interphase architecture in human B-lymphocytes and sperm revealed differences of localization of acrocentric chromosomes. We speculate, that the latter might be related to the fact that the nucleolus organizing region is not active in sperm.
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P266

Small supernumerary marker chromosomes (sSMC) in humans - are there B chromosomes hidden among them?

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Small supernumerary marker chromosomes (sSMC) are a heterogeneous group concerning their chromosomal origin, their shape and structure and their clinical effects. Among sSMC-carriers only about 70% show clinical signs. B chromosomes are additional passengers found in the karyotypes of about 15% of eukaryote species. They are best understood as genome parasites exploiting the host genome. B chromosomes were described for plants, insects and vertebrates including mammals. The question as to whether sSMC can be interpreted as in some way equivalent to the B chromosomes reported in other species has been the subject of debate and discussion. However, in summary, it is thought that (most) sSMC are not B chromosomes. Nonetheless, there are at least two potential candidates which may already be, or may in future evolve into B chromosomes: (1) acrocentric-derived inverted duplication sSMC without associated clinical phenotype = sSMC(acro) and (2) sSMC stainable only by DNA derived from itself. (Ad 1) It is known that there is an yet unexplained doubled transmittance-rate of sSMC via the maternal compared to the paternal line. If sSMC(acro) really would tend to develop to something like 'human B-chromosomes' they should be more stable in their inheritance throughout the generations than sSMC(n-acro): sSMC(n-acro) are transmitted 3.8-fold less frequently via paternal than maternal line, while in sSMC(acro) this rate is only 2.1-fold diminished. Thus, there could exist a subset of familial sSMC(acro) already behaving in a similar way to B-chromosomes and hence beginning to spread in the population. (Add 2) No conclusions are yet possible about the size of the second suggested group for B chromosome carriers in human, as only two such cases are known as yet. Further studies are necessary to come to final conclusions here.

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P267

Comparison of human and non-human primate methylation status of CpG islands in the promoter region of CCRK

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Little is known about how the human brain differs from that of our closest relatives, although humans and primates share a high extent of DNA sequence homology. One explanation are species differences in the regulation of gene expression. We focused our attention on differences in promoter DNA methylation in human and non-human primate brains. Three study genes were selected because bioinformatic analysis of published microarray expression data suggested differential expression between humans and chimpanzee. For comparative methylation analysis, we performed bisulphite sequencing of DNA from frontal cortex of 11 humans, one chimpanzee, two baboons, and one Rhesus monkey. Species-specific methylation patterns were found for the cell-cycle related kinase (CCRK) gene that activates CDK2 and is indispensable for cell growth. CCRK has an intermediate CpG promoter with tendency to high-CpG promoter. In the analyzed CpG island we could distinguish three different regions, two that are conserved between the analyzed species and one with a different conservation status. The first region, an ALU-Sg repeat, was almost completely methylated in all human and primate samples. The second region, a block of 6 CpGs at the end of the ALU-Sg repeat, was mostly unmethylated in the 11 humans and Rhesus monkey, but highly methylated in chimpanzee and the two baboons. The third region, corresponding to the end of the CpG island, was completely unmethylated in all human and primate samples. We conclude that the methylation status of the second region varies between human and Rhesus monkey on the one hand and chimpanzee and baboon on the other hand. To test whether the differentially methylated CpG positions have an impact on gene expression, we are currently performing quantitative real-time RT PCR expression analyses of human and primate brains. Our preliminary results suggest that methylation of the second CpG block is inversely correlated with mRNA expression of CCRK.

P268

Correlation between inter-individual variations in DNA methylation and polymorphisms in DNMT genes: A tendency toward higher methylation levels in males

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In human, reasons for inter-individual variations in DNA methylation are not well investigated. To examine the contribution of polymorphisms in DNMT genes to methylation variations we performed a search for polymorphisms in coding regions of all known human DNA methyltransferases genes (DNMT1, 3A, 3B, 3L and 2=TRDMT1) in 96 normal males and 96 normal females. Global methylation was estimated by studying two repetitive DNA elements, namely Line-1 and Alu repeats, while single loci were investigated at three differentially methylated regions: PEG3, NESP55 and H19 imprinted genes; two additional single loci were also studied at Xq28 and 19q13.4. We observed inter-individual correlations in the degree of methylation between Alu and L1 repeats. Moreover, all studied CpGs showed a slightly higher methylation in males ($P<0.0003$ to 0.0381), with the exception of imprinted genes ($P=0.0342$ to 0.9616) which were almost equally methylated in both sexes with only a small tendency towards higher methylation in males. One hundred and eleven DNA polymorphisms in the DNMT genes were detected, 24 of these are in coding regions of which 10 induce an amino acid change that could affect the protein structure/function. Correlation analysis between detected DNA polymorphisms and quantitative methylation data, based on alleles, genotypes or haplotypes, did not reveal significant associations. DNA samples with polymorphisms that induce an amino acid change were further investigated for global methylation patterns by differential methylation hybridization

(DMH). This analysis revealed that two samples that are heterozygous for either a G to A at DNMT3L (R271Q) or a T to C at DNMT2 (Y101H) showed variation from normal patterns; this was also shown by MeDIP based micro arrays. All together our data show that DNMT genes are more polymorphic than expected but that major polymorphism/haplotypes do not have a considerable influence on methylation levels/patterns in normal healthy individual.

P269

Positive selection in non-coding sequences of DNA repair genes during primate evolution

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Differences between humans and chimpanzees do not only exist in morphology and cognitive abilities, but also in their susceptibility to neoplasms. These differences appear to be due to changes in gene regulation rather than structural changes in the gene products. Here we compared the genomic sequences of DNA repair genes, which are responsible for maintaining genome integrity. Conservation and divergence of necessary gene sequence structures can be considered as a hint for negative and positive selection, respectively. First we applied the dN/dS method to analyze open reading frames (ORF) of "master" transcripts of 100 DNA repair genes. Four percent of the analyzed genes showed positive selection in coding sequences. This corresponds to the average number of positively selected genes in the human genome. We then compared non-coding sequences, in particular the promoters, introns, 3'-UTRs and 5'-UTRs, of the same gene set. When considering a nucleotide sequence divergence of more than 5% (gap- and length-filtered) as conspicuous, 7.5% of the analyzed 3'-UTRs, 8.8% of 5'-UTRs, 25.5% of promoter regions, and 45.9% of introns showed evidence for positive selection. The sequence divergence of introns is even higher if one takes length differences of homologous DNA segments into account. The 7.5-45.9% genes with positive selection in a non-coding sequence element(s) are in striking contrast to the 4% genes with positively selected ORFs. In addition, we used HAPMAP data to analyze intraspecific sequence evolution among humans. Two genes, TNKS and RAD9, showed intraspecific positive selection in ORFs and interspecific accelerated evolution of non-coding sequences. Collectively, our results suggest that positive selection in non-coding sequences, in particular introns may affect gene regulation and contribute to phenotypic differences in DNA repair and tumor susceptibility between humans and chimpanzees.

P270

Evolutionary dynamics of human Y-chromosomal segmental duplications

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The male-specific region (MSY) of the human Y chromosome is basically composed of three discrete classes of euchromatic sequence: X-transposed, X-degenerate, and ampliconic. We have identified and analyzed a fourth discrete sequence class: the autosomal-duplicated sequences. Several computational methods were applied to systematically decipher the segmental duplication architecture of these euchromatic segments and determine their ancestral chromosomal origin. Using comparative FISH we reconstructed the evolutionary history of the duplicated sequences on the primate Y chromosome and provide first insights into the development of species-specific Y-chromosomal and autosomal duplicons. Phylogenetic analysis shows that duplicons of a defined euchromatic segment from the human Yp11.2/Yp11.1 region already present multiple paralogs predominantly located in subtelomeric regions in Old World monkeys. Our data indicate a change of the chromosomal position bias to accept duplicative transpositions at the time period of separation of the Old World monkeys from the human/ape lineage. Comparative inference based on de novo acquisitions and/or extensive chromosomal reconstruction of autosomal-duplicated sequences as demonstrated on the pygmy chimpanzee Y chromosome offer not only a valuable taxonomic tool on the genomic level, but also support a model where substantial reorganization and amplification of duplicated sequences contribute to speciation.

P271

Pooled DNA in whole genome association studies with single nucleotide polymorphism (SNP) arrays

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The high cost associated with whole genome association (WGA) studies limits their application to only the most serious pathologies. Thus experimental designs using pooled DNA samples have gained much interest as a potential alternative to individual genotyping.
We first explored this approach in a pilot study using cystic fibrosis (CF) as a model. We pooled DNA aliquots of 96 German CF patients and controls, each and hybridized each pool to 500K SNP arrays (Affymetrix). Array probe intensity data was analyzed and visualized using newly developed software tools GPFrontend and GPGraphics in conjunction with the program GenePool (see abstract by Uebe et al.) as a surrogate marker for allele frequency. A block of SNPs on 7q around the CFTR gene was genomewide the only locus showing marked difference in hybridization intensities.
We next studied optical disc diameter (ODD), a highly heritable quantitative trait with normal distribution in humans and unknown genetic basis. We pooled DNAs from both phenotypical extremes of a cohort of 787 ophthalmologically investigated individuals. As expected, no single locus explained the difference between both groups, but many chromosomal regions showed marked differences in hybridization intensities. When ranking 500K SNPs we observed 35 clusters of high-ranking SNPs composed of at least 3 SNPs ranked among the top 1,000. SNPs in these clusters were in high LD and all localized next to a gene. Genotyping each individual with one SNP at each locus confirmed significant differences (p-value < 0.05) in allelic frequency between both groups at 28 of 34 SNPs tested, confirming the results of the arrays.
We conclude that DNA pooling is an efficient method to identify SNPs showing marked allelic differences between groups and may serve as substitute for individual genotyping in WGA studies. Furthermore, our data suggest that the genetic architecture of ODD is highly complex.

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Mechanisms underlying the microinversions that serve to distinguish the human and chimpanzee genomes

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Submicroscopic inversions contribute significantly to the divergence of human and chimpanzee genomes. The underlying mechanisms of mutagenesis are, however, not fully understood. In this study, we analysed the breakpoints of 35 inversions in the size range 50bp to 22kb that distinguish the human and chimpanzee genomes. All 35 inversions were confirmed experimentally by PCR. Using the macaque genome as an outgroup, the ancestry of the inversions was inferred: 24 inversions were identified as being human-specific whereas 11 inversions were assigned to the chimpanzee lineage. Sequences flanking the inversion breakpoints were screened for the presence of specific recombination-associated motifs (≥5bp in length) and sequences capable of forming non-B DNA (such as direct repeats, inverted repeats and symmetric elements). The breakpoint flanking regions in the inferred human or chimpanzee ancestral sequences which were altered by microinversion were combined into one dataset. A second dataset comprised those sequences which flanked the deduced inversion breakpoints. Macaque sequences orthologous to these breakpoint regions were considered as a separate dataset. Several motifs known to be associated with site-specific cleavage/recombination, high frequency mutation and gene rearrangement were found to be overrepresented ($p \leq 0.01$) within the human/chimpanzee ancestral sequences and the orthologous macaque sequences but not within the set of sequences flanking the human/chimpanzee microinversions. Sequences capable of slipped and triplex non-B DNA structure formation were also significantly overrepresented ($p < 0.01$) within both ancestral and inverted sequences but not in the orthologous macaque sequences. These findings imply that sequences which have the potential to

form non-B DNA structures and to induce (or at least facilitate) double strand breaks, have promoted microinversions in human and chimpanzee lineages since their separation 5-6 MYA.

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Identification and characterization of PMS2-PMS2CL-"hybrid"-alleles

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Although deficiency of the mismatch repair (MMR) gene PMS2 has been shown to account for a substantial fraction of microsatellite instable colorectal cancers, pathogenic PMS2 alleles have been documented so far in only a very limited number of patients with hereditary non-polyposis colon cancer (HNPCC) or MMR-deficiency syndrome. This is largely explained by the difficulties in detecting PMS2 mutations which arise from the fact that a PMS2 pseudogene, PMS2CL, comprising the C-terminal region of the functional gene is embedded in an inverted duplication centromeric to PMS2. Due to sequence transfer between PMS2 and PMS2CL that leads to PMS2 “hybrid”-alleles, containing PMS2-specific sequence variants at the 5’- and PMS2CL-specific variants at the 3’-end of the functional gene, the reference genomic sequences cannot be fully relied upon to distinguish between gene and pseudogene. We developed an RNA-based assay that is based on direct sequencing of long-range RT-PCR-products. This assay allows the effective and reliable mutation analysis in the PMS2 gene as well as the unequivocal identification of PMS2 “hybrid”-alleles. Here we will report on the identification and characterisation of four common PMS2 “hybrid”-alleles that represented a quarter (21/86) of the alleles in 43 control individuals. Three of these functional PMS2 “hybrid”-alleles contained PMS2CL specific sequences restricted to the terminal exon 15 and one consisted of PMS2CL specific sequences in exons 13-15. By characterizing the breakpoints of the sequence exchange between PMS2 and PMS2CL in the latter “hybrid”-allele we were able to develop a simple and reliable genomic DNA-PCR assay to screen for carriers of this “hybrid”-allele in diagnostic and research settings. Using this assay we will test the frequency of this “hybrid”-allele containing a missense alteration of so far unknown functional significance in the normal population and compare it to its incidence in HNPCC and other cancer populations.

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Copy number variations in the NF1 gene region are infrequent and do not predispose to recurrent type-1 deletions

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Gross deletions of the NF1 gene at 17q11.2 belong to the group of genomic disorders characterized by the local sequence architecture that predisposes to genomic rearrangements. Segmental duplications within regions associated with genomic disorders are prone to non-allelic homologous recombination (NAHR), which mediates gross rearrangements such as deletions or duplications. Copy number variants (CNVs) without obvious phenotypic consequences also occur frequently in regions of genomic disorders. In the NF1 gene region, putative CNVs have been reportedly detected by array comparative genomic hybridization (aCGH). These variants include duplications and deletions within the NF1 gene itself (CNV1) and a duplication which encompasses the SUZ12 gene, the distal NF1-REPc repeat and the RHOT1 gene (CNV2). In order to explore the possibility that these CNVs could have played a role in promoting deletion mutagenesis in type-1 deletions (the most common type of gross NF1 deletion), non-affected transmitting parents of patients with type-1 NF1 deletions were investigated by multiplex ligation-dependent probe amplification (MLPA). However, neither CNV1 nor CNV2 were detected. This would appear to exclude these variants as frequent mediators of NAHR giving rise to type-1 deletions. Using MLPA, we were also unable to confirm CNV1 in healthy controls as previously reported. We conclude that locus-specific techniques should be used to independently confirm putative CNVs, originally detected by aCGH, in order to avoid false positive results. In one patient with an atypical deletion, a duplication in the region of CNV2 was noted. This duplication could have occurred concomitantly with the deletion as part of a complex rearrangement or may alternatively have preceded the deletion.

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Whole genome comparisons between different vertebrates provide new insights into breakage and fusion events during mammalian karyotype evolution

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Genome comparisons not only allow one to reconstruct the eutherian ancestral karyotype but also to deduce the inter-relationship of the different eutherian orders within the mammalian phylogenetic tree. Such comparisons have the potential (i) to reveal the DNA sequences present within the evolutionary breakpoint regions and (ii) to ascertain whether or not the evolutionary breakpoints occur preferentially in certain genomic regions. To address these questions, we used gene synteny analysis (E-painting) in six different mammalian species and chicken. E-painting greatly reduces the complexity of comparative genome sequence analysis and can yield valuable insights into the dynamics of eutherian genome evolution. We identified a total of 526 evolutionary breakpoint intervals and mapped them to a median resolution of 120 kb, the highest so far obtained. Evolutionary breakpoints were found disproportionately in regions of high gene density but did not co-locate with common fragile sites or cancer-associated breakpoints. Primate-specific breakpoints were found to occur preferentially in regions characterized by segmental duplications and copy number variants. Breakpoint 'reuse' was noted in certain genomic regions indicating that some genomic regions are prone to recurrent breakage. The structure of the ancestral eutherian karyotype, reconstructed here through E-painting, was strongly supported by comparison with the genome of the opossum (a marsupial) which belongs to the Metatheria, the sister group of the Eutheria.

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Four major haplotype groups in the phenylalanine hydroxylase gene

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Mutations and haplotypes at the phenylalanine hydroxylase (PAH) gene have attracted considerable attention as a source of population genetic information. The gene contains a range of polymorphic markers including several single nucleotide polymorphisms (SNPs), a VNTR minisatellite and an STR microsatellite. We determined modified haplotypes consisting of seven SNPs and the VNTR and STR polymorphisms in 546 independent phenylketonuria (PKU) chromosomes from Germany and found a small number of distinct haplotype groups linked to specific mutations. For a more detailed analysis we subsequently developed a method for molecular haplotyping of all 22 common SNPs in the 3' half of the PAH gene (Introns 5-12, 15 kb) based on allele-specific long-range PCR. Analysis of 86 independent alleles associated with wild type (19 alleles) or PKU mutations (21 mutations reflecting at least 23 different mutation events) revealed four distinct haplotype constellations that differed from each other in 8 to 16 markers. Our combined data indicate that there are four major haplotype groups in the PAH gene that are difficult to relate to each other and must have been present in Europe before the population expansion in the Neolithic period. The haplotype groups are found in association with various different mutations in chromosomes of differing ethnic origin. The exclusive association of common, ancient PKU mutations with specific haplotypes and the apparent rarity of recombination over 100 kb of the PAH gene indicate a haplotype block of remarkable stability throughout the history of Europe.

P277

Low dose irradiation increases the rate of nondisjunction in man

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Trisomy 21 is a major cause of human prenatal and postnatal morbidity and mortality. It can be diagnosed unequivocally and thus offers important prerequisites for epidemiological studies. Nonetheless, even despite decades of research, apart from maternal age and altered levels of

recombination, no single exogenous or endogenous factor leading to trisomy 21 has been unambiguously identified. Most cases result from maternal meiotic nondisjunction, which happens around conception. Maternal age distribution and selective abortion after prenatal diagnosis have the strongest influence on its frequency. If these variables remain constant, then any sudden increase in frequency must be due either to chance or to an environmental factor. Based on time trend analyses allowing for jumps, significant and abrupt increases of trisomy 21 were observed in January 1987 in West-Berlin and in Belarus. In both areas, ascertainment of all (pre- and) postnatally diagnosed cases of trisomy 21 between 1980 and 1992 can be considered complete. In both cases, the most relevant exposure, explaining the January 1987 peak, was the inhalation of iodine-131 (physical half live about 8 days) due to the Chernobyl reactor accident, exactly nine months earlier. In addition, a long-term effect was also observed in several European countries. This is explained by the total long-term exposure, especially due to Cs-137. These coherent observations, which also fulfil the Bradford Hill criteria, prove that the increase in trisomy 21 was not a chance event but is causally related to low dose irradiation. Thus, maternal meiosis is an error prone process that is highly sensitive to the effect of exogenous factors, particularly around conception. This conclusion has not only practical consequences for genetic counselling under the aspect of risk avoidance and primary prevention but also theoretical implications concerning the physiology of the meiotic process.

P278

Risk constellations of patients with coronary artery disease originating from Sachsen-Anhalt

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Until now the population of Sachsen-Anhalt (SH) has been characterized by one of the highest ratios of fatal myocardial infarction (MI) and cardiovascular disease in Germany.
Method: The background of this study was a coronary-risk screening of 989 European Caucasians with origin from SH. Early-onset coronary macroangiopathy and MI (<56y) were related to clinical, biochemical, genetic, and environmental factors. Here we report on a strongly age and gender matched subgroup of 198 (62 female, 52.8y SD 7,3) probands pair-wise matched according their angiographically proven coronary state. We searched for independent coronary risk factors including genetic variants of pro-inflammatory cytokines TNFalpha and TNFß.
Results: Within the whole subgroup smoking (OR 3,9, p<0,001), food nutrition (OR 4,1, p<0,001), and the AA genotype of the T60N-TNFß-SNP (cases 0.184, controls 0.069, OR 4,0, p=0,010) were identified as independent risk markers related to angiographically proven coronary macroangiopathy. But Lp(a) (OR 2,8, p=0,01) hypercholesterolemia (OR 2,7, p=0,035), hyperkinesia (OR 2,5, p=0,019), and a positive familial MI anamnesis (OR 5,8, p=0,001) were only found to be positively correlated in the male subgroup (bivariate analysis). However, investigating possible confounding effects of the common risk factors hypercholesterolemia, hypertension, HDL cholesterol, Diabetes mellitus, smoking as well as the T60N-TNFß-SNP, only smoking (OR 4,1, p=0,001) and the homozygous mutated genotype (OR 3,6, p=0,03) turned out to be significantly associated with coronary macroangiopathy (binary logistic regression).
Conclusions: The frequencies of T60N-TNFß genotypes were comparable with those of other populations (Ozaki 2002, Koch 2001). However, besides smoking the AA genotype was the only one independent risk marker strongly associated with coronary macroangiopathy. These results suggest a possible modified impact of this genetic marker on coronary risk in the population of SH.

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Heritability of facial traits in a Caucasian sample

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Facial traits are an example of a highly multivariate trait that shows considerable variation in the normal population. Association analysis can only be conducted on sub-traits due to dimensionality. It is desirable to define sub-traits with high heritability for ensuing association analysis. In this study, we have analyzed a sample of 570 randomly sampled individuals, including 110 sibships. Frontal and sideview pictures of faces were analyzed by a graph based analysis that describes a face based on

graph coordinates and a local wavelet decomposition. We conduct a heritability decomposition of this data set by defining a linear combination of the traits that exhibits maximal heritability and successively extracting more components after removing previous heritability components. By means of caricatures, we can demonstrate that the first two components define traits related to the nose and eyes, respectively, and have heritabilities of ~80%. By means of bootstrap estimation we explore the confidence regions of the components.
In summary, highly heritable sub-traits can be found in the face even in pictures of the face that are expected to have higher variability as compared to anthropometric measurements.

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Evolutionary analysis of the highly dynamic CHEK2-duplicon in human and higher primates

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We analyzed an evolutionary highly dynamic duplicon embedded in a segmental duplication cluster located in the Yq11.1/q11.21 transition region. The ancestral duplicon spans a substantial portion of the CHEK2 (cell cycle checkpoint kinase 2) gene located in 22q12.1. FISH and in silico analysis identified at least 15 paralogous loci in the human genome. Cross-species FISH revealed an highly amplified state of the duplicon in african great apes, as well. In contrast, the orang-utan showed no amplification of the CHEK2-duplicon. FISH-mapping in two Old World monkeys (OWM), the rhesus macaque and the pig-tailed macaque, consistently showed signals in close proximity to the NOR on the OWM “marker-chromosome”. Surprisingly, both investigated OWM species differed significantly in signal intensity. While the rhesus macaque seems to have a single copy of the duplicon, FISH analysis of the pig-tailed macaque suggests a significant intrachromosomal gain of copy number. Signal intensity in the marmoset again pointed to a single-copy of the duplicon in this outgroup species. Assuming a single copy status in orang-utan, rhesus macaque and marmoset the CHEK2-duplicon must have underwent at least two independent initial amplification events. The “pericentromeric-seeding” of the ancestral CHEK2-duplicon must have occurred in a common ancestor of the african great apes (7-13 Mya) and a more recent independent intrachromosomal amplification of the duplicon must have taken place in the pig-tailed macaque genome (5 Mya). Whereas the duplicon is already amplified both inter- and intrachromosomaly in african great apes, the ancestral duplicon has not yet passed the “pericentromeric swapping” event in the pig-tailed macaque. Our research on the evolution of the CHEK2-duplicon reveals the existence of CNV in OWMs and furthermore demonstrates that highly dynamic duplicons may be important in driving primate evolution.

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Unusual pattern of SNPs in two genomic regions flanking the copy number variation in the LPA gene in African populations

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The apolipoprotein(a) gene locus on chromosome 6q26-27 (LPA, MIM 152200) harbours a transcribed intra-gene copy number variation (CNV) formed by a 5.5 kb Kringle IV-2 repeat (KIV-2-VNTR; 1 to app 42 copies). We have resequenced the genomic regions directly flanking this KIV-2-VNTR in 20 individuals each from 3 world populations (represented by Gabonese, Austrians, and Chinese). This resulted in the detection of two clusters of triallelic SNPs (3 triallelic positions in a 550 bp, resp. 2 in a 20 bp fragment) directly up- and downstream of the intra-gene CNV. The two regions were also hypervariable for biallelic SNPs (e.g. >20 SNPs within 550 bp), mainly in the Africans. The KIV-2-VNTR allowed to directly assess SNP haplotypes by cutting and sequencing single apo(a) alleles after separating the two alleles of individuals heterozygous at the KIV-2-VNTR, using pulsed field gel electrophoresis after endonuclease digestion of genomic DNA.
The third variants of the SNPs were only observed in Africans. None of these triallelic variants is annotated as triallelic in the public databases. However, some of the third variants are carrying the ancestral allele, as defined by the respective chimpanzee locus. Extending the analyses to 20 individuals each of 3 more African populations (Khoi San, South African Bantu, and Egyptians) and Indians confirmed our results and showed that apo(a) haplotypes carrying the third SNP variants were shared across all African populations, with allele frequencies up to 25%.

While the mechanism causing this clustering of triallelic SNPs remains speculative, as is the fact why such variants were only observed in, but conserved among very distant African populations, our results are interesting beyond LPA itself. CNVs have become a major focus of genomic research, and large scale CNV-trait association studies with SNP microarrays have begun. Hence undetected (and possibly population specific) hypervariable regions at CNVs can bias such studies.

P282

Construction of a genomic bacterial artificial chromosome library of the mole vole *Ellobius lutescens*

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The mole vole *Ellobius lutescens* is an exceptional mammal that is known for its odd number of diploid chromosomes (2 n = 17, X) with a single X chromosome in males and females.
An *Ellobius lutescens* bacterial artificial chromosome (BAC) library was constructed with genomic DNA from kidney cells of one male specimen. We used the Copy Control™ pCC1 BAC™ Vector (Epicentre) with an insert capacity up to 200 kb. The library consists of about 32.600 clones and is deposited in 86 microtiter plates with 384 wells. The library was estimated to cover about 1,5 times the haploid Ellobius genome (based on the fact that is has about the same size as the human genome and based on the determined size of the insert DNA). All clones were gridded onto nylon membranes and thus are available for screening of genomic regions of interest such as candidate genes for sex determination in this species. The BAC library was constructed for screening with probes of flow-sorted *Ellobius lutescens* chromosomes in order to create a panel of chromosome-specific markers. For that purpose the DNA of flow-sorted chromosomes is amplified by DOP-PCR and hybridized onto nylon filters gridded with BAC clones. Positive clones will be screened for $(CA)_n$ microsatellite markers that will be characterized by cloning into a plasmid vector with subsequent sequencing. The resulting panel of chromosome-specific microsatellite markers will assist in the mapping of the male sex determining gene in *Ellobius lutescens*.

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The first serotonin receptor allelic variant database

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Serotonin (5-hydroxytryptamine, 5-HT) controls a variety of physiological functions. 5-HT receptor subtypes mediating serotonin action can be divided into seven main classes (5-HT1R to 5-HT7R). A multitude of candidate gene screenings has been published during the last years. We have started to structure this information in the first serotonin receptor allelic variant database using LOVD (Leiden Open Source Variation Database). Up to now, the database comprises data of 5-HT3 receptor subunits. To date, five different human subunits are known (5-HT3A-E), which are encoded by the serotonin receptor genes HTR3A, HTR3B, HTR3C, HTR3D and HTR3E. Different receptor subtypes seem to be involved in chemotherapy induced nausea and vomiting (CINV), irritable bowel syndrome (IBS) and psychiatric disorders. During the last years HTR3 case-control and pharmacogenetic studies indicated that HTR3A and HTR3B polymorphisms may contribute to the etiology of psychiatric disorders and may predict CINV and medical treatment of psychiatric patients. Currently, the database is subdivided into five sub-databases, referring to the serotonin receptor genes. This database will successively be extended by data of additional serotonin receptor genes. Within each sub-database we are collecting mutations, polymorphisms, demographic information as well as pharmacogenetic data. Every sub-database includes general information about the respective gene and is linked to other resources such as OMIM, GDB, HGMD and HAPMAP. The remote user is able to search the data and to submit new data into the database. This central information pool should help clinicians as well as scientists to evaluate their findings and to use the information for subsequent studies. Data about functional consequences of variants will be integrated in future as well to enable specific drug design in the therapy of respective conditions.

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Identification of protein-protein interactions in vivo by a proteomic approach

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Proteins may exist in several complexes in a spatial and temporal manner to accomplish distinct biological functions. The analysis of interacting partners will provide a strong insight into the physiological role of a particular protein. For this reason, we developed a protein-protein interaction assay comprising SELDI-based mass spectrometry coupled with immunological techniques. Hereby, we investigated protein complexes captured from crude cell extracts using antibody-coupled beads regarding the protein composition of these complexes by SELDI MS. To avoid the tendency to detect false positive protein-protein interactions of examinations performed in vitro, we assessed interacting partners of solely endogenously expressed proteins. Using this specific proteomic approach, we detected and identified in several assays interacting partners of different members of the S100 Ca2+-binding protein family as well as protein interactions between E2F transcription factors and the corepressor Alien. Hereby, we showed that E2F-1 and Alien interact in a functional manner as Alien repressed the transcriptional activity of E2F-1 and inhibited the cell proliferation. Beside this result, we discovered in another assay a complex containing S100A11 and Rad54B, a DNA dependent ATPase, which is involved in homologous recombination repair of DNA damages. Hereby, we showed that Rad54B targeting to DNA double-strand break repair sites requires protein interaction with S100A11.

P285

A multiplexed assay for DNA methylation testing of over 300 methylation candidate markers enables convenient methylation analyses for tumor-marker definition

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Here we present the design principle and performance of a combined multiplex-PCR and microarray hybridization technique for multiplexed methylation testing.
Targeting 323 published DNA regions hypermethylated in several neoplasias, methylation analysis is performed via methylation dependent restriction enzyme (MSRE) digestion of 500ng of starting DNA. A combination of several MSREs warrants complete digestion of unmethylated DNA. All targeted DNA regions have been selected in that way that sequences containing multiple MSRE sites must be flanked by methylation independent restriction enzyme sites. This strategy enables pre-amplification of the methylated DNA fraction before methylation analyses. Thus the design and pre-amplification would enable methylation testing on serum, urine, stool etc when DNA is limiting.
When testing DNA without pre-amplification upon digestion of 500ng the methylated DNA fraction is amplified within 16 multiplex PCRs and detected via microarray hybridization. Within these 16 multiplex-PCR reactions 358 different human DNA products can be amplified. From these about 20 amplicons serve as digestion & amplification controls and are either derived from known differentially methylated human DNA regions, or from several regions without any sites of MSREs used in this system. The primer set (every reverse primer is biotinylated) used is targeting 347 different sites located in the 5'UTR of 323 gene regions. After PCR amplicons are pooled and positives are detected using strepavidin-Cy3 via microarray hybridization. Although the melting temperature of CpG rich DNA is very high, primer and probe-design as well hybridization conditions have been optimized, thus this assay enables unequivocal multiplexed methylation testing of human samples. The assay has been designed that 6 samples can be run in parallel using 96well PCR plates. Handling 12-18 DNA samples in parallel can be easily performed enabling completion of analyses within 1-2 days.

P286

Comparison of genotyping consistency between genomic and whole-genome amplified DNA using the Illumina GoldenGate and Infinium-II assays

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High-throughput SNP genotyping has become an important research strategy in human genetics. Although most genotyping assays require minimal amounts of DNA, repeated use often leads to depletion of the sometimes irreplaceable samples. To address this problem whole-genome amplification technologies have been developed in the last years and are meanwhile commercially available. Albeit the amplification seems to be mostly successful, it is controversially discussed whether the whole genome amplified DNA (wgaDNA) represents an exact copy of the genomic DNA (gDNA) template. In the present study, we aimed to assess the genotyping consistency between 45 wgaDNAs (generated using the REPLI-g DNA Amplification Kit, Qiagen, Hilden) and their corresponding gDNA samples. The gDNAs were of different age and quality. To compare genotype consistency between wga and gDNA, 20 high quality sample pairs were genotyped using Illumina's HumanHap550V3 BeadChips (565.000 SNPs). 25 sample pairs of different DNA quality were genotyped for 384 SNPs using Illumina's GoldenGate assays. All samples genotyped on the HumanHap500 BeadChips performed well, with average call rates of >99%. The average consistency between gDNA and wgaDNA was 99.99% when comparing SNPs successfully genotyped in the corresponding samples. Of the 25 sample pairs genotyped with GoldenGate assays, 22 performed well with average call rates >99% (gDNA) and >98% (wgaDNA). Genotype consistency between wga and gDNA was 100% for all SNPs successfully genotyped in both corresponding samples. The remaining 3 sample pairs showed noticeably worse results with an average genotype call frequency of 99.8% (gDNA) versus 60.1% (wgaDNA) and a genotype consistency of only 89%. Possible explanations for the observed discrepancies include the age of gDNA, the extraction method as well as the presence of unknown inhibitors interfering with the amplification process.

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Divergent transcriptional control of the AZFa gene DBY (DDX3Y) and its X- homologue DBX (DDX3X) by allel-specific promoter structures

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DBY (DDX3Y) and its paralogue DBX (DDX3X), members of the DEAD-Box family of RNA-helicases, are master regulators of human spermatogenesis. Deletions of the AZFa interval of the Y-chromosome, in which the DBY gene is located, are causing a complete absence of germ cells, called Sertoli-Cell-Only syndrome. The two paralogue genes on X- and Y-chromosome are highly conserved in their amino acid sequences (94%), providing strong indication that both copies are under functional selective constraints. In contrast, the 5'UTRs and the promoter regions of both genes have undergone dramatic chromosome-specific changes, probably evolved after the lack of recombination during evolution of mammalian sex chromosomes. This caused the development of a complex testis-specific transcriptional and translational control of DBY and DBX, observable in testis-specific transcriptional start sites (TSS) and alternative splicings. Moreover, DBY and DBX are translated in different male germ cells.
Comparative in-silico analysis of the DBY and DBX promoter sequences revealed distinct patterns of putative TF (Transcription Factor) binding sites. Most striking is the amplification of a minisatellite sequence MSY2 (DYS440), composed of four repeat units and only located upstream of the testis-specific DBY TSS. These transcripts encompass an additional 5´exon (Exon-T) which is alternatively spliced to the DBY exon 1. Exclusive to the DBX promoter are five blocks with repetitive TF binding sites (POZ-TFs; ZnF-TFs; GC/GT-; TC-; AG-rich). Surprisingly we also found some conserved TF-binding sites, namely for Sox5, YY1, SP1 and RFX which are known to be implicated in testis-specific gene expression. By functional luciferase-reporter assays we mapped the strongest DBY and DBX promoter activities to different promoter domains which include activating and repressing sequence modules. This suggests a complex chromosome-specific promoter architecture of the functional homologous DBY and DBX genes.

P288

High-resolution breakpoint mapping of human chromosome 21 segmental aneuploidies for genotype-phenotype correlation and identification of underlying genomic architecture

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As part of the European FP6-sponsored AnEUploidy consortium we are involved with work package 2: Genotype-phenotype correlations in human aneuploidies. The objective of this work package is to study the phenotypic consequences of gene dosage imbalance in the human population.
One of our roles in this project is the characterization of segmental aneuploidies of HSA 21. Until now we collected 19 cases with detailed clinical information, enabled by using a standardized phenotypic list. Cell lines and/or DNA are available for all patients. Karyotyping as well as additional analysis (FISH, microarray-based high-resolution genome profiling) were performed for the majority of cases. We have used a chromosome 21 specific oligo-array with 385,000 oligonucleotides to further delineate the genomic rearrangements in this cohort. Breakpoint fine mapping of approximately 1kb accuracy has been performed for the most cases, enabling breakpoint sequencing as a next step. For one case with a partial chromosome 21 deletion (46,XY,del(21)(q11.2q21.3)) the rearrangement coincide with bordering segmental duplications (SDs) that have identical orientation and high (>95%) similarity. This suggests that recurring deletions and/or corresponding duplications of similar size that are mediated by NAHR (non-allelic homologous recombination) may exist. These analysis of the underlying genomic-architecture are enabled by in-house developed software tools.
Detailed genotype-phenotype correlations are ongoing for all patients to get a better insight into the underlying gene dosage imbalances. Expansion of the patient cohort and transcriptome analysis are planned as joined efforts of the consortium.

P289

Quantitative SNP genotyping is a robust and accurate tool for validation and fine-mapping of copy number changes detected by Array-CGH analysis

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Copy number changes (CNVs) detected by Array-CGH usually have to be validated, especially when the changes are suspected to be of clinical relevance. CNV validation may be done by FISH or MLPA. FISH, however, is unsuitable in cases where genomic material is duplicated in tandem. MLPA, on the other hand, depends on the availability of probes which may be a problem in case of genomic regions that do not contain genes or CNV hot spots. Furthermore, MLPA probes are expensive and purchase is advisable only if repeated use is intended.
Quantitative SNP genotyping: We observed that the height of peaks at heterozygous positions in sequence electropherograms is determined almost exclusively by the allele ratio: a 1:1 ratio of SNP alleles results in two peaks at the same position with the same height. Artificial mixtures of two different alleles in ratios 1:2, 1:3, 1:4 etc. result in the corresponding peak ratios. Thus, allele ratios can be deduced from the peak ratios at heterozygous genomic positions.
Application: Array-CGH analysis using the DNA of a patient with short stature, microcephaly, severe mental retardation, epilepsy, Dandy-Walker malfor¬ma¬tion, delayed myelinization, cutaneous syn¬dactyly and joint laxity revealed a gain of 500 kb of genomic material from 17q25. Since FISH did not result in an additional signal elsewhere in the genome, the duplication is likely to have occurred in tandem. In order to validate the Array-CGH results and to identify the genes which contribute to the patient's phenotype, we performed fine-mapping of the duplication borders by quantitative SNP genotyping. The initial resolution of the breakpoint mapping was approximately 50 kb. By quantitative SNP genotyping we could pinpoint the border of the duplication event to a region of 1.5 kb. Thereby we confirmed the duplication by an independent method and also excluded several genes, which otherwise would have to be considered as positional candidates for the phenotype of our patient.

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Evaluation methods of whole genome methylation arrays

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DNA methylation changes are early events in neoplastic transformation and distinct patterns have a great potential as biomarkers. Methylation patterns thus might correlate with different types of cancers and provide useful information for diagnosis and prognosis of neoplastic diseases.
To investigate methylation changes on a genome-wide scale, microarrays are an effective tool. Nevertheless, evaluation of the quality of microarray results and determination of suitable parameters for data analysis are essential to guarantee reliable results.
In this study we performed several whole genome methylation screenings using the “Agilent Human CpG Island microarray” (covering 27 800 CpG-islands with more than 237 000 probes) and evaluated our results with different approaches. These investigations included different “spike-in”- experiments (e.g. using BAC-clones), selective examinations of particular probes specific for genes with known methylation status and confirmation of the chip data using a PCR based control system.
This latter system, based on several multiplex-PCR-amplifications followed by a microarray hybridisation, is able to determine the methylation status of 300 cancer-relevant marker genes.
Several controls and analysis strategies will be discussed to finally produce reliable methylation data from microarray hybridisations.

P291

Trans allelic interaction at the mouse scapinin (Phactr3) locus

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The effect described here is very similar to Paramutation-like phenomena which have been extensively studied in plants and so far described for a very few engineered loci in the mouse. Here we report an allele specific expression analysis of the Phactr3 (phosphatase and actin regulator 3) locus identifying the first internal mouse transcripts with a paramutation-like effect not associated with transgenic or knockout mice. In our previous work, we showed that the Phactr3 gene was mainly transcribed in the brain, exhibiting a complex genomic organisation with four alternatively spliced leader exons. Due to the location of the Phactr3-gene in the distal imprinting region on mouse chromosome 2, we generated a mouse model to investigate the possible parental influence on the allelic expression pattern by reciprocally mating NMRI mice and Mus musculus castaneus. We were able to identify a SNP in leader exon 1C representing a restriction fragment length polymorphism (RFLP). After RT-PCR NMRI as well as Mus m. castaneus showed a homozygous restriction pattern according to their genotype. Unlike this RT-PCR products of the F1 hybrids of both crosses were transcribed from the NMRI allele only. Therefore, the Phactr3 exon 1C splice variant is potentially strain specific regulated, leading to the expression of only one allele of the reciprocal crosses. So far this has not yet been described for an internal mouse gene. Such a paramutation-like trans-allele phenomenon could be of particular importance as shown for the insulin gene (INS) in human and its relevance for type 1 diabetes. These results potentially provide new insight into non-Mendelian inheritance in mammals and may serve also as a model to investigate the regulation of allele specific expression.

P292

Biomedical applications of high throughput sequencing in human genetics

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Since its introduction in 2006 by 454 Life Science - Roche, Next-Generation-Sequencing has entered many fields of biomedical research. With many applications in microbiology, cell biology, but most of all genomics and genetics, it has become one of the methods with highest potential to allow novel scientific approaches, and offer new routes of analysis.
At the Genomics Core Facilities of DKFZ we apply Next-Generation-Sequencing, to address questions of
i. host-pathogen interaction,
ii. identification of integration sites of gene therapy vectors within the human genome,
iii. de novo sequencing of micro RNAs and
iv. genomic re-sequencing.

Although the technology allows unparalleled throughput, it is still not possible to produce full genomic coverage of individual patients at reasonable cost. Therefore we have started to develop novel strategies to extract defined regions from patient DNA. We are aiming for enrichment and sequencing of all exons of candidate genes for specific tumor entities and heart disease. Such an approach might contain up to 6.500 exons in a single selection and sequencing step. In a nano-bisulfite sequencing set-up of approx. 600 promotors of cancer-related genes, we want to identify specific methylation changes in tumor samples. Finally, we intend to re-sequence large genomic regions that show association with defined diseases, and hope to identify somatic variations and/or mutations causing the respective disease.
Current projects and ongoing developments will be presented.

P293

Genome-wide analysis of copy number variation (CNV) in a population-based sample of the German population

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Copy-number variation (CNV) is the most prevalent type of structural variation in the human genome. It has already been recognized that some CNVs can contribute to human phenotype and strong efforts are currently made to determine their influence on susceptibility to common diseases.
In the context of the German “National Genome Research Network” (NGFN) we have recently performed genome-wide association studies (GWAS) in neuropsychiatric disorders, including bipolar disorder, schizophrenia, and alcoholism, using Illumina HumanHap 550 arrays. Apart from SNP genotype analysis, we envisage CNV analyses to in-depth analyze these data sets. Currently, systematic identification of CNVs is not routine. In the present study, we set out to develop a suitable procedure to systematically screen for CNVs and gain insight into their prevalence, structure and distribution in the German population. As screening sample, we used 383 individuals from the Heinz Nixdorf Recall study.
To detect CNVs, we analyzed the data format provided by Illumina’s BeadStudio software. Each data point (i.e. SNP genotype) read from the HH550 arrays is output as the log ratio (logR) of the intensities of two fluorescent dyes. We developed a program to screen for windows of n consecutive heterozygous genotypes that show strong logR ratio deviations from the expected values, suggesting the presence of more (duplications) or less (deletions) than 2 genomic copies. We analyzed the intensity data for each sample independently and also compared each individual to the mean value of a control group (the latter to reduce the possible influence of non-CNV related reasons for logR deviations). Analysis of the data sets is underway and results will be presented. An overview of the prevalence of CNVs gained by this approach will provide the basis to screen for disease-related CNVs in our GWAS data for different psychiatric phenotypes.

P294

The impact of ovarian stimulation on the cellular epigenome in preimplantation mouse embryos

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Ovarian stimulation which is an integral component of assisted reproduction seems to impair genome-wide methylation reprogramming, implantation and fetal development in mice and to increase the risk for imprinting disorders in humans. To reveal the impact of ovarian stimulation on imprinted gene methylation, we analyzed the differentially methylated regions of H19 and Snrpn by conventional bisulphite sequencing as well as bisulphite pyrosequencing in mouse 4-cell, 8-cell, and morula stage embryos derived from superovulated and non-superovulated matings of C57BL/6J females with either C57BL/6J or Mus musculus castaneus (CAST/Ei) males. In preimplantation embryos from C57BL/6J inbred matings, a significant loss of methylation of H19 and Snrpn was found after superovulation. In

contrast, our H19 methylation analysis of preimplantation embryos derived from superovulated and non-superovulated intersubspecific (C57BL/6J x CAST/Ei) matings, which allowed discrimination of parental alleles by a SNP, revealed no dramatic effect of ovarian stimulation, but very similar methylation levels and expected methylation patterns with the paternal allele predominantly methylated and the maternal allele predominantly unmethylated. However, a significant percentage of both superovulated and nonsuperovulated intersubspecific morula stage embryos displayed aberrant methylation on the maternal H19 allele. The observed discrepancy in methylation levels between superovulated embryos from inbred and intersubspecific matings may be due to the action of complex modifiers acting in the intersubspecific genetic background ("hybrid effects"). On the other hand, in inbred embryos one can not distinguish between paternal and maternal alleles and, therefore, not exclude a PCR amplification bias due to the very small number of analyzed cells. To avoid the influence of these factors, we will continue our analysis only with C57BL/6J inbred embryos and increase the number of analyzed cells.

P295

Meiotic studies in fertile males - preliminary results

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Meiotic studies were carried out in testicular biopsies from 8 males of proven fertility. Samples were processed using the air-drying technique and spermatogenic cells were stained with DAPI. Spermatocyte distribution at pachytene, metaphase I and metaphase II stages was studied counting 1,000 pachytene spermatocytes per individual. The proportion of metaphase II to metaphase I was higher than 0.5, indicating a normal meiotic progression in all individuals. Chiasma count and chromosome abnormalities were analyzed in metaphase I spermatocytes. The total mean chiasma frequency was 51.0, with individual means ranging from 48.7 to 53.0. Only one of the 643 spermatocytes I evaluated (0.2%) was hyperploid, showing one extra univalent of C group. Structural chromosome abnormalities were detected in seven of the eight individuals studied, and ranged from 0% to 5.7%. The percentage of dissociated sex chromosomes at metaphase I was 20.1%. Synaptic abnormalities affecting autosomes were found in 5 spermatocytes I (0.8%), and 2 of them (0.3%) showed one bivalent as two separated univalents. Both frequencies of chromosomal and synaptic abnormalities described in this work, as well as spermatocyte distribution and chiasma count, could be used as reference frequencies in further studies on males with idiopathic infertility, in order to ascertain the causes of their infertility.

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P296

Disomy/Tetrasomy 21 mosaicism in CVS

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We report on a case of discordant cytogenetic findings following chorionic villi sampling (CVS) in the 13th gestational week. Chromosome analysis after short term cell culture showed a normal chromosome complement, 46,XX. In the long term cell culture, karyotyping revealed a tetrasomy 21 in all analysed cells. FISH analysis with a chromosome 21 specific painting probe confirmed the tetrasomy 21. One metaphase with 46 chromosomes and two regularly painted chromosomes 21 was also found. Interphase FISH on long term culture with locus specific probe for chromosome 21 showed four signals in 78%, three signals in 3% and two signals in 17% of the nuclei. For further analysis, amniocentesis was performed at 16 weeks of gestation. Interphase FISH on uncultured amniocytes with probes for chromosome 13, 18, 21, X and Y revealed in 199 nuclei a normal signal distribution. Two nuclei with four signals for chromosome 21 were observed. The chromosome analysis of cultured amniocytes and of fetal blood after cordocentesis resulted in normal karyotypes, 46,XX. Molecular genetic analysis on long term cell culture of chorionic villi with the microsatellite marker D21S11 showed an abnormal triallelic peak pattern with one allele in double dose. This excludes a cell culture artefact in the long term culture. Marker analysis of placental tissue obtained from the cord insertion site after delivery showed a mosaic consisting of a normal biallelic and to a lesser extent a triallelic cell

line.
Meiotic nondisjunction followed by a mitotic error and rescue of a diploid cell line and a tetraploid cell line can explain the findings in this case.

P297

Polar body analysis by nested multiplex PCR - a novel method demonstrating high sensitivity in detecting chromosome aneuplodies

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Chromosome aneuploidies in oocytes are the main maternal factors affecting artificial reproduction, in particular in older women. About 70% of the oocytes of women older than 40 years show aneuploidies. Several techniques have been employed to select for oocytes in the past, among them blastomere biopsy and polar body analysis. Since blastomere biopsy is considered illegal in Germany, polar body analysis remains the only option in this context. Molecular cytogenetic techniques (FISH) have the disadvantage, that only a very limited number of chromosomes can be analyzed, that the results are difficult to interprete, and that reproducibility is apparently low. Here, we present a novel method for numerical chromosome analysis based on a nested multiplex PCR.
Individual polar bodies are placed on a chemically structured microscope slide. A nested multiplex PCR is carried out, amplifying 5 loci of each chromosome. Detection of the amplification products is achieved by conventional agarose gel electrophoresis. Presence and absence of all 23 chromosomes can be validated this way. In our experience, the analysis shows high reproducibility and sensitivity and is extremely robust. The first data regarding clinical outcome will be presented.
To determine the sensitivity, we analyzed the data derived by Sher G et al. (Fert Steril 87: 1033-1040, 2007). In this study, comparative genome hybridization was used to analyze polar bodies and blastomere biopsies. It was shown, that aneuplodies show an almost even distribution among all chromosomes, and that oocytes show on average three to four distinct aneuploidies. Data comparison showed a sensitivity of the PCR method at least twofold higher than 6-probes FISH techniques.

P298

Conventional cytogenetics and CGH: a strong team for the analysis of spontaneous abortions

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Spontaneous abortions occur in about 15% of all recognized pregnancies. Chromosome aberrations are the most frequent findings and make up more than 50% of all abortions. We here present a protocol for genetic analysis of first trimester abortions that involves cytogenetic analysis and comparative genomic hybridisation (CGH). In a pilot study we investigated trophoblast and/or embryonic tissue from 42 abortions. Parts of the tissue samples were cultured for chromosome analysis and whenever possible the rest was used for DNA extraction.
Fourteen samples (33%) could not be cultured and 9 of them were therefore analyzed by CGH only. Of the 28 successfully karyotyped cases 9 (32%) were normal and 19 (68%) had chromosome abnormalities (13 aneuploidies, 3 triploidies, 2 mosaics, 1 structural aberration).
CGH was performed in 9 cases. Five samples were normal, 3 trisomic, and 1 had a deletion. Furthermore, CGH revealed a pure trisomy 22 in a poorly growing sample that had appeared to be mosaic composed of three different cell lines by cytogenetic analysis. In addition, a cytogenetically detected deletion 13q14-qter was identified as a derivative chromosome der(13)t(11;13)(p15.3;q14.1) by CGH.
The study suggests that CGH should be included in the routine work-up of abortions. While conventional cytogenetics gave results in 76% of our cases, application og CGH increased this percentage to 88. Besides, CGH has the potential of further resolving structural aberrations and to sort out culture artefacts. Moreover, rare trisomies, as neglected by the QF-PCR standard protocol can be detected.

P299

The kind of licence of a patient's doctor and the calculation software used are all-dominant factors for the individual risk of fetal trisomy 21/18/13 after combined first-trimester screening

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As a result of a specific national development of events, the German gynaecologists have various options to perform first-trimester screening. They may now acquire licences of the Fetal Medicine Foundation (FMF) England or the FMF Germany in order to benefit from the regulations, quality control, and calculation software of either organisation. Alternatively they may renounce all kind of certification and use a German Internet programme for risk calculation. Depending on the attended doctor and the respective screening programme a patient's risk may then be as different as for example 1:3 and 1:6000, or 1:2 and 1:1500 etc. in spite of identical analytical values (NT-, PAPP-A-, and free ßhCG-levels) and identical demographic data. Rationally, genetic counselling in case of suspect first-trimester screening results must consider information about the attended doctor (kind of licence) and the choice of calculation software. Differences between risk software packages, flaws, systematical and severe errors of included algorithms are described.

P300

Improvement of chances by Preimplantation Genetic Screening (PGS)

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Introduction: Preimplantation genetic screening (PGS) wants to improve artificial reproductive technologies (ART) by raising the rates of pregnancy, implantation and birth, decreasing the rates of abortion, multiple pregnancies and malformation and detecting patients which should retain from homologous IVF but opt for oocyte donation. We conduct a cohort study with 100 patients offering it to all patients with a minimum of 8 oocytes. After 72 patients we re-evaluated the mathematical chances of oocyte selection.
Methods: On day 0 laser biopsy of the first polar body and FISH with chromosomes 13, 16, 18, 21, 22 (Vysis) were performed. The probability to perform an embryo transfer with two intended embryos detected euploid for these chromosomes was calculated based on the composition of euploid (eufert), aneuploid (anfert) and not detected (ndfert) fertilised oocytes in each case. The a priori probability (pa) of a random selection without the PGS information was related to the a posteriori probability (pp) with this information. The improvement of the chance (pp/pa) was multiplied with the known pregnancy rates of the German national IVF register (DIR) for two parameters (NPR 2): age and amount of transferred embryos.
Results: In three calculations, dependent on the assumptions of the ndfert to be in reality eufert or anfert (probability between 0 and 1), and averaging these two calculations, we obtained for pa 0.20, for pp 1 and for the improvement factor (likelihood ratio) 8.18. Based on an average NPR 2 of 26 %, a theoretical pregnancy rate (TPR) was calculated of 59 %.
Conclusions: As a doubling globally is found by no one (and our PGS results correspond to NPR 2), there must be an intrinsic handicap in PGS, either in the biopsy procedure or the genetic diagnosis. We need to improve the PGS procedure and our indications, the latter by calculating individual cut-off levels in order to move towards the TPR.

P301

Paternal exposure to drugs - Is there an increased risk for congenital abnormalities? Experience of the Berlin Institute of clinical teratology and drug risk assessment during pregnancy

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There is often uncertainty concerning prenatal developmental toxicity of paternal exposure to drugs or chemical substances. Three possible mechanisms are discussed: direct mutagenic effects, impairment of spermatogenesis and seminal transfer of chemicals. Review of the literature on paternal exposure is mostly based on case reports or retrospective analyses and might lead to an overestimation of possible risks. For example, conflicting results were reported in 3 recent studies with small case numbers for paternal use of azathioprine (Weber-Schöndorfer, 2005).

Our institute has answered about 85.000 requests for drug risk assessment in pregnancy and lactation, the follow-up rate is approximately 30%. However, only 2% of all requests regard paternal exposure. Interestingly, most inquiries for paternal exposure come from geneticists (48%), followed by gynaecologists (35%), patients and their relatives (24%). Most of the information requests for paternal exposure concerned immunomodulators.
So far, we have completed follow-up of 363 pregnancies with paternal exposure. No specific adverse effects were observed after paternal immunomodulatory therapy (56 exposures to azathioprine, 55 to methotrexate, 47 to interferones, and 28 with other immunomodulators). The same applied to ribavirine (33 exposures) and colchicine (11 exposures). However, case numbers are still too small to rule out a risk.
At present, there is no evidence that justifies elective termination of pregnancy (ETOP) because of suspected paternal teratogenicity. There is an ongoing debate on the necessity of invasive prenatal diagnostic after paternal exposure to immunosuppressives, chemotherapy or colchicine. Routine amniocentesis is not indicated yet according to the recommendations of the European Network of Teratology Information Services (ENTIS). However, level II ultrasound examination should be offered to couples with paternal exposure to drugs suspected as teratogenic or mutagenic.

P302

Genetic susceptibility testing and screening in common disorders

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Dealing with an increasing number of new technologies, the impact of genetic susceptibility testing on public health issues gains in importance. Especially regarding common complex disorders it has to be considered whether or not susceptibility tests with high clinical utility should be widely available or implemented nationwide.
Based on a systematic research, various reports and literature on epidemiological, ethical, social, and health economic as well as regulatory and policy issues have been considered. Several frameworks for the assessment of genetic testing services have been identified that do not only regard disease and test specific characteristics but treatment options and health system issues as well. Especially the varying health systems and the respective cost considerations show differences that have to be taken into account while comparing screening programmes across Europe and deciding about a test´s implementation being worthwhile or not.
Given the vast heterogeneity in genetic testing and screening, future efforts have to focus on gene-disease associations and the impact of disease-causing environmental factors. Only if the analytic and the clinical validity are specified, health economic considerations are reasonable to determine the public health impact. In this way, target populations can be identified more effectively and genetic screening programmes could be more efficiently implemented. Until then, the family history might prove the most effective tool to determine whether an individual should undergo genetic testing or not.

P303

From scientific program to clinical service: a two years single center experience of genetic counseling, testing and surveillance for familial breast-/ovarian cancer

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From 1998 to 2005 the German Cancer Aid supported a multi center study on familial breast-/ovarian cancer, in which more than 10.000 persons have been genetically counseled and 3150 women from 2471 families have been genetically tested. Since 2005, the program was taken over by public health insurances. Since then, 285 patients with breast-/ovarian cancer and 207 persons at risk of developing breast-/ovarian cancer from 243 families attended the Center for Familial Breast-/Ovarian Cancer in Kiel for genetic counseling. Most patients were referred by gynecologists (52.8%) and other specialists (18.4%), whereas only 15.8% attended due to information on public media, health insurance (0.8%) or

family members included in the German Cancer Aid study (12.1%). In addition, 294 families asked for genetic testing by phone, of which 164 families met the inclusion criteria (but were not yet or no more interested in the program) and 130 families did not. Altogether 158 families have been genetically tested, of which 20.3% carried a pathogenic mutation in the BRCA1 gene and 2.5% in the BRCA2 gene. Furthermore, 3.2% of the families carried an unclassified variant (UV) in the BRCA1 gene and 7.5% in the BRCA2 gene. The highest mutation frequency was observed in families with breast- and ovarian cancer (nearly 60%). Intensive surveillance program including ultrasound, mammography and MRI was offered to 158 women at risk, of which only 56.9% made use of (44 breast-/ovarian cancer patients and 46 healthy women). Prophylactic surgery was performed in 10 and planned in 4 women. In conclusion, two years after the genetic counseling, testing and surveillance program was overtaken by health insurances from the German Cancer Aid the demand on genetic counseling and testing is still unbowed. The mutation detection rate remains unchanged (appr. 23%). Unfortunately, the demand on intensive surveillance program is lower then expected.

P304

k-MED: Multimedia education in human genetics

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k-MED (Knowledge in Medical Education) is a web based medical e-learning project funded by the German federal initiative for new media in education, the Hessian Ministry of Science and Arts and the participating universities. E-learning provides effective strategies for medical universities to meet the demands of the new German legislation for undergraduate medical education. The k-MED e-learning management system (ILIAS) supports online courses as well as online evaluation, online communication, and online exams (all in the same design, structure of web pages, access, navigation etc.).
Courses are held at the partner universities of k-MED: mainly at the medical schools of Marburg, Giessen, Frankfurt, and Hannover. Medical subjects include biochemistry, physiology, anatomy/histology, infectious diseases/immunology, pharmacology, dermatology, radiology/nuclear medicine, anaesthesia, and human genetics. Here, we present our teaching material currently organized in five e-learning modules: chromosomal aberrations, formal genetics, molecular diagnostics, cytogenetics, and congenital abnormalities and syndromes. These are basic courses intended to do the educational groundwork, which will enable academic teachers to concentrate on more sophisticated topics during their lectures.
The courses have been evaluated by a large group of about 2500 students during three years at the Faculty of Medicine in Marburg. The group consists of scientists (human biology) and medical students in the basic study period or the clinical period, respectively. The results of the online-evaluation and their significance for the further development of k-MED are discussed.

P305

Genetic testing for spinal muscular atrophy (SMA) - lessons learned from artificial-insemination-by-donor (AID) practice in Germany?

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Introduction: In Germany, an estimated 1000 children are conceived annually through artificial insemination by donor (AID). In the absence of any binding recommendations, donors are variably screened for chromosomal aberrations and carrier status, e.g. for cystic fibrosis, in addition to ensuring a negative family history for inherited disorders. According to current recommendations the number of lifebirths produced with sperm from a single donor should not exceed 15.
Case: A couple, after the loss of their child with spinal muscular atrophy (SMA), were confirmed to be heterozygotes for the SMN1 deletion and opted for AID. SMA is caused by homozygous absence of SMN1 in ~95%, and the carrier frequency is 1:35. The potential donor was identified as SMA carrier by real-time quantitative SMN1 analysis. Advice was sought from the "Kommission für Grundpositionen und ethische Fragen" (Committee on Policy Statements and Ethical Issues) of the GfH, but no specific guidelines have been established so far for how to proceed with respect to the families that had already conceived a pregnancy through AID from this donor. Subsequently, the donor and the families

involved were informed separately by the institution that had performed the AID. The reactions of the families involved, partly predictable, partly unexpected, will be discussed.
Conclusions: 1.) No firm guidelines currently exist for genetic testing prior to sperm donation, either from the "Arbeitskreis Donogene Insemination" or from the GfH.
2.) No specific guidelines exist how to communicate results of genetic donor testing to the families that are retrospectively found to be involved.
3.) Sperm donors are by definition a healthy subpopulation in which genetic screening for selected genetic conditions could be piloted. At least four aspects will have to be considered: autosomal-recessive inheritance, disease frequency, disease severity, and economic costs of a donor screening program.

P306

DNA-based testing for heritable disorders in Europe

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Objectives: Regarding recent attention to develop policies in the field of provision of clinical genetic testing services this document intends to investigate access to, acceptance, utilisation and regulation of genetic services in selected European countries as well as one non-European country.
Methods: This document was assembled on the basis of relevant international reports, of sources accessible via the internet, of data from self-designed, internationally administered surveys, and with the help of a panel of experts from European countries contributing in several workshops, and from National Societies of Human Genetics from several European countries.
Results: A selection of divergent health care systems was reviewed and compared such as Finland, Germany, Portugal, Sweden, UK, France, Italy, Spain, Czech Republic, Lithuania and Serbia/Montenegro. Background information for evaluation of clinical validity and utility of genetic testing with focus on DNA-based testing for heritable disorders with a strong genetic component (usually due to the action of a single gene) was provided.
Conclusions: There is a great heterogeneity in genetic testing services among the countries surveyed. It is premature to mandate that genetic testing in clinical services meets professional standards of clinical validity and utility, because there is as yet no consensus within the scientific community and among health care providers to what extent clinical validity and utility can and need to be assessed. Points to consider in the process of developing such standards are proposed.

P307

Disruption of erythroid K-Cl cotransporters alters erythrocyte volume and partially rescues erythrocyte dehydration in a mouse model for sickle cell anemia

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Red blood cell (RBC) K-Cl cotransport activity is a major determinant of RBC volume and density. Pathologic activation of erythroid K-Cl cotransport activity in sickle cell disease contributes to RBC dehydration and cell sickling. To address the roles of individual K-Cl cotransporter isoforms in RBC volume homeostasis, we disrupted the Kcc1 and Kcc3 genes in mice. As RBC K-Cl cotransport activity was undiminished in Kcc1-/- mice, decreased in Kcc3-/- mice, and almost completely abolished in mice lacking both isoforms, RBC K-Cl cotransport activity is largely mediated by KCC3. Whereas RBCs of either Kcc1-/- or Kcc3-/- mice were of normal density, RBCs of Kcc1-/-/Kcc3-/- mice exhibited defective volume regulation including increased mean corpuscular volume, decreased densities, and increased susceptibility to osmotic lysis. The increased K-Cl cotransport activity of SAD mice transgenic for a hypersickling human hemoglobin S variant correlated with upregulation of both KCC1 and KCC3 RBC protein levels. RBCs of SADKcc1-/-/Kcc3-/- mice lack nearly all K-Cl

cotransport activity and exhibit normalized values of mean corpuscular volume, corpuscular hemoglobin concentration mean and K+ content. These findings suggest that inhibitors of RBC K-Cl cotransport may prove useful for the therapy of sickle cell disease.

P308

SMN2 gene promoter methylation: Implications for progression and therapy of Spinal Muscular Atrophy (SMA)

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The disease determining survival motor neuron gene 1 (SMN1) is homozygously deleted in ~96% of SMA patients. Within the SMA region on chromosome 5q, the SMN genes exist in two almost identical copies (SMN1, SMN2) which are ubiquitously expressed and encode identical proteins. Since all SMA patients lacking SMN1 carry at least one SMN2 copy, transcriptional activation of the SMN2 gene is likely to be clinically beneficial. We have shown that several histone deacetylase inhibitors (HDACis) increase SMN2 expression in fibroblasts derived from SMA patients. Here, we show that the DNA demethylating agent 5-aza-2´-deoxycytidine increases SMN2 transcript- and protein levels in SMN1-deleted fibroblasts. This finding indicates that both, histone acetylation and DNA methylation, regulate SMN2 gene activity. By DNA methylation analysis we identified a highly conserved SMN2 methylation pattern in fibroblasts and blood samples from SMA patients. Comparison of the methylation patterns of type I and type III SMA patients carrying 2 SMN2 copies revealed significant differences in cytosine methylation at 7 specific sites, suggesting that SMN2 promoter methylation modulates the disease severity. Two of the differentially methylated cytosines are located close to a transcriptional start silenced by DNA methylation. Strikingly, pan-HDACis such as SAHA and FK-228 are able to overcome SMN2 silencing by DNA methylation, whereas other isoenzyme selective HDACis such as MS-275 and VPA do not. We suggest that SMN2 promoter methylation modulates the disease severity which may in part explains the variable phenotypes in the presence of identical SMN2 copy numbers. Moreover, our analyses favour pan-HDACis such as SAHA and FK-228 for SMA therapy due to their propensity to counteract SMN2 gene silencing by DNA methylation. These results may improve the understanding of how the SMN2 gene expression is regulated and might have clinical implications for the SMA treatment with HDACis.

P309

Simultaneous targeted exchange of two nucleotides by single-stranded oligonucleotides clusters within a region of about fourteen nucleotides

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Transfection of cells with gene-specific, single-stranded oligonucleotides can induce the targeted exchange of one or two nucleotides in the targeted gene. To characterize the features of the DNA-repair mechanisms involved, we examined the maximal distance for the simultaneous exchange of two nucleotides by a single-stranded oligonucleotide. The chosen experimental system was the correction of a hprt-point mutation in a hamster cell line, the generation of an additional nucleotide exchange at a variable distance from the first exchange position and the investigation of the rate of simultaneous nucleotide exchanges. The smaller the distance between the two exchange positions, the higher was the probability of a simultaneous exchange. The detected simultaneous nucleotide exchanges were found to cluster in a region of about fourteen nucleotides upstream and downstream from the first exchange position. We suggest that the mechanism involved in the repair of the targeted DNA strand utilizes only a short sequence of the single-stranded oligonucleotide, which may be physically incorporated into the DNA or be used as a matrix for a repair process.

P310

Inhibition of telomerase expression by androgens: A novel role of Androgen receptor mutations for prostate cancer development

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Androgens play a central role in prostate development and prostate cancer proliferation. Induction of telomerase is an early event in prostate carcinogenesis and is considered as a marker for both primary tumors and metastases. Interestingly, several reports suggest that telomerase activity is regulated by androgens in vivo. Here, we show that the wildtype human androgen receptor (AR) inhibits the expression of the catalytic subunit of human telomerase (hTERT) and telomerase activity via inhibition of hTERT promoter activity in the presence of androgen receptor agonists. However, the androgen-mediated repression of hTERT is abrogated in LNCaP prostate cancer cells that express a mutant AR (T877A) frequently occurring in prostate cancer. We reveal that this single amino acid exchange is sufficient for the lack of transrepression. Interestingly, chromatin immunoprecipitation (ChIP) data suggest that in contrast to the wild-type AR, the mutant AR is recruited less efficiently to the hTERT promoter in vivo indicating that loss of transrepression is due to reduced chromatin recruitment. Thus, our findings suggest that the wild-type AR inhibits expression of hTERT, which is indicative of a protective mechanism, whereas the T877A mutation of AR not only broadens the ligand spectrum of the receptor but abrogate this inhibitory mechanism in prostate cancer cells, revealing a novel role of AR mutations in prostate cancer development and suggests to search for new AR antagonists that inhibit transactivation but allow transrepression.

P311

Repression of the human Androgen Receptor (AR) by Corepressors (CoRs) and a new strategy to inhibit AR in prostate cancer cells using peptide aptamers

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The androgen receptor (AR) plays an important role in male development and prostate cell proliferation. The AR is involved in various diseases like the androgen insensitivity syndrome, Kennedys disease, and also Prostate Cancer (PCa), which is one of the most often diagnosed malignancies in men.
The AR is activated by androgens and regulates the proliferation of prostate cancer cells. PCa is treated through the ablation of androgens and application of antiandrogens (AR antagonists), which function by recruiting Corepressors (CoRs) to the AR leading to transcriptional silencing on epigenetic level. This therapy is at first successful, but eventually PCa proliferation becomes independent of androgens.
Crucially, the AR can also be activated in a ligand-independent manner through the signal transduction machinery, such as Protein Kinase A (PKA). Interestingly, activation of these signalling pathways inactivate CoRs and therefore significantly contribute to the androgen refractory PCa proliferation.
We have identified new binding motifs through a peptide aptamer screen in yeast for the antagonist-bound AR, that interact independently of the signal transduction machinery. The binding of the peptides to AR was confirmed in mammalian cells. The fusion of these AR-interacting peptides to the potent transcriptional silencing domain SUMOG97A led to novel CoRs being highly specific for the AR. The new CoRs repress the human AR and also inhibit the expression of the endogenous PSA gene. Importantly the new CoRs also inhibit the growth of LNCaP prostate cancer cells. Furthermore the interaction between the AR and the new CoRs is not reduced by PKA signalling.
Taken together these data suggest that AR interacting peptide aptamers fused to SUMOG97A are potent, specific and PKA independent CoRs of the AR.
Therefore, these novel CoRs represent a new strategy to treat PCa, also in the androgen-independent and refractory stages.

P312

PPAR-gamma activators: Off-target against TGF-ß mediated glioma cell migration and brain invasion

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Gliomas are the most common primary tumors of the central nervous system, with glioblastomas as the most malignant entity. Rapid proliferation and diffuse brain invasion of these tumors are likely to determine the unfavourable prognosis. Considering its pro-migratory properties, the transforming growth factor ß (TGF-ß) signaling pathway has become a major therapeutic target. Analyses of resected glioma tissues revealed an intriguing correlation between tumor grade and the expression of TGF-ß1-3. Using an organotypic glioma invasion model, we identified the peroxisome proliferator-activated receptor gamma (PPAR-gamma) agonist troglitazone (TRO), which has been developed for the treatment of type II diabetes mellitus, as a potent inhibitor of TGF-ß mediated glioma migration and brain invasion. The anti-migratory property of TRO occurred at clinically achievable levels and was mediated by transcriptional repression of TGF-ß1-3, resulting in dramatically reduced TGF-ß secretion. To date, TRO appears to be the most potent inhibitor of TGF-ß expression and may thus represent a promising drug for adjuvant glioma therapy. Here, we show that the PPAR-gamma inactive TRO derivative delta 2-TRO antagonized TGF-ß signaling and ex vivo glioma invasion similar to TRO, giving clear evidence that the anti-glioma properties of TRO occur in a PPAR-gamma independent manner. The identification of the yet unknown anti-migratory target protein of (delta 2)-TRO may provide new therapeutic strategies for adjuvant therapy of glioma and other highly migratory tumor entities

P313

Studies on uptake of peptide nucleic acids (PNA) in mitochondria

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Homoplasmic single base pair exchanges are frequently found in the mitochondrial DNA (mtDNA) of malignant cancer cells in vitro and in vivo. We are interested in targeting these mtDNA sequences specific for these cancer cells in vitro by oligomers consisting of synthetic peptide nucleic acids (PNA). PNAs are uncharged and are able to invade a DNA-double strand. We studied the binding affinity of 125I-labeled PNAs and 32P-DNA oligonucleotides to PCR products from mtDNA sequences from these cancer cells and controls. In addition, we studied the uptake of PNAs labeled by Biotin in mitochondria of tumor cells. To ameliorate the uptake in mitochondria we modified the PNAs by TPP (Triphenylphosphonium ion) or peptides. We suggest that targeting of mtDNA sequences found in cancer cells by sequence specific PNAs represents an approach to influence the behavior of these cells.

P314

Lovastain treatment accelerated the delayed cortical bone injury repair in the Nf1Prx1 mice

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We have recently generated a mouse model of the Neurofibromatosis type 1 associated bone dysplasia by conditionally inactivating both Nf1 alleles in the developing limbs -Nf1Prx1. Despite tibia bowing, which is collectively caused by decreased bone mineralization and increased bone vascularization, spontaneous fractures do not occur in Nf1Prx1 mice probably due to the relatively low mechanical load. Here we are reporting results of the artificially induced Nf1Prx1 tibia cortical bone injury, which simulates aspects of the NF1 pseudarthrosis.
During cortical bone repair in the wt mice mineralization of extracellular matrix closely follows the migration of mesenchymal progenitor cells from the activated periosteum, which results in the new bone formation in the bone marrow cavity. We could show that these early steps of cortical bone repair process are impaired in Nf1Prx1 mice. The mineralization process is delayed which coincides with a fibroblast-like and/or cartilaginous phenotype persistence at the injury site. Additionally, the bone surrounding the drilling hole is massively penetrated by blood vessel and fibrous tissue, which is associated with partial demineralization of the old bone.
All of the above mentioned changes are alleviated by the high dose systemic Lovastatin treatment. The Lovastatin treated animals exhibit better quality of cortical bone with no signs of demineralization of the injury surrounding bone. Additionally a striking increase of the new bone formation within the bone marrow cavity is observed. Consequently the cortical bone repair process is accelerated and reaches the control values on the day 14-post injury.
Presented experimental model is ideal for the pre-clinical stage testing of the candidate drugs,

targeting Nf1 associated bone dysplasia and the Lovastatin test data encourage further exploration of this drug group applicability in the NF1 treatment.