Topics

Symposia

S 1 Next Generation Sequencing
S 2 Brain Development and Malformations
S 3 Allelic and Non-allelic Modifiers of Disease phenotype
S 4 Structural Variation
S 5 Behavioural Phenotypes
S 6 Genome-wide Studies of Gene Regulation

SEL Selected Presentations

Workshops

W 1 Cancer Genetics
W 2 Functional Analysis I
W 3 Clinical Genetics
W 4 Methods
W 5 Evolution / Functional Analysis II
W 6 Epigenetics
W 7 Complex Diseases
W 8 Mental Redardation
W 9 Neurogenetics
W 10 Molecular Basis of Disease
W 11 Developmental Genetics

Educational Sessions

EDU 1 Genetische Prädiktion bei multifaktoriellen Krankheiten
EDU 2 Mikrodeletionssyndrome
EDU 3 Familiäre Tumorerkrankungen
EDU 4 Neuromuskuläre Erkrankungen

Poster

Poster Session I

P-CancG-001–32	**Cancer genetics**
P-ClinG-033–109	**Clinical genetics**
P-Compl-110–126	**Complex genetics/complex diseases**
P-CytoG-127–158	**Cytogenetics**

Poster Session II

P-Prenat-159–169	**Prenatal diagnosis, reproductive medicine**
P-MoleG-170–245	**Molecular and biochemical basis of disease**
P-Neuro-246–277	**Neurogenetics**
P-Devel-278–282	**Developmental genetics,**
P-Techno-283–296	**Genomics, technology and bioinformatics**
P-Therap-297–300	**Therapy for genetic diseases**
P-Counse-301–306	**Genetic counselling, education, genetic services, public policy**
P-Popul-307–309	**Population genetics, genetic epidemiology, evolutionary genetics**

Vorträge/Lectures

SYMPOSIA

S1-01
Next generation sequencing in clinical diagnostics
Dr. Bernd Timmermann, Next Generation Sequencing Group, Max Planck Institute for Molecular Genetics, Berlin bio.logis GmbH, Frankfurt

Next generation sequencing techniques allow to analyze large genomic regions within a reasonable time-frame. Selective DNA enrichment techniques by hybridization have become the tools of choice to lower the burden of time and cost even further. With the combination of these technologies it is now practical feasible to sequence hundreds or even thousands of genes in a single individual with a suspected genetic disease or complex disease predisposition. Currently there are three next generation sequencing platforms available: The Roche FLX Genome Sequencer, Illuminas Genome Analyzers and Lifetechnologies SOLiD system. Those machines are widely distributed in academic labs, and their performance has been well characterized. In brief, FLX produces long reads (average 400 bases), but the number per run is moderate (up to 1,500,000). An important advantage is the short runtime. Illuminas GA and SOLiD produces short reads (100 versus 50 bases), but are characterized by the large number of reads per run (several hundreds of millions). The performance of the short read technologies is increasing rapidly. For most diagnostic applications a targeted analysis of a limited number of genes is currently preferred, as this will alleviate functional interpretation and overcome limitations in computational power as well as ethical restrictions. The low-scale gene analysis by Sanger sequencing that currently dominates the diagnostic field will ultimately be replaced by massively parallel sequencing of entire gene pathways or networks.

S1-02
Interrogating the architecture of cancer genomes
Dr. Peter Campbell, Cancer Genome Project, Wellcome Trust Sanger Institute

Cancer is driven by mutation. Using massively parallel sequencing technology, we can now sequence the entire genome of cancer samples, allowing the generation of comprehensive catalogues of somatic mutations of all classes. Bespoke algorithms have been developed to identify somatically acquired point mutations, copy number changes and genomic rearrangements, which require extensive validation by confirmatory testing. The findings from our first handful of genomes illustrate the potential for next-generation sequencing to provide unprecedented insights into mutational processes, cellular repair pathways and gene networks associated with cancer development. I will also review possible applications of these technologies in a diagnostic and clinical setting, and the potential routes for translation.

S1-03
Mendelian genetics by exome sequencing
Mike Bamshad, University of Washington, USA

Researchers, health-care providers and the public alike are interested in knowing to what extent a person's genetic makeup can help guide decisions about individual health and medical care. But finding genetic variants that influence human diseases has been a challenging task and remains one of the major obstacles to realizing the goal of personalized medicine. Over the past few years, great strides have been made toward developing new DNA sequencing technologies and computational tools

for analysis of DNA sequence to help in the search for disease-causing variants throughout the human genome. To this end, it has recently become possible and affordable to sequence all the protein-coding exons of each gene in the human genome, otherwise known as the "exome," in a single experiment. We recently demonstrated the first successful application of exome sequencing to discover the gene for a rare, Mendelian disorder of unknown cause, Miller syndrome (OMIM %263750). For four affected individuals in three independent families, we captured and sequenced coding regions to a mean coverage of 40X, and sufficient depth to call variants at ~97% of each targeted exome. Filtering against public SNP databases and a small number of HapMap exomes for genes with two novel variants in each of the four cases identified a single candidate gene, DHODH, which encodes a key enzyme in the pyrimidine de novo biosynthesis pathway. Sanger sequencing confirmed the presence of DHODH mutations in three additional families with Miller syndrome. This result demonstrated that exome sequencing of a small number of unrelated, affected individuals can be a powerful, efficient strategy for identifying the genes underlying rare Mendelian disorders that will likely transform the genetic analysis of monogenic traits. Subsequently, we are adapting exome sequencing to search for rare DNA sequence variants underlying the heritability of complex traits. This poses far more challenges. Use of exome sequencing for genetic analysis raises a number of important ethical questions: How many clinically relevant mutations reside in the exome of every individual? Should information about these mutations be given to each patient or research participant? How should decisions about the clinical relevance of mutations be made? And who should make these decisions? Developing a framework to study what are the best answers to these questions will be critical as exome, and eventually whole genome, sequencing becomes a standard instrument of scientists and clinicians.

S2-01

Human evolution from a chimpanzee's perspective

Wolfgang Enard, Max-Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany enard@eva.mpg.de

With the completion of the human genome sequence and the advent of technologies to study functional aspects of genomes, molecular comparisons between humans and their closest relatives, the chimpanzees, have gained momentum. In particular, the available sequence of the chimpanzee genome defines a catalog of some 40 million genetic differences that contains essentially all changes (~ 20 million) that led to the genetic basis of human-specific biology during the last 6 million years. The challenge ahead is to extract meaningful insights from this catalog. I will discuss how additional genome-wide data, such as the Neanderthal genome or primate gene expression patterns, can be informative and how particular hypotheses, as for example the link between genetic changes in FOXP2 and the evolution of speech and language, can be further interrogated.

S2-02

The genetic basis of human brain malformations and related developmental disorders

William B. Dobyns, Departments of Human Genetics, Neurology and Pediatrics, The University of Chicago

The past decade has seen rapid advances in the genetic basis of several different classes of brain malformations as well as disorders with similar phenotypes but apparently normal brain imaging. Overall, more than xx genes developmental genes have been associated with one or more brain malformations. The most important disorders of forebrain development include holoprosencephaly (9+ genes), agenesis-hypogenesis of the corpus callosum (8+ genes), and septo-optic dysplasia (1 gene). The most important mid-hindbrain malformations include pontocerebellar hypoplasia (4+ genes), diffuse cerebellar hypoplasia (4+ genes), the Dandy-Walker spectrum which includes classic Dandy-Walker malformation, mega-cisterna magna and cerebellar vermis hypoplasia (3+ genes), and the molar tooth-cilia dysfunction group (10+ genes). Several developmental genes have been associated with disorders of brain size including severe congenital microcephaly (6+ genes), genetic postnatal microcephaly (3+ genes) and congenital or postnatal megalencephaly (2+ genes). The most important malformations of cortical development include lissencephaly (6+ genes), periventricular nodular heterotopia (2+ genes), cobblestone malformations (9+ genes) and polymicrogyria (6+ genes) although the known polymicrogyria genes all represent very rare forms. As the number of genes associated with brain malformations has increased, several patterns have begun to emerge showing that mutations of some of these important developmental genes may present with more than one brain malformation, or as developmental encephalopathies (Angelman or Rett-like syndromes, infantile seizure disorders) with apparently normal brain structures. Good examples include ARX and SHANK3. Similarly, mutations of genes first identified in children with complex developmental encephalopathies may be associated with brain malformations, such as FOXG1 and MECP2. Collectively, these data suggest that human brain malformations represent the "tip of the iceberg" of the overall disease burden resulting from mutations of overlapping networks of developmental genes.

S2-03

Mouse models of neuronal migration disorders

Fiona Francis ('Cytoskeleton and neuronal migration disorders', INSERM UMR-S 839, F75005, Paris, France; Université Pierre et Marie Curie, F75005, Paris; Institut du Fer à Moulin, F75005, Paris)

Four genes have been identified for different forms of type I lissencephaly, LIS1, DCX, TUBA1A and Reelin, which code for proteins in two functional categories. Mouse mutants for the first three cytoskeletal genes have a predominantly hippocampal phenotype showing a fragmented pyramidal cell layer indicative of radial migration abnormalities in this structure. Epilepsy and cognitive deficits are obvious in these models despite the fact that the isocortex is remarkably preserved. Reeler mice, mutant for an extracellular matrix protein, differ because they have a severer disorganization of both the isocortex and the hippocampus and thus resemble most closely the human disorder. Band heterotopia has up till recently been difficult to reproduce in the mouse. Paradoxically, RNA interference for Lis1 and Dcx genes produces such a phenotype in the rat and there exists one genetic rat model, tish. Furthermore two recently identified mouse models, HeCo and a conditional knockout of RA-GEF1, show band heterotopia and epilepsy. Identification of the genes mutated in all these models and a study of the interactions between them, should aid a comprehension of the pathophysiology of this intriguing disorder. Important for neuronal migration, radial glial cell integrity is also an emerging theme in diverse cortical disorders. Mouse studies of cortical malformation genes are thus greatly aiding our comprehension of important mechanisms for corticogenesis.

S3-01

Genetic modifiers of risk in Mendelian cancer syndromes: common breast and ovarian cancer susceptibility variants and cancer risks for BRCA1 and BRCA2 mutation carriers as an example

Antonis C. Antoniou, Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, UK

Several lines of evidence suggest that genetic factors modify the cancer risks for carriers of mutations in Mendelian cancer syndromes. Past studies concentrated on variants in candidate genes thought to be functionally relevant to the diseases. However, these have not been very successful and most studies were too small to provide enough power

to detect the modest associations that are likely to be present. Furthermore, the analysis of genetic modifiers of risk for genetically susceptible individuals poses several analytical issues. The talk will review the latest evidence for genetic modifiers of risk for Mendelian cancer syndromes with a particular focus on BRCA1/2.
Recently, polymorphisms identified through genome-wide association studies of unselected cancer patients and controls have been shown to be associated with cancer risk in studies of large numbers of mutation carriers. This approach identified seven genetic variants (in FGFR2, TOX3, MAP3K1, LSP1,2q35, 5p12 and SLC4A7) that are associated with the risk of breast cancer and one variant (in BNC2) that is associated with ovarian cancer risk for BRCA1 and/or BRCA2 mutation carriers participating in the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) studies. Differential associations were found between these variants and breast cancer risk for BRCA1 and BRCA2 mutation carriers. These are in line with differences observed in the associations between these polymorphisms and different disease subtypes in the general population and suggest that studies of mutation carriers may be useful for identifying genetic variants associated with different disease subtypes in the general population.
These genetic variants appear to interact multiplicatively on breast cancer risk for BRCA2 mutation carriers. Based on the joint genotype distribution of the 7 risk associated polymorphisms in BRCA2 mutation carriers, the 5% of BRCA2 mutation carriers at lowest risk are predicted to have a probability of 42-50% of developing the disease by age 80. In comparison, the corresponding probability is 80-96% for the 5% of BRCA2 mutation carriers at highest risk. Such risk differences may be sufficient to influence the clinical management of mutation carriers and suggest that this is may be one of the first clinically useful impact of common, low penetrance variants identified through genome wide association studies.

S3-02

Gene modifiers in cystic fibrosis: a consortium approach

Michael Knowles, M.D.*, Cystic Fibrosis/Pulmonary Research and Treatment Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Cystic fibrosis (CF) is a recessive "monogenic" genetic disorder caused by mutations in CFTR. However, there is a wide range of organ involvement and disease severity in CF, even for patients who are homozygous ΔF508; therefore, there must be other non-CFTR genetic variants and/or environmental effects that account for this disease heterogeneity. Two studies of twins and sibs have assessed environmental versus genetic influences, and both concluded that genetic factors play a major role in lung disease severity (Mekus, F. et al; Hum Genet 2003; Vanscoy, LL, et al, Am J Respir Crit Care Med, 2007).
The search for gene modifiers in CF has been underway for more than a decade, using candidate gene approaches. Early studies were hampered by small sample size, and limitations of study design and phenotyping. These limitations have largely been overcome by 3 large studies in North America: 1) UNC/CWRU (case-control, extremes-of-phenotype design); 2) Johns Hopkins (twin/sib, family-based design), and 3) Canada (study of all Canadian CF patients). These 3 groups now utilize standardized measures of lung function to more accurately define pulmonary phenotype. These groups have also reported heritability and/or modifiers of lung and other phenotypes using standardized methodologies. (Drumm, M, et al, 2005, N.Engl.J.Med; Bremer, LA, et al, 2008, Hum Mol Genet 2008; Collaco, JM, et al, 2008, JAMA; Dorfman, R, et al, 2008, JCI)
To overcome the limitations of the candidate gene approach, a Consortium to conduct genome-wide association studies (GWAS) has been formed by investigators at UNC/CWRU, Hopkins, and Canada. A GWAS has been completed in >4,200 CF patients, plus ~1,000 parental samples, using the Illumina 610K Quad platform. Analyses of the GWAS data are ongoing for different phenotypes. Preliminary results suggest strong associations of lung disease severity to several genomic regions. Because of the collaborative and complementary nature of studies being undertaken in CF, there is reason to be optimistic that more participants will be added, and emerging data will drive progress for better prognostic and therapeutic approaches.
This study is supported by U.S. CFF CUTTING06P0; CFF KNOWLE00A0; NIH R01HL068927; NIH R01HL68890, NIH RR00046, NIH R01DK66368; R37DK044003; Canadian CFF; Genome Canada.
*Reporting for the GMS investigators and the North American CF Gene Modifier Consortium.

S3-03

Identifying a modifying gene in a polycystic kidney disease rat model resulting in a new therapeutic target

Norbert Gretz, Sonja Spahn, Medical Research Centre, Heidelberg University, Mannheim

We performed gene expression profiling in a rat strain of polycystic kidney disease (PKD). This strain (cy-rat) had developed a phenotypic difference in its disease severity in 2 highly inbred substrains. We identified COMT as a significantly differentially expressed gene. Using a COMT inhibitor (Tolcapone) we could reduce disease severity in one substrain to the level of the "lower" expressing rat strain. This approach was also tested in other rat models of PKD (PKD2, ARPKD) and proved partially effective. No adverse effects of the treatment could be observed.

S4-02

Copy-number variants in neuropsychiatric disorders

Sven Cichon, Institute of Neurosciences and Medicine (INM-1), Research Center Juelich and Institute of Human Genetics, University of Bonn, Institute of Neurosciences and Medicine (INM-1), Research Center Juelich, Institute of Human Genetics, Department of Genomics, Life & Brain Center, University of Bonn

Copy-number variants (CNVs) are the most prevalent types of structural variations in the human genome. They contribute significantly to inter-individual genetic variability and their importance in modulating human diseases is increasingly being recognized. Genome-wide approaches have revealed rare, highly penetrant CNVs that play a role in the development of neuropsychiatric disorders such as schizophrenia and autism spectrum disorders in a proportion of disease cases. There is evidence that some of these CNVs underlie negative selection. The existence of rare CNVs with high penetrance offers opportunities to model some relevant pathogenic processes, although specific inferences may be limited by the fact that some of the specific CNVs span multiple genes. A further challenge in the interpretation of current CNV studies is the broadness of phenotypes that can be related to the same CNV, suggesting the presence of pleiotropic effects.
It is expected that more disease-associated CNVs in neuropsychiatric disorders will be identified with the use of technologies with higher resolution and that are better designed to investigate common CNVs reliably.

S5-01

Genetic findings for nicotine dependence: A convergence with genetic findings for lung cancer and chronic obstructive pulmonary disease

Laura J. Bierut and COGEND colleagues, Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA

Despite reductions in smoking in the U.S and Europe, smoking remains one of the leading contributors to death. Lung cancer is the most striking disease associated with smoking, and its prevalence over time directly mirrors changes in smoking behavior. Chronic obstructive

pulmonary disease is another illness closely related to smoking behavior and lung cancer. Recent genetic studies have identified genetic factors that contribute to smoking quantity and nicotine dependence, and the most compelling evidence of risk is on chromosome 15 in the region that includes the α5-α3-β4 nicotinic receptor genes. Genetic association findings for lung cancer and chronic obstructive pulmonary disease converge on this same region.
Further dissection of this associated region demonstrates that there are at least two distinct genetic variants that are contributing to the risk for nicotine dependence. The next step to understand the underlying biology requires investigation of the genetic variants that potentially alter biologic function. In this region of association, a SNP (rs16969968) that causes an amino acid change in the α5 nicotinic receptor is a compelling functional variant. This genetic variant results in a change from aspartate to asparagine in the α5 protein subunit, and in α5 containing receptors, nicotinic agonist evoked responses show significant differences. Distinct from this amino acid change, non-coding genetic variants also contribute to differences in α5 receptor subunit expression in the brain. These two biological mechanisms (amino acid change and expression level differences) are associated with altered risk for nicotine dependence and lung cancer, and this work implicates the α5 receptor subunit as a major genetic contributor to the development of these disorders.

S5-02
Systems-level mechanisms mediating genetic risk for the psychoses
Andreas Meyer-Lindenberg, Chair of Psychiatry, University of Heidelberg, Director, Central Institute of Mental Health, Mannheim, Germany

Background: Recently, genome-wide significant risk variants for psychotic disorders have been discovered that afford an opportunity to establish neural mechanisms linked to genetic risk for schizophrenia and bipolar disorder through an imaging genetics approach.
Methods: We use an imaging genetics approach in a sample of healthy German volunteers of German descent and a replication sample of similar ethnicity, recruited and assessed with a cognitive and resting fMRI and structural imaging battery in the context of the BMBF-funded MooDs project.
Results: ZNF804A and CACNA1C impact on cortical-subcortical connectivity with emphasis on hippocampus and prefrontal cortex. Cognitively specific and global abnormalities can be dissociated, indicating structural (maturational?) components and connectivity aspects relationg to the specific type of information processed (working memory, theory of mind, episodic memory, emotional regulation). These subcomponents can again be mapped onto clinical features of schizophrenia and bipolar disorder, respectively.
Discussion: Genome-wide significant variants impact on the brain connectome, partially recapitulating phenotypes observed in patients at risk and with overt disease. Disturbances of connectivity are likely part of the core neurogenetic risk architecture of psychosis across the Kraepelinian divide.

S5-03
Polygenic obesity
Johannes Hebebrand, Anke Hinney, Department of Child and Adolescent Psychiatry, University of Duisburg-Essen, Essen

Over the last fifteen years, the genetic analysis of obesity led to the identification of confirmed major genes. While such major genes have a clear influence on the development of the phenotype, they are however rare and thus of minor clinical importance. Polygenic effects concern a clearly larger number of affected subjects. The first truly validated polygenic variant with an influence on body weight pertains to the V103I polymorphism in the melanocortin-4 receptor gene. The I103 variant leads to a mean BMI reduction in the magnitude of 0.5 kgm^2; approximately 4% of the German population is heterozygous. Genome wide association studies (GWAS) have made it possible to detect several more polygenes underlying obesity. The FTO (fat mass and obesity associated) gene was found to be associated with type 2 diabetes mellitus as based on a GWAS; statistical adjustment for BMI revealed that this association actually reflects an association with obesity. This finding has subsequently been replicated in large independent study groups. In the first genome wide association study for obesity, which was based on 487 obese children and adolescents and 442 lean adult controls a FTO SNP was significantly associated with obesity after correction for multiple testing. Mean BMI increases by 1.2 kg per allele. Recently a variant downstream of the melanocortin-4 receptor gene was also identified as having an influence on body weight; the mean effect of a single allele corresponds to +0.22 kg/m^2. Three recent GWAS have led to the detection of several more SNPs. In total, genetic variation at 17 loci has been found to be associated with obesity. Functional studies have been initiated; noteworthy is the Fto knock-out mouse which is underweight. Specific copy number variants have been identified more frequently in obese patients than in controls; however, the children were enriched for associated developmental delay. In conclusion, the advent of GWAS has led to the detection of several SNPs associated with obesity; effect sizes in terms of grams per allele are small; the BMI variance explained by these associations is in the magnitude of 2% and thus substantially lower than predicted as based on current heritability estimates for body weight in the magnitude of 0.5 to 0.7.

S6-01
The connectivity of genomic elements
Stylianos E Antonarakis, University of Geneva, Switzerland

The understanding of the pathogenesis of genetic disorders requires not only the elucidation of the function of each nucleotide and each genomic variant, but also the „connectivity" of genomic elements. Three examples of genomic connectivity will be presented: i. the long-range extension of transcripts; ii. the cis and trans control of gene expression variation; and iii. the physical interaction of conserved non-coding sequences in chromatin.

S6-02
Alternative splicing networks in nervous system development and disease
Benjamin J. Blencowe, Centre for Cellular and Biomolecular Research and Department of Molecular Genetics. University of Toronto

Recent studies employing microarray profiling and high throughput sequencing of RNA (RNA-Seq) have revealed a multitude of alternative splicing events that are regulated in a cell and tissue type-dependent manner (1,2). Similar to earlier observations showing that transcriptionally co-regulated genes tend to belong to shared functional categories, genes with co-regulated alternative exons also tend to operate in common functional pathways and biological processes (3). An important current goal is to understand how these "networks" of co-regulated exons are controlled, their specific functions, and how their disruption contributes to human disease.
We have recently discovered and characterized a novel nervous system- and vertebrate lineage-specific alternative splicing regulator, the neural-specific SR-related protein of 100 kDa (nSR100/SRRM4) (4). This protein regulates a network of nervous system-specific exons in genes with functions associated with cytoskeletal organization and other aspects of neuronal differentiation. Disruption of nSR100 prevents neuronal differentiation in vitro and in vivo. To investigate functions of the nSR100-regulated exon network, we are employing a high-throughput immunoprecipitation screen to assess the roles of specific exons in the network at the protein level. This screen has identified exons that function in modulating protein-protein interactions involving nSR100 regulated genes. These results thus provide evidence for an important

role of a coordinated exon network in the regulation of protein-protein interactions associated with development of the nervous system.
Using RNA-Seq, we are comparing the transcriptomes of human brain tissues from normal individuals and individuals with Alzheimer's Disease. Transcripts with pronounced alternative splicing level changes in Alzheimer's Disease patients are enriched in genes with cytoskeletal associated functions, several of which have previously been implicated in the disease but not known to be affected at the splicing level. The emerging results suggest that disruption of an alternative exon network in genes with cytoskeletal functions may play an important role in Alzheimer's Disease.

References
(1) Pan et al. Nature Genetics. 40:1413-1415. 2008
(2) Wang et al. Nature. 456:470-476. 2008
(3) Fagnani et al. Genome Biology. 8, R108. 2007
(4) Calarco et al. Cell. 138:898-910.2009

S6-03
MicroRNAs, RNA binding proteins, and post-transcriptional gene regulation
Nikolaus Rajewsky, Systems Biology, Max Delbruck Center for Molecular Medicine Berlin-Buch, Germany

In recent years it has become apparent that a large fraction of all genes in animals is post-transcriptionally regulated by microRNAs. microRNAs are thought to be attractive candidates for novel diagnostic and therapeutic approaches in many diseases.
Furthermore, animal genomes contain hundreds of genes with RNA binding domains. It is clear that many of these proteins have important functions in mRNA localization, stability and in regulating protein production. Furthermore, numerous RNA binding proteins have been linked to various diseases. However, only recently technologies have become available to probe post-transcriptional regulatory networks on a genome-wide scale.
I will briefly review previous efforts to understand more about the function of microRNAs. I will then present ongoing work where we use high throughput quantitative proteomics, next generation sequencing and computational approaches to unravel the function of small RNAs and RNA binding proteins in well defined in-vivo systems.

Selected Presentations

SEL-01
Mutations in FAM134B, encoding a newly identified Golgi protein, cause autosomal recessive severe sensory and autonomic neuropathy
Kurth I.[1], Pamminger T.[2], Hennings JC.[2], Soehendra D.[1], Huebner AK.[2], Rotthier A.[3], Baets J.[3], Senderek J.[4], Topaloglu H.[5], Farrell SA.[6], Nürnberg G.[7], Nürnberg P.[7], de Jonghe P.[3], Gal A.[1], Kaether C.[8], Timmerman V.[3], Hübner CA.[2]
[1]Department of Human Genetics University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Department of Clinical Chemistry, Jena, Germany, [3]VIB Department of Molecular Genetics, Antwerp, Belgium, [4]Institute of Cell Biology, Zürich, Switzerland, [5]Department of Pediatric Neurology, Ankara, Turkey, [6]Department of Laboratory Medicine, Ontario, Canada, [7]Cologne Center for Genomics and Institute for Genetics, Cologne, Germany, [8]Leibniz Institute for Age Research, Jena, Germany

Sensory and autonomic innervation is important to protect the body from tissue damage. This is highlighted by hereditary sensory and autonomic neuropathy type 2 (HSAN 2B, MIM 613115) which is characterized by severe mutilations due to impaired nociception and autonomic dysfunction. In a consanguineous family, we mapped the locus of this autosomal recessive condition to chromosome 5p15.1 and subsequently identified homozygous loss of function mutations in FAM134B. Analysis of unrelated patients with clinical symptoms compatible with HSAN also revealed homozygous loss-of-function mutations in FAM134B in three additional families. In all affected individuals, onset was early with impaired nociception complicated by ulcerations of hands and feet and chronic osteomyelitis leading to progressive acro-osteolysis.
FAM134B belongs to a family of three uncharacterized genes. We showed that Fam134b encodes a cis-Golgi protein of sensory and autonomic ganglia. RNAi mediated knockdown of Fam134b by a lentiviral approach resulted in structural alterations of the cis-Golgi compartment and induced apoptosis in a subset of murine primary dorsal root ganglia neurons. The structure of the mammalian Golgi apparatus, composed of numerous membrane stacks, is necessary to maintain its function as the central organelle for modification and distribution of newly synthesized lipids and proteins within the cell. Our findings support a critical role of FAM134B in maintaing the elaborate architecture of the organelle and show the significance of the protein in the long term survival of nociceptive and autonomic ganglia neurons.

SEL-02
Homozygous disruption of an extracellular matrix component cause Temtamy preaxial brachydactyly syndrome
Li Y.[1], Laue K.[2], Yigit G.[3], Temtamy SA.[4], Aglan M.[5], Pawlik B.[3], Nürnberg G.[6], Wakeling EL.[7], Quarrel OW.[8], Nürnberg P.[9], Topaloglu KA.[10], Hammerschmidt M.[2], Wollnik B.[11]
[1]Institute of Human Genetics an Center for Molecular Medicine Cologne, Cologne, Germany, [2]Institute for Developmental Biology, Cologne, Germany, [3]Institute of Human Genetics and Center for Molecular Medicine Cologne, Cologne, Germany, [4]Department of Clinical Genetics and Division of Human Genetics, Cairo, Egypt, [5]Department of Clinical Genetics and Division of Human Genetics, Cairo, Egypt, [6]Cologne Centre for Genomics and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany, [7]North West London Regional Genetics Centre, London, UK, [8]Sheffield Clinical Genetics Service at Sheffield Children's Hospital, Sheffield, UK, [9]Cologne Centre for Genomics and Center for Molecular Medicine Cologne and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany, [10]Pediatric Endocrinology and Metabolism, Adana, Turkey, [11]Institute of Human Genetics and Center for Molecular Medicine Cologne and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany

Temtamy preaxial brachydactyly syndrome (TPBS, OMIM 605282) is an autosomal recessively inherited congenital syndrome characterized by bilateral symmetrical preaxial brachydactyly and hyperphalangism of digits, multiple congenital anomalies, mental retardation, sensorineural deafness, and growth retardation. Skeletal anomalies in TPBS patients include progressive kyphoscoliosis and pectus excavatum.
Here we used a homozygosity mapping strategy in two consanguineous Egyptian families with TPBS, including the original family described by Temtamy, to map the TPBS locus. Linkage analysis using the Affymetrix 250K SNP array in both families revealed a locus with a homozygous critical region of 2 Mb on chromosome 15q26. We tested several highly relevant positional candidate genes within this region and identified two different homozygous mutations in one of these genes, named here TPBS1. A homozygous 1-bp deletion, c.14delG, was found in one family, while the index patient of the second family carried a homozygous 30-bp deletion, c.44_73del30. Furthermore, we found disease causing mutations in the TPBS1 gene in three additional TPBS families. The encoded protein is an extracellular matrix (ECM) component involved in the regulation of cartilage growth activity. We used a morpholino knockdown strategy in zebrafish for further functional characterization. Interestingly, zebrafish morphants showed an overlapping phenotype compared to TPBS and we could show that BMB signaling is altered in this zebrafish model.

In summary, our findings provide novel insights into the molecular pathogenesis of Temtamy preaxial brachydactyly syndrome.

SEL-03

A truncating mutation in the BOD1 gene leads to mental retardation and oligomenorrhea

Esmaeeli-Nieh S.[1], Porter I. M.[2], Motazacker M. M.[1], Jensen L. R.[1], Kahrizi K.[3], Goswami C.[1], Behjati F.[3], Khodai H.[4], Dehgani A.[4], Tzschach A.[1], Garshasbi M.[1], Swedlow J. R.[2], Ropers H. H.[1], Najmabadi H.[3], Kuss A. W.[1]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany, [2]Wellcome Trust Centre for Gene Regulation & Expression, University of Dundee, UK, [3]Genetics Research Center of the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, [4]Welfare Rehabilitation Center, Yazed, Iran

In the course of a large systematic clinical and molecular study to identify mental retardation (MR) causing defects with an autosomal recessive mode of inheritance (ARMR), we found a single 4.3 Mbp interval on chromosome 5q with a LOD score of 3.8 by autozygosity mapping in a family with four females affected by mild MR and oligomenorrhea. By sequencing the coding regions of all 27 genes within this locus we discovered a nonsense mutation in exon 2 of the BOD1 gene. This defect co-segregated with the disease and was not found in 720 control chromosomes. All other genes inside the interval were not affected by nucleotide changes. BOD1 is expressed in a wide range of tissues, including brain and ovary. By RT-PCR, we identified two hitherto unknown transcripts of BOD1 in control fibroblasts and showed expression of all four transcripts in a variety of brain tissues. Quantitative RT-PCR revealed loss of all BOD1 isoforms in patient fibroblasts, including splice variants that did not contain exon 2. This seems to be due to nonsense mediated decay, as it could be abrogated by cycloheximide treatment of the cells. The absence of BOD1 protein in patient cells was confirmed by Western blotting experiments. Using live cell imaging we observed several abnormalities in patient cell division. These findings are in keeping with a previous report that describes severe biorientation defects due to BOD1 depletion in Hela cells (Porter et al. J. Cell Biol. 179:187-197, 2007) and may also provide an explanation for the oligomenorrhea observed in all the patients of this family. Furthermore, pull-down and mass spectrometry experiments enabled us to identify an interacting protein that links BOD1 to gene regulation, and overexpression studies in primary murine neurons indicate an extra-nuclear localization of BOD1 during interphase. The latter indicates that BOD1 might also play a role in neuronal information processing and can provide a lead to the origin of MR in this condition.

SEL-04

Genome-wide association study identifies a novel susceptibility gene for bipolar disorder and schizophrenia: neurocan (NCAN)

Mühleisen TW.[1,22], Degenhardt F.[1,22], Mattheisen M.[1,4,22], Miro X.[2], Strohmaier J.[3], Steffens M.[4], Herms S.[1,22], Weingarten M.[1,22], Priebe L.[1,22], Haenisch B.[1,22], Alexander M.[1,22], Vollmer J.[1,22], Breuer R.[3], Schmael C.[3], Tessmann P.[1,22], Moebus S.[5], Wichmann HE.[6], Schreiber S.[7], Müller-Myhsok B.[8], Lucae S.[8], Muglia P.[9], Jamain S.[10], Leboyer M.[10], Heath S.[11], Kapur-Pojskic L.[12], Oruc L.[13], Rivas F.[14], Mayoral F.[14], Babadjanova G.[15], Grigoroiu-Serbanescu M.[16], Diaconu CC.[17], Czerski PM.[18], Hauser J.[18], Zimmer A.[2], Lathrop M.[19], Schulze TG.[20], Wienker TF.[4], Schumacher J.[1, 23], Maier W.[21], Propping P.[1], Rietschel M.[3], Nöthen MM.[1,22], Cichon S.[1,22]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Institute of Molecular Psychiatry, Bonn, Germany, [3]Department of Genetic Epidemiology in Psychiatry at the Central Institute of Mental Health, Mannheim, Germany, [4]Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany, [5]Institute of Medical Informatics, Biometry and Epidemiology, Essen, Germany, [6]Institute of Epidemiology at the German Research Center for Environmental Health, Neuherberg, Germany, Institute of Epidemiology at the Helmholtz Center Munich, German, Research Center for Environmental Health, Neuherberg, Germany, Institute of Medical Informatics, Biometry, and Epidemiology, Munich, Germany, Klinikum Grosshadern, Munich, Germany, [7]Institute of Clinical Molecular Biology, Kiel, Germany, Department of General Internal Medicine, Kiel, Germany, [8]Max Planck Institute of Psychiatry, Munich, Germany, [9]Neurosciences Centre of Excellence in Drug Discovery at GlaxoSmithKline, Verona, Italy, [10]INSERM U 955 IMRB Psychiatry Genetics, Créteil, France, University of Paris Faculty of Medicine IRF[10], Creteil, France, AP-HP Henri Mondor-Albert Chenevier Group at the Department of Psychiatry, Creteil, France, [11]Centre National de Génotypage, Evry, France, [12]Laboratory for Human Genetics at the INGEB, Sarajevo, Bosnia and Herzegovina, [13]Psychiatric Clinic at the Clinical Center, Sarajevo, Bosnia and Herzegovina, [14]Civil Hospital Carlos Haya, Malaga, Spain, [15]Russian State Medical University, Moscow, Russia, [16]Biometric Psychiatric Genetics Research Unit at the Alexandru Obregia Clinical Psychiatric Hospital, Bucharest, Romania, [17]Stefan S Nicolau Institute of Virology at the Romanian Academy, Bucharest, Romania, [18]Department of Psychiatry, Poznan, Poland, [19]Centre National de Genotypage, Evry, France, [20]Unit on the Genetic Basis of Mood and Anxiety Disorders at the National Institute of Mental Health, Bethesda, USA, [21]Department of Psychiatry, Bonn, Germany, [22]Department of Genomics at the Life and Brain Center, University of Bonn, Germany, [23]Unit on the Genetic Basis of Mood and Anxiety Disorders at the National Institute of Mental Health, Bethesda, USA

Bipolar disorder (BD) is a highly heritable and common neuropsychiatric disease of mood. It is characterized by recurrent episodes of mania and depression which are accompanied by disturbances in thinking and behavior. We conducted a genome-wide association study (GWAS) of BD in a sample of 682 German patients and 1,300 controls using about 500,000 quality-controlled single-nucleotide polymorphisms (SNPs) from Illumina's HumanHap550 BeadArrays. The top association signals from the autosomes and the X-chromosome were tested in six BD samples from different European countries comprising 1,729 patients and 2,313 controls.
A SNP in neurocan (NCAN) on chromosome 19p13.11 was associated with BD across all follow-up samples and showed the most significant replication result (P = 2.31E-4). A combined analysis of the GWAS and the replication samples led to a genome-wide significant P-value of 3.02E-8. To test previous hypotheses of a genetic overlap between BD and schizophrenia (SCZ), we tested this SNP in a SCZ sample of 466 German patients and 866 independent controls. We found association with SCZ for the same risk allele that was discovered in the BD samples. A combined analysis of all BD samples and the SCZ sample increased the P-value to 5.74E-10.
NCAN encodes a chondroitin sulfate proteoglycan of the extracellular matrix supposed to be involved in cell adhesion and migration. To map the gene's spatiotemporal expression, we performed whole mount-in situ hybridizations in embryonic and postnatal wildtype mice. We ob-

served transcript expression patterns in cortical and hippocampal areas co-localized with brain areas in which neuroimaging studies identified abnormalities in BD patients that may be indicative of disturbances in key neuronal circuits.

W1 CANCER GENETICS

W1-01

A novel gene for breast and ovarian cancer.

Meindl A.[1], Hellebrand H.[1], Wiek C.[2], Erven V.[2], Wappenschmidt B.[3], Niederacher D.[2], Mathew CG.[4], Schindler D.[5], Schmutzler RK.[3], Hanenberg H.[2]
[1]Klinikum rechts der Isar, Munich, Germany, [2]Heinrich-Heine University, Düsseldorf, Germany, [3]University Hospital, Cologne, Germany, [4]Kings Colllege, London, URK, [5]Department of Human Genetics at the University, Würzburg, Germany

Germ-line mutations in a number of genes involved in the recombinational repair of DNA double-strand breaks are associated with a predisposition to breast and ovarian cancer. We identified a novel gene which is localized within this pathway and mutated primarily in families presenting with breast and/or ovarian cancer. In index cases from 1100 German families with gynecological malignancies, we identified six monoallelic pathogenic mutations that confer an increased risk for breast and ovarian (BC/OC) cancer. We identified two frameshift-causing insertions, two splice site mutations and two missense alterations, which proved to be non-functional in two independent in vitro assays. These mutations were exclusively found within 480 pedigrees with the occurrence of both tumor entities (1.3%), but not in 620 pedigrees with breast cancer only or in 2912 healthy German controls. In addition, we found a more frequent missense variant (2%) that might confer at least moderate risks for BC/OC families. Clinical and histopathological analyses revealed that the associated carcinomas develop as early as BRCA1-associated carcinomas, but may represent a distinct entity with more favourable prognosis. In summary, the results demonstrate that this gene is a novel tumor suppressor gene and supports the hypothesis that most of the familial cases showing no mutations in BRCA1/2, are linked to yet unknown genes, which are also highly penetrant, but infrequently mutated.

W1-02

Single cell copy number screen of novel tumor suppressor gene candidates in classical Hodgkin lymphoma

Giefing M.[1,4], Zühlke-Jenisch R.[1], Massow A.[1], Gesk S.[1], Klapper W.[2], Vater I.[1], Küppers R.[3], Siebert R.[1]
[1]Institute of Human Genetics Christian-Albrechts University, Kiel, Germany, [2]Department of Pathology Christian-Albrechts University, Kiel, Germany, [3]Institute for Cell Biology University Duisburg-Essen, Essen, Germany, [4]Institute of Human Genetics, Polish Academy of Sciences, 60-479 Poznań, Poland

By analyzing classical Hodgkin lymphoma cell lines with combined high resolution array-CGH and expression profiling we recently identified several novel tumor suppressor gene candidates. In order to further investigate the role of these genes (CD70, CHD2, CYBB, GNG7, SEPT9, TNFSF9, TRAF3) in primary tissue we analyzed a cohort of 21 cryo-sections of lymph-nodes of Hodgkin lymphoma patients on single cell level using the FICTION technique. The FICTION approach combines immunostaining for CD30+ to detect Hodgkin and Reed-Sternberg cells with fluorescent in situ hybridization (FISH) using probes (BAC clones) specific for the analyzed genes loci and a set of commercial centromere probes as a reference. The median signal number of the centromere probes allowed to assess the overall ploidy level of each analyzed sample. Thus, the copy number of the analyzed genes was calculated not only in comparison to the number of centromere signals in a particular tumor cell but also in comparison to the ploidy of the case. For each hybridization 10 to 40 CD30+ tumor cells were evaluated by two independent observers. A deletion was scored if at least 30% of the cells showed lower number of signals for the gene specific probe than for the centromere probe and the ploidy level.

Gene copy number losses in the primary samples were identified with the following frequencies: CYBB 6/18 (33%); CHD2 5/19 (26%); TRAF3 5/20 (25%); GNG7 2/18 (11%); TNFSF9 2/18 (11%); CD70 2/18 (11%); SEPT9 0/17 (0%). No homozygous deletions have been identified.
Highly recurrent losses were detected for the CYBB, CHD2 and TRAF3 genes. CYBB (cytochrome b-245, beta polypeptide) encodes the catalytic subunit of the cytochrome b558 - a part of the NADPH (Nox2) oxydase. Recent findings show Nox2 as a key factor in the intrinsic cell death pathway that involves the release of apoptotic intermediates from mitochondria. CHD2 (chromodomain helicase DNA binding protein 2) controls chromatin condensation and gene expression via the organization modifier and the SNF2 (helicase) domains. Recently, it has been demonstrated that Chd2 deficiency leads to lymphomas in 44% of heterozygous mice. The TNF receptor associated factor ($TRAF_3$) is involved in signal transduction of CD40, a TNFR family member important for the activation of the immune response. This protein is involved in the induction of NFkB and cell death.
Recurrent deletions, together with literature data further support the hypothesis that CYBB, CHD2 and TRAF3 are putative new tumor suppressor genes involved in the pathogenesis of classical Hodgkin lymphoma.

W1-03

Recurrent translocations targeting the IRF4 oncogene identify a novel subtype of mature B-cell lymphoma affecting predominantly children

Salaverria I.[1], Philipp C.[2], Oschlies I.[3], Kohler C.[4], Gesk S.[1], Trautmann H.[5], Szczepanowski M.[3], Spang R.[4], Kreuz M.[6], Burkhardt B.[7], Pott C.[8], Reiter A.[7], Küppers R.[2], Klapper W.[3], Siebert R.[1]
[1]Institute of Human Genetics Christian-Albrechts University, Kiel, Germany, [2]Institute of Cell Biology University of Duisburg-Essen, Essen, Germany, [3]Department of Pathology Christian-Albrechts University, Kiel, Germany, [4]Institute of Functional Genomics University of Regensburg, Regensburg, Germany, [5]Second Medical Department Christian-Albrechts University Kiel, Kiel, Germany, [6]Institute for Medical Informatics Statistics and Epidemiology University of Leipzig, Leipzig, Germany, [7]Department of Pediatric Hematology and Oncology Justus-Liebig University, Giessen, Germany, [8]Second Medical Department Christian-Albrechts University, Kiel, Germany

Within the German Network "Molecular Mechanisms in Malignant Lymphomas" (MMML), we performed systematic FISH-screening followed by long-distance inverse polymerase chain reaction (LDI-PCR)-based molecular characterization of translocations affecting the immunoglobulin heavy chain (IGH) locus in diffuse large B-cell lymphoma (DLBCL). By this approach a t(6;14)(p25;q32) juxtaposing the IRF4 oncogene with the IGH-locus was cloned, which has not yet been described as recurrent aberration in DLBCL. Subsequent FISH analyses of 457 mature B-cell lymphomas from pediatric and adult trials for IRF4 translocations identified 17 cases with IGH-IRF4, 2 cases with IGL-IRF4 and 1 case with IGK-IRF4 fusion. In 2 cases the IRF4 partner remains unidentified. IRF4 break positive cases shared overexpression of MUM1/IRF4 at RNA and protein level. Histologically, they were predominantly DLBCL of GCB-type and follicular lymphoma Grade 3 expressing BCL6. All cases lacked a t(14;18) translocation and/or BCL2 breaks, whereas seven cases presented BCL6 breaks and one case a MYC break. The number of secondary genetic alterations detected by array-CGH in IRF4 positive lymphomas was lower than in other DLBCL, with 11q gains being recurrent. Gene expression profiling using Affymetrix U133A arrays revealed a specific expression signature of the IRF4 break positive cases. Indeed, a combination of 28 probesets/21

genes distinguishes IRF4 break positive cases from the other subtypes of DLBCL. Clinically, the IRF4 break positive cases were associated with lower age at diagnosis and more favorable prognosis than other DLBCL. Our results suggest IRF4 translocations to be the primary genetic alterations in a novel molecularly defined subset of mature B-cell lymphomas predominantly affecting children.

W1-04

IDH1 mutations are detected in 9.3% of all AML and are strongly associated with intermediate risk karyotype and unfavourable prognosis: a study of 999 patients

Schnittger S., Haferlach C., Ulke M., Kaya L., Weiss T., Kern W., Haferlach T.
MLL Münchner Leukämie Labor, München, Germany

Introduction: IDH1 is the gene coding for the soluble isocitrate dehydrogenease 1 (NADP+), which catalyzes the oxidative decaroxylation of isocitrate to 2-oxoglutarate. The gene has been shown to be frequently mutated in high-grade gliomas at residue p.R132, which is located in the substrate binding site of IDH1. So far, several other tumors have been analyzed without detection of the respective mutation (Bleeker et al., Human Mutation 2009). However, recently a next generation sequencing project found IDH1 mutated in 8.5% of AML with normal karyotype (Mardis et al., NEJM, 2009). Aim: To further evaluate the importance of IDH1R132 (IDH1mut) in AML we have analyzed a cohort of 999 comprehensively characterized AML cases.
Methods: The respective base exchange was analysed by a LightCycler based melting curve assay with subsequent sequencing of the positive samples.
Results: The cohort was comprised of 536 male and 463 female patients (median age: 65.9 years; range: 17.1- 93.3 years). 833 had de novo AML (83.4%), 122 AML following MDS (s-AML,12.1%) and 44 AML after previous treatment of different malignancies (t-AML, 4.4%). Karyotype was available in all cases: 681 had a normal karyotype (NK) AML, and 319 had chromosomal aberrations (t(15;17): n=29; inv(16): n=12, t(8;21): n=23, t(11q23): n=10, t(6;9): n=4, inv(3): n=3; -7: n=27, +8: n=29, +13: n=11, -Y: n=4; complex aberrant: n=60, others: n=106). Overall, in 93 pts IDH1 p.R132 mutations were detected (9.3%). Five different amino acid exchanges were observed: R132C (n=49), R132L (n=22), R132 H and G (n=7, each), and R132S (n=5). With respect to history of the patient IDH1mut were found in 80/833 of de novo AML (9.6%), 11/122 (9.0%) of s-AML, and 2/44 (4.5%) of t-AML, respectively (n.s.). More females (57/463, 12.3%) than males (36/536; 6.7%) had IDH1mut (p=0.003). Age was slightly higher in the mutated cases (63.9 vs. 61.9 years, n.s.). No differences were found for WBC count. IDH1mut were distributed differently between karyotypes: in NK 69/681 (10.1%) and in aberrant karyotypes 24/318 (7.5%). However, IDH1 was never mutated in inv(16), t(8;21), t(6;9), t(11q23), inv(3), or in complex aberrant karyotypes (n=112). In 2 of 27 cases (7.4%) with t(15;17) an IDH1 mutation was detected. Thus, the IDH1 mututations clustered in the intermediate risk karyotype group in comparison to the good or poor risk groups (91/771; 11.8% vs 2/134 (1.5%), p<0.001). The cohort was also characterized for several other molecular mutations. FLT3-ITD was present in 22% (212/954), FLT3-TKD in 6.7% (33/496), NPM1 in 35.4% (329/929), NRAS in 14.6% (48/328), MLL-PTD in 6.9% (64/932), CEBPA mutations in 7.4% (48/645) and RUNX1 mutations in 33.0% (99/299) of analysed cases, respectively. IHD1 mutations were found to be more frequent in NPM1 mutated than in NPM1wt cases (41/329; 12.4% vs 48/598; 8.0%, p= 0.019) and in those with MLL-PTD (11/64; 17.2% vs 77/867; 8.9%, p= 0.031). With lower frequencies IDH1mut were also detected together with RUNX1 mutations (n=8/99), CEBPA mutations (n=2/48), NRAS mutations (n=7/48), and FLT3-TKD (n=1/33). IDH1 was similarly distributed between FLT3-ITD mutated and unmutated cases (18/212; 8.5% vs. 72/744; 9.7%). In 22 (23.7%) of all IDH1mut AML no additional mutation was detected, whereas in 48 (51.6%) one additional, in 22 (23.7%) two additional and in one case three additional mutations were found. An unfavourable effect of IDH1mut on event free survival (EFS) was observed in the total group (median: 272 vs. 456 days; p=0.007) as well as in those with intermediate risk karyotype (median: 272 vs. 449 days; p=0.008). A shorter EFS of the IDH1mut was particularly seen in the NPM1wt cohort (median: 244 vs. 375 days; p=0.038) with a strong trend for an independent effect in a multivariate analysis (p=0.089). Conclusions: IDH1 mutations are frequent in AML and are prognostically unfavourable especially in the NPM1wt cohort. IDH1 mutations seem to be a new class of mutation probably complementing with the classical type 1 and type 2 mutations.

W1-05

Germline nonsense mutation and somatic inactivation of SMARCA4/BRG1 in a family with Rhabdoid Tumor Predisposition Syndrome not linked to SMARCB1/INI1

Gesk S.[1], Schneppenheim R.[2], Frühwald M.C.[3], Hasselblatt A.[4], Jeibmann A.[4], Kordes U.[2], Kreuz M.[5], Leuschner I.[6], Martin-Subero J.I.[7], Obser T.[8], Oyen F.[8], Vater I.[7], Siebert R.[7]
[1]Institute of Human Genetics Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein Campus Kiel, Schwanenweg 24 24105 Kiel, Germany, [2]Department of Pediatric Hematology and Oncology University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany, [3]Department of Pediatric Hematology and Oncology University Children's Hospital Muenster Albert-Schweitzer-Strasse 33, 48149 Muenster, Germany, [4]Institute of Neuropathology University Hospital Muenster Domagkstrasse 17, 48149 Muenster, Germany, [5]Institute of Medical Informatics Statistics and Epidemiology, University of Leipzig, Germany, [6]Institute of Paidopathology Pediatric Tumor Registry, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein Campus Kiel, Germany, [7]Institute of Human Genetics Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Campus Kiel Schwanenweg 24, 24105 Kiel Germany, [8]Department of Pediatric Hematology and Oncology University Medical Center Hamburg-Eppendorf Martinistrasse 52, 20246 Hamburg, Germany

Rhabdoid Tumors (RT) of early infancy are highly aggressive tumors with poor prognosis, predominantly presenting in kidney and CNS. Most RT show inactivation of the SMARCB1/INI1 tumor suppressor, a core member of the ATP dependent SWI/SNF chromatin remodeling complex. Familial cases, described as RT Predisposition Syndrome (RTPS), have been linked to heterozygous SMARCB1/INI1 germline mutations. Among 50 patients within the German RT registry, a germline mutation of SMARCB1/INI1 was identified in 10 and was associated with a lower median age at diagnosis and a higher rate of multicentric CNS disease or other synchronous RT. None of the patients with germline SMARCB1/INI1 mutation survived two years or more. Remarkably, in one family two sisters were diagnosed with RT but lacked SMARCB1/INI1 mutations. Moreover, the RT showed INI1-expression by immunohistochemistry and linkage to the SMARCB1/INI1 locus was ruled out. We therefore performed mutation analysis of other members of the SWI/SNF complex in this family and identified the heterozygous germline nonsense mutation c.3565C>T; (p.Arg1189X) in SMARCA4/BRG1, which was also detected in the healthy father. The RT cells showed loss of the wild-type allele which was in agreement with SNP array analysis identifying partial uniparental disomy of the paternal allele in 19p13 including SMARCA4. Immunohistochemistry with an anti-SMARCA4/BRG1 antibody revealed complete absence of detectable SMARCA4 protein in the RTs in this family, whereas the protein was present in all 14 randomly selected atypical teratoid rhabdoid tumors (ATRT) and in a panel of other embryonal tumors. Expression analyses in a lymphoblastoid cell line of one of the patients by RT-PCR and Western blot indicated nonsense-mediated decay of the mutant allele. Transient recombinant over-expression identified the truncated SMARCA4 protein, however at a lower concentration compared to the wild-type protein. These findings indicate SMARCA4 to be a second member of the SWI/SNF complex involved in cancer predisposition.

W1-06

Genomic loss of the putative tumor suppressor gene E2A promotes cutaneous T-cell lymphoma in human

Steininger A.[1], Möbs M.[2], Ullmann R.[1], Kreher S.[3], Anagnostopoulos I.[2], Janz M.[3], Schröck E.[4], Dörken B.[3], Sterry W.[2], Mathas S.[3], Assaf C.[2]
[1]Max Planck Institute for Molecular Genetics, Berlin, Germany, [2]Charité-Universitätsmedizin Berlin, Berlin, Germany, [3]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, [4]Institute for Clinical Genetics, Dresden, Germany

Primary cutaneous T-cell lymphoma (CTCL) is characterized by the presence of neoplastic T cells primarily in the skin. The most common variants of CTCL are mycosis fungoides (MF) and the Sézary syndrome (SS). Whereas in MF malignant T cells are localised mainly in the skin, the more aggressive SS is characterized by tumor cells in the skin and the blood. In a genome wide analysis of DNA copy number changes in leukemic cells from 22 SS patients and skin- tumor samples of 8 MF patients by high resolution array comparative genome hybridization (array CGH) we identified a deletion at 19p13.3 in 13/22 patients with SS, which was not found in any of the patients with MF. The minimal region of overlap at 19p13.3 includes the gene E2A. Deletion of this gene was verified by FISH on highly purified SS leukemic cells in 10 out of 12 cases tested. Concomitant with the genomic loss of E2A, immunohistochemistry demonstrated a reduced protein level in SS tumor cells in the skin samples.
Apart from its essential function for lymphocyte development, E2a has been discussed as a tumor suppressor gene in mice, most likely acting via regulating the expression of Myc and Cdk6. In order to test whether this signaling pathway is relevant also for SS, we transiently transfected an E2A-deficiant Sézary cell line with E2A expression constructs and measured the effect on the levels of CDK6 and MYC, and cellular proliferation.
Our data strongly suggest that loss of 19p13.3 including the E2A locus is centrally involved in SS pathogenesis. We provide experimental evidence that loss of E2A promotes proliferation of SS tumor cells and that it disrupts cell cycle control by a lost suppression of MYC and CDK6.

W2 FUNCTIONAL ANALYSIS I

W2-01

Crx ChIP-Seq analysis reveals an in vivo photoreceptor regulatory network and defines novel candidate genes for inherited retinal diseases

Lawrence K.[1], Karlstetter M.[2], Weigelt K.[3], Seifert M.[4], Benes V.[5], Stoehr H.[2], Weber BHF.[2], Corbo JC.[1], Langmann T.[2]
[1]Department of Pathology and Immunology, St. Louis, USA, [2]Institute of Human Genetics, Regensburg, Germany, [3]Department of Immunology, Rotterdam, The Netherlands, [4]Genomatix GmbH, München, Germany, [5]European Molecular Biology Laboratory, Heidelberg, Germany

Photoreceptor cells of the retina are highly susceptible to mutations in a plethora of distinct genes and thus are associated with a large number of inherited diseases. Consequently, the vast majority of the more than 150 cloned human genes causing retinal diseases are highly or specifically expressed in photoreceptors. The transcription factor, cone-rod homeobox (Crx), is expressed in both rods and cones. Crx controls the transcription of numerous photoreceptor genes including many known retinal disease genes. In order to establish the first genome-wide, in vivo framework of Crx-regulated gene expression in the mouse retina, we have performed Crx chromatin immunoprecipitation coupled to Solexa massive parallel sequencing (ChIP-Seq). Mapping and clustering of over 8 million ChIP-Seq reads identified more than 5.700 replicated Crx-bound regions (CBRs). Hypergeometric distribution analysis showed a strong correlation between assigned CBRs and genes dysregulated in Crx-/- retinas as revealed by gene expression microarray analyses. A large number of novel Crx target genes were identified, including other transcription factors such as Nrl, Nr2e3, Ror?, Mef2c, Otx2 and regulatory miRNAs. The cis-regulatory activity of selected newly identified CBRs was verified by luciferase assays and electroporation of CBR-driven DsRed reporters into live, explanted mouse retinas. In depth analysis of highly CBR-covered genomic regions showed that photoreceptor cis-regulatory regions aggregate in the vicinity of transcription start sites. Moreover, individual Crx sites within CBRs interact and cooperate at defined sequence spacing. Evolutionarily conserved CBRs in highly expressed genes, including the rhodopsin locus, are often multi-partite and act in a combinatorial fashion. We used this information to develop a strategy for the efficient identification of novel retinal disease genes. All conserved mouse CBRs were mapped to the human orthologous genomic regions and an alignment with intervals containing uncloned retinal disease genes was performed. Candidate genes with multiple combinatorial CBRs of high confidence were identified for 17 different retinal disease loci. Taken together, we report the first ChIP-seq analysis for a retinal transcription factor and provide in vivo insight into the organization of photoreceptor transcriptional regulation. This ChIP-seq dataset reveals targets for focused re-sequencing of mapped retinal disease loci and helps to prioritize non-coding regulatory regions of established genes for further screening.

W2-02

Fail-Safe mRNA/microRNA Network Motif is Associated with Down-Regulation of Elastin in Postnatal Aortic Development

Ott CE.[1], Grünhagen J.[1], Jäger M.[1], König J.[2], Schmidt-von Kegler M.[2], Horbelt D.[3], Schwill S.[4], Kallenbach K.[4], Guo G.[1], Manke T.[5], Knaus P.[3], Seemann P.[2], Mundlos S.[1], Robinson PN.[1]
[1]Institute for Medical Genetics - Charité-Universitätsmedizin Berlin, Berlin, Germany, [2]Berlin-Brandenburg Center for Regenerative Therapies - Charité-Universitätsmedizin Berlin, Berlin, Germany, [3]Institute for Biochemistry - Freie Universität Berlin, Berlin, Germany, [4]Department of Cardiac Surgery - University Hospital Heidelberg, Heidelberg, Germany, [5]Max Planck Institute for Molecular Genetics, Berlin, Germany

Elastin production is characteristically turned off in the course of maturation of elastin-rich organs such as the aorta. Although a number of transcriptional and posttranscriptional regulatory mechanisms for elastin expression have been identified, the factors associated with the dramatic down-regulation of elastin expression in the adult aorta have remained largely unknown.
We used expression profiling, cell culture and zebrafish experiments, and a number of bioinformatics analyses in order to develop a comprehensive genomics analysis of elastin silencing in the developing aorta. Expression profiling of mRNA as well as microRNA (miRNA) revealed marked up-regulation of miR-29 family members with commensurate down-regulation of miR-29 target genes such as elastin and other extracellular matrix (ECM) genes. In addition to multiple miRNA regulatory elements (MRE) for miR-29 in elastin's 3' UTR, we identified numerous miR-29 and miR-15 MREs in the coding sequence and demonstrated activity using in vitro reporter assays. All publically available miRNA databases concentrate on MREs in the 3' UTR of target genes, and this is probably one reason why most publications about miRNAs have analyzed only 3' UTR MREs. After our initial results suggested that coding sequence MREs in elastin could be important, we developed our own programs to perform a comprehensive analysis of MREs in the CDS and 3' UTR of the extracellular matrix genes such as elastin and many collagens that are down-regulated in the adult aorta. To further elucidate the role of miR-29 and miR-15 family in developmental processes, we investigated the effect of miRNA precursors on zebrafish embryos. Injection of miR-29 or miR-15 family miRNA precursors led to dose-dependent morphogenesis defects during zebrafish development. Closer inspection 48 hours post fertilization revealed an altered heart morphology with pericardial edema and malformation of the tail fin with reduced blood circulation in the tail artery.

Transforming growth factor-beta (TGF-beta) was found to be active in the neonatal but not in the adult aortic samples on the basis of phospho-SMAD assays, and was shown to downregulate miR-29 expression in aortic smooth muscle cells. On the basis of the fact that TGF-beta up-regulates a number of ECM genes including elastin, that are miR-29 targets, we propose that a coherent mRNA/miRNA feedforward loop may represent a fail-safe mechanism to repress expression of these genes in the mature aorta.

W2-03

ARHGEF7 (Beta-Pix) acts as guanine nucleotide exchange factor for LRRK2

Haebig K.[1], Gloeckner CJ.[2], Riess O.[1], Ueffing M.[2], Biskup S.[1], Bonin M.[1]
[1]Institute of Human Genetics Department of Medical Genetics, University of Tuebingen, Germany, [2]Department of Protein Science Helmholtz-Zentrum München, Munich, Germany

Mutations in the Parkinson's disease (PD) key player LRRK2 (leucine-rich repeat kinase 2) accounts for 5-6% of familial and 1-2% of sporadic PD cases. The multidomain protein LRRK2 exhibits overall low GTPase and kinase activity in vitro. Initial expression analysis of LRRK2 siRNA transfected human dopaminergic cells reveal the prominent upregulation of the guanine nucleotide exchange factor ARHGEF7. The co-immunoprecipitation of LRRK2 and ARHGEF7 in HEK293 as well as SH-SY5Y cells show their interaction in vitro. By means of pulldown assays in whole brain lysate and the co-localisation analysis in differentiated SH-SY5Y cells the in vivo relevance of the interaction is emphasized. The co-immunoprecipitation analyses with different LRRK2 mutants (G2019S, K1906M, R1441C, T1348N) highlight that the GTP hydrolysis impaired R1441C LRRK2 mutation leads to reduced binding to ARHGEF7 and the presence of ARHGEF7 even increases binding of mutant LRRK2 to GTP. We show that ARHGEF7 might act as a guanine nucleotide exchange factor for LRRK2 by using a GTP competition assay. Additionally kinase assays demonstrate that LRRK2 is able to phosphorylate ARHGEF7 in vitro at previously unknown phosphorylation sites. Downstream effects of phosphorylation of ARHGEF7 through LRRK2 could be (i) a feedback control mechanism for LRRK2 activity as well as (ii) an impact of LRRK2 on actin cytoskeleton regulation.

W2-04

The role of matrix metalloproteinases in growth and endochondral ossification

Lausch E.[1], Keppler R.[1], Steinwender C.[1], Unger S.[2], Spranger J.[1], Superti-Furga A.[1], Zabel B.[3]
[1]Zentrum für Kinderheilkunde und Jugendmedizin, Freiburg, Germany, [2]Institut für Humangenetik, Freiburg, Germany, [3]Zentrum für Kinderheilkunde und Jugendmedizin und Institut für Humangenetik, Freiburg, Germany

Matrix metalloproteinases (MMPs)are believed to be key factors in development and homoeostasis of the skeletal tissues. Of those highly expressed in bone, MMP9 and MMP13 appear to be essential for endochondral ossification, bone remodelling, and repair. In mice, both enzymes degrade native collagens and other extracellular matrix (ECM) components in the growth plate, and cleave or release biologically active molecules stored in the ECM, thereby regulating proliferation, differentiation, and apoptosis of different cell types in skeletal development. Targeted ablation of either Mmp9, Mmp13, or both, causes severe alterations of the growth plate which are, however, transient, and only apparent during embryonic development and early postnatal growth. Adult animals have a normal or subtle phenotype with a slight reduction of body length.
Guided by the striking similarity of clinical and radiographic findings in Mmp9 and Mmp13 knock-out mice to a rare human genetic disorder, Metaphyseal Anadysplasia (MANDP, MIM 309645), we here describe mutations in either of the two genes as molecular basis of the disease in familial and sporadic cases. Functional studies resolve the controversial issue of inheritance, as dominant MANDP is associated with heterozygous missense mutations in the prodomain of MMP13 resulting in aberrant autoactivation of the enzyme, while homozygous loss of either MMP9 or MMP13 function causes recessive MANDP.
In dominant MANDP, MMP9 appears to be degraded intracellularly by autoactivated MMP13, thus providing an explanation for the more severe clinical picture. Nevertheless, as adult patients affected by MANDP are of average height and do not show any obvious signs of skeletal pathology, our results suggest that neither MMP9, nor MMP13 are undispensable for skeletal development and homeostasis in man.

W2-05

The putative pyrophosphate channel ANK controls endosome function

Kühnisch J.[1], Seifert W.[2], Mundlos S.[1], Nürnberg P.[2], Kornak U.[1]
[1]Institut für Medizinische Genetik, Charité - Universitätsmedizin Berlin, Germany, [2]Cologne Center for Genomics, Universität zu Köln, Germany

Mutations in the putative pyrosphosphate (PPi) transporter ANK cause mineralization disorders of bone and cartilage such as craniometaphyseal dysplasia (CMD) and chondrocalcinosis (CC). Although a considerable body of genetic evidence supports the importance of ANK in pathological mineralization a detailed analysis of its subcellular distribution and cell biological function has not been performed.
We showed by immunofluorescence staining that endogenous ANK is enriched at the Golgi apparatus in various cell types. Accordingly, sucrose gradient centrifugation of mouse liver homogenates detected Ank in co-localization with markers for early endosomes and the Golgi compartment. Overexpression of ANK in Hela cells confirmed the Golgi localization but also resulted in an accumulation in plasma membrane ruffles. This subcellular distribution suggests an important function of ANK in post-Golgi and endosome protein trafficking. Therefore, endosome function was investigated by uptake experiments in Hela cells after siRNA knock-down of ANK. While a 50% reduction was observed after 10 min of transferrin uptake EGF pulse-chase experiments revealed no delay in uptake, but diminished degradation after 60 min of chase. Moreover, loss of ANK induced perinuclear accumulation of early and recycling endosomes. Primary sequence analysis of ANK predicted a number of Yxx-motifs known to be utilized for adaptor protein complex mediated protein sorting. Upon knockdown of µ1A ANK was dispersed from the Golgi compartment to peripheral vesicles supporting the important role of ANK for endosome function.
This study indicates a role of the putative PPi channel ANK as a regulator of endosomal function. Our results raise the hypothesis that PPi not only plays a role in extracellular mineralization but also in intracellular sorting and that a defect within the endosomal pathway might play a role in ANK-associated human disorders.

W2-06

Missense mutations affecting the N-terminal region of NIPBL almost completely abolish adherin complex formation with the cohesin-associated protein MAU-2

Braunholz D.[1], Albrecht M.[1], Gil-Rodríguez M.C.[1], Gillessen-Kaesbach G.[1], Wilde J.[2], Deardorff M.[2], Kaiser F.J.[1]
[1]Institute of Human Genetics,Lübeck, Germany, [2]Children`s Hospital, Philadelphia, USA

Cornelia de Lange syndrome (CdLS) is a dominantly inherited genetic disorder with multiple organ system and developmental abnormalities. The phenotype is classically characterized by typical facial features, upper limb defects, hirsutism, gastrointestinal system involvement as well as growth and mental retardation. Mutations in three genes encoding

the cohesin associated factor NIPBL or the highly conserved structural components of the cohesin ring SMC1A and SMC3 have been demonstrated in about 60% of the individuals with CdLS.
Although the cohesin complex was originally described and is best known for its role in sister chromatid cohesion, accumulating evidence suggest that cohesin is also involved in DNA repairing mechanisms and in the regulation of gene expression.
Cohesins have been proposed to form a ring structure which embraces sister chromatids. The loading and unloading of cohesin from chromatin is regulated by a heterodimeric protein complex called adherin, consisting of NIPBL which directly binds to MAU-2, the human ortholog of yeast Scc4.
In our study we wanted to characterize the formation of this adherin complex by analyzing the interaction of NIPBL with MAU-2. By the use of various fragments encoding different parts of NIPBL and MAU-2 in yeast-two-hybrid assays, we could narrow down the MAU-2 interacting region to the N-terminal 300 residues of NIPBL and the NIPBL-binding region within the N-terminal part of MAU-2. Furthermore, we analyzed additional small protein fragments of MAU-2 in ß-galactosidase assays to map the most critical region to amino acids 30 to 70.
In a parallel approach the N-terminal encoding region of the NIPBL gene was sequenced in a cohort of about 100 patients with CdLS. By this, six new missense mutations (G15R, S111T, A179T, P192L, L254V and S262A) were identified. In addition to the yeast-two-hybrid experiments we used a mammalian-two-hybrid assay to further characterize the effects of these amino acid substitutions on the interaction of NIPBL with MAU-2. Site-directed PCR-based mutagenesis was performed to integrate all six plus two recently described missense mutations into NIPBL expression constructs. Different mammalian cells lines were transiently transfected, cell extracts generated and dual luciferase reporter assays performed. Whereas six of the eight mutations analyzed do not significantly alter the reporter gene expression, amino acid substitutions G15R and P29Q almost completely abolish the interaction of NIPBL with MAU-2.
While no detailed clinical data was available for patient P29Q, patient G15R is severely mentally retarded, shows a typical CdLS phenotype without any abnormalities of the arms.
Our data show for the first time that specific missense mutations affecting the N-terminal region of NIPBL can almost completely abolish adherin complex formation. Whether these mutations result in an altered cohesion loading or unloading to distinct gene loci which maybe modify the expression of specific genes is currently under investigation.

W3 CLINICAL GENETICS

W3-01
Mutations in SHOC2 cause a homogeneous phenotype out of the neuro-cardio-facial-cutaneous syndrome spectrum

Lorenz S.[1], Bartholdi D.[2], Ceylaner S.[3], Goecke T.O.[4], Korenke G.C.[5], Luckner D.[6], Morlot M.[7], Morlot S.[8], Tartaglia M.[9], Kutsche K.[1], Zenker M.[10], Zenker M.[11]
[1]Institut für Humangenetik, Hamburg, Germany, [2]Institut für Medizinische Genetik, Zürich, Switzerland, [3]Intergen, Ankara, Turkey, [4]Institut für Humangenetik und Anthropologie, Düsseldorf, Germany, [5]Zentrum für Kinder- und Jugendmedizin, Oldenburg, Germany, [6]Universitätsklinik für Kinder- und Jugendheilkunde, Wien, Austria, [7]Endokrinologikum, Hannover, Germany, [8]MVZ wagnerstibbe, Hannover, Germany, [9]Dipartimento di Ematologia Oncologia e Medicina Molecolare, Rome, Italy, [10]Humangenetisches Institut, Erlangen, Germany, [11]Institut für Humangenetik, Magdeburg, Germany

Noonan, cardio-facio-cutaneous (CFC), and Costello syndrome belong to a group of developmental disorders characterized by facial dysmorphism, congenital cardiac defects, reduced postnatal growth, variable cognitive impairment and tumor predisposition. The term neuro-cardio-facial-cutaneous (NCFC) syndrome has recently been proposed for these and related diseases. These mendelian traits are caused by mutations in genes encoding RAS proteins (KRAS, HRAS, and NRAS), downstream transducers (RAF1, BRAF, MEK1 and MEK2) or pathway regulators (PTPN11, SOS1, NF1, and SPRED1). The common biological consequence of mutations associated with this group of disorders is dysregulated signal traffic through the RAS-MAPK pathway. To rationalize further candidate gene approaches to NCFC syndrome gene discovery, the group of Marco Tartaglia used a systems biology approach based on in silico protein network analysis and identified SHOC2 as new disease gene. The recurrent SHOC2 missense mutation c.4A>G (p.S2G) has been detected in individuals with a consistent phenotype, previously termed Noonan-like syndrome with loose anagen hair. We screened exon 1 of SHOC2 in 167 mutation-negative patients out of the NCFC syndrome spectrum and identified 15 individuals with the c.4A>G transition. The patients show a distinctive phenotype with macrocephaly, high forehead, hypertelorism, ptosis, and low-set and posteriorly rotated ears. They have ectodermal anomalies such as dark-pigmented skin and easily pluckable, sparse, thin and slow-growing hair and skeletal abnormalities which include short neck and pectus anomalies. Most of the patients have a congenital heart defect and variable degree of mental retardation. Several of the patients had features suggestive of Costello or CFC syndrome. Based on the characteristic combination of features we anticipate that individuals with a similar phenotype can readily be identified in the future and tested specifically for SHOC2.

W3-02
Clinical characterization of 29 neurofibromatosis type-1 patients with molecularly ascertained 1.4 Mb type-1 NF1 deletions

Kehrer-Sawatzki H.[1], Kluwe L.[2], Roehl A.[1], Bammert S.[1], Mautner V.M.[2]
[1]Institute of Human Genetics, Ulm, Germany, [2]Department of Maxillofacial Surgery, Hamburg, Germany

In 5% of patients with neurofibromatosis type-1 (NF1), large deletions of the NF1 gene region have been observed. Patients with large NF1 deletions have been reported to suffer frequently from a particularly severe manifestations of the disease. However, until now, a comprehensive study of the genotype-phenotype relationship in patients harbouring large NF1 gene deletions of comparable extent, associated with haploinsufficiency of the same set of genes, has not been performed. In this study, we have analysed 29 patients with precisely determined type-1 NF1 deletions of 1.4 Mb, and breakpoints located within the NF1-REPs, to assess comprehensively the clinical phenotype associated with this specific type of deletion. We observed that type-1 NF1 deletions are frequently associated with facial dysmorphic features (noted in 90% of patients), tall stature (46%), large hands and feet (46%), scoliosis (43%), hyperflexibility of joints (72%), significant delay of cognitive development and/or learning disabilities (93%), and mental retardation (IQ<70, 38%). All these features were observed at significantly higher frequencies than in the general NF1 patient population. We also confirmed an increased frequency of MPNSTs (21%) in patients with type-1 NF1 deletions. Even more remarkably, 50% of the adult patients exhibited a very high burden of cutaneous neurofibromas (more than 1000 tumours). Novel clinical features that we observed at increased frequency in patients with type-1 NF1 deletions include pes cavus (noted in 17% of patients), bone cysts (50% of patients), attention deficits (73%), hypotonia (45%) and speech difficulties (48%). Importantly, 76% of the patients harbouring large NF1 deletions developed plexiform neurofibromas, a rate significantly higher than in the general NF1 population. Furthermore, the frequency of subcutaneous neurofibromas (76%) was also found to be significantly increased in patients with type-1 NF1 deletions. The high frequency of subcutaneous or deep-seated neurofibromas associated with type-1 NF1 deletions is important because these neurofibromas are specifically associated with premature mortality in NF1. These novel findings highlight the importance of deletion analysis and diagnosis in NF1 since the severe and complex manifestations as-

sociated with large NF1 deletions (and particularly type-1 deletions), require the comprehensive clinical investigation and early treatment of patients. The high benign tumour load and the consequent increased risk of malignancy noted in deletion patients renders the frequent monitoring of tumour presence and growth by MRI mandatory, since early surgical tumour removal is likely to increase the survival rate of NF1 patients very significantly.

W3-03

A risk is a risk is a risk: On the current biostatistical practise(s) in first trimester screening.

Krawczak M.[1], Fritzer E.[1], Freitag-Wolf S.[1]
[1]Institute of Medical Informatics and Statistics, Kiel, Germany

Considerable heterogeneity exists regarding the algorithms and data bases used for risk calculations in first trimester screening in Germany and elsewhere. While some of the available computer programmes make use of covariates such as maternal weight and smoking status, others even ignore maternal age. This discrepancy is somewhat surprising since clear and generally accepted rules exist in statistical modelling to decide which covariates should be taken into account under which circumstances. Furthermore, the provision of risk figures to pregnant women that sometimes differ by orders of magnitude is unethical. We will reiterate some of the basic principles of risk calculation, including the notion that no posterior risk can be calculated without making assumptions about prior risks, and that a deliberate neglect of available information is usually unlikely to improve the outcome of risk calculations. These issues will be exemplified by three currently proposed approaches to numerical risk assessment, namely those of the FMF UK (http://www.fetalmedicine.com/), the FMF Germany (http://www.fmf-deutschland.info/de/), and the so-called 'advanced first trimester screening' (http://www.firsttrimester.net/).

W3-04

European external quality assessment (EQA) for Constitutional molecular karyotyping: Experiences from the EMQN/CEQA pilot scheme.

Gabriel H.D.[1], Vermeesch J.R.[2], Patton S.J.[3], Elles R.[3], Hasting R.J.[4]
[1]ZMG Osnabrueck, Osnabrueck, Germany, [2]Center for Genetics, Leuven, Belgium, [3]National Genetics Reference Laboratory, Manchester, UK, [4]John Radcliffe Hospital, Oxford, UK

Constitutional molecular karyotyping has become the „gold standard" in detection of chromosomal imbalances in the last years. This new technique offers a fast and powerful method to scan the whole genome for gains and losses of chromosomal material in a resolution up to 1-10kb.
So far, published Best Practice Guidelines are limited. The European Molecular Genetics Quality Network (EMQN) and the Cytogenetics European Quality Assessment scheme (CEQA) are the largest providers for external quality assessment (EQA) schemes in Europe. In 2008/2009 EMQN and CEQA initiated a joint pilot scheme for molecular karyotyping.
Based on a questionnaire 30 labs were selected to participate in the scheme. Criteria for selection were methodology, samples/year, turnaround-time and diagnostic vs. research setting. In brief, 10 labs were selected for each method (BAC-ArrayCGH, Oligo-ArrayCGH, SNP-Array). All labs offer constitutional molecular karyotyping as a diagnostic tool.
10 ugDNA isolated from a cell line (J.Vermeesch, Leuven) was sent out, showing two clinical significant aberrations: a 1.7 Mb subtelomeric deletion at 20p and a 9.1 Mb interstitial duplication at 18p11.32p11.22. A 30 year old male patient with obesity, microgenitalia, no philtrum and mental retardation was presented as a case scenario.
Participating labs were ask to proceed according to their standard methods and to return the results (genotype and interpretation) in their normal report format (in English).
All selected labs returned their report via a web-based interface. For the pilot study no marking criteria were defined. One lab failed to perform testing (Oligo-Array-CGH). Six labs made significant genotyping errors (21%). Interpretation of the results was considered to be of equal importance. In an ideal report the following points should be aimed: Major aspects: 1) genotype-phenotype correlation according to references and knowledge 2) requesting parental samples 3) comments on balanced rearrangement e.g. further testing. Minor aspects: 1) comment on validation procedures 2) recommendation of genetic counselling 3) providing references for the interpretation.
According to these criteria 24 labs (83%) failed to provide an adequate interpretation. Moreover, 7 of these labs (24%) provided no interpretation at all.
Only 3 labs (10%) fulfilled the required criteria (correct genotype and major aspects of the interpretation).
In general, it became clear that many labs have still great problems with the new technique particularly with interpretation of the results. The scheme demonstrated the necessity of significant improvement in the performance of constitutional molecular karyotyping in most labs. The aim of the following regular schemes is to raise standards in constitutional molecular karyotyping in all aspects. Individual, fully interpreted, concise and informative reports should be the standard practice.

W3-05

Clinical Diagnostics in Human Genetics with Semantic Similarity Searches in the Human Phenotype Ontology

Köhler S.[1], Schulz M.H.[2], Krawitz P.[1], Bauer S.[1], Dölken S.[1], Ott C.E.[1], Mundlos C.[3], Horn D.[1], Mundlos S.[1], Robinson P.N.[1]
[1]Charité - Universitätsmedizin Berlin Institut für Medizinische Genetik, Berlin, Germany, [2]Max-Planck-Institut für Molekulargenetik, Berlin, Germany, [3]Allianz Chronischer Seltener Erkrankungen, Berlin, Germany

The differential diagnostic process attempts to identify candidate diseases that best explain a set of clinical features. This process can be complicated by the fact that the features can have varying degrees of specificity, and by the presence of features unrelated to the disease itself. Depending on the experience of the physician and the availability of laboratory tests, clinical abnormalities may be described in greater or lesser detail.
Geneticists have traditionally used programs such as POSSUM or London-Dysmorphology Database (LDDB) to help with the differential diagnosis. Clinicians choose a list of clinical features from these databases that best match the phenotypic features observed in their patients, and the programs return a list of diseases characterized by the features, or which have at least a certain number of them. While these programs are undoubtedly extremely helpful, the programs in many cases return long, unranked lists on the one hand, and do not recognize semantically similar concepts (e.g., muscle atrophy and amyotrophy).
We have adapted semantic similarity metrics to measure phenotypic similarity between queries and hereditary diseases annotated using the Human Phenotype Ontology (HPO, http://www.human-phenotype-ontology.org). We show that our approach outperforms simpler term-matching approaches that do not take the semantic interrelationships between terms into account. The advantage of our approach was greater for queries containing phenotypic "noise" or imprecise clinical descriptions. Our methods provide a ranked list of differential diagnoses, and also provide a cutoff which tells users whether a given combination of clinical features is per se enough to justify any diagnosis. The semantic network defined by the HPO can be used to refine the differential diagnosis by suggesting clinical features that if present best differentiate among the candidate diagnoses. Semantic similarity searches in ontologies represent a novel and useful way of harnessing the semantic structure of human phenotypic abnormalities to help with the differential diagnosis. We have implemented our methods in a freely available web-application for the field of human Mendelian disorders available at http://compbio.charite.de/phenomizer.

W3-06

Clinical and molecular findings on 20 patients with fibrodysplasia ossificans progressiva

Stefanova I.[1], Grünberg C.[2], Gillessen-Kaesbach G.[1]
[1]Institut für Humangenetik, Universität zu Lübeck, Germany, [2]Klinik für Orthopädie und Unfallchirurgie, Allg. Krankenhaus Hagen, Germany

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

We report on the clinical and radiological findings of 20 patients (10 female and 10 male patients at ages between eight months and 38 years) with FOP and on molecular findings of 13 of these patients. Most of the patients show a typical hypoplasia or aplasia and valgus deviation of the first toe (17/20 patients). A common symptom, often leading to the diagnosis are painful swellings on shoulders and face beginning to arise approximately at an age of three years (15/20). Hand anomalies, such as thumb hypoplasia (18/20) and clinodactyly of the fifth finger (15/20) are common. Eight of 20 patients have hypoacusis. Further clinical signs are restricted mobility of the cervical spine and progressing scoliosis, restriction of mobility as well as contractures of other joints as a result of heterotopic ossifications. Some of the patients show typical craniofacial features like hypomimia, sparse eyebrows and hair as well as teeth anomalies.

Molecular investigations were performed on 13/20 patients. Ten patients show the typical mutation R206H in the ACVR1 gene. Two patients with a rather atypical phenotype show different mutations in the ACVR1 gene (G328W and G356D).

We obtained X-rays of the left hand from 7 patients and performed analysis of the metacarpophalangeal profile (MCPP). We suggest that there is a typical pattern in classical FOP, therefore MCPP could be used as an additional diagnostic tool.

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

W4 METHODS

W4-01

Analysis of H19 and Snrpn Methylation Imprints in In Vitro Grown and Vitrified Oocytes via Limiting Dilution Bisulfite Pyrosequencing

El Hajj N.[1], Zechner U.[2], Linke M.[2], May A.[2], Traphoff T.[3], Eichenlaub-Ritter U.[3], Haaf T.[1]
[1]Institute of Human Genetics, Wuerzburg, Germany, [2]Institute of Human Genetics, Mainz, Germany, [3]Institute of Gene Technology/Microbiology, Bielefeld, Germany

Cryopreservation of ovarian tissue is an emerging technology for fertility preservation in chemotherapy patients. Maternal methylation imprints are acquired during oogenesis and oocyte growth. To study the possible effects of cryopreservation by vitrification of follicles on imprint establishment and maintenance during in vitro growth (IVG) of mouse oocytes in follicle culture, we developed multiplex limiting dilution analysis of a few bisulfite treated cells. In brief, pools of 10 germinal vesicle oocytes each were treated with bisulfite, diluted 1:20, and distributed in 20 PCR tubes. Six negative controls were run to exclude DNA contamination. After the first-round multiplex PCR reaction, a second round of nested PCRs with specific primers for either Snrpn or H19 was performed, and methylation levels were determined by pyrosequencing. Methylation reprogramming defects were studied at the level of individual CpG sites (stochastic errors) and the entire allele (imprinting mutations). For Snrpn the rate of stochastic methylation errors in in vivo grown control (IVC) oocytes, and IVG and vitrified oocytes was 3.32%, 3.25%, and 6.14%, repectively. For H19, the error rate was 0%, 1.11%, and 2.36%. Stochastic methylation errors (abnormal methylation of a single CpG site in a larger CpG island) are most likely without functional consequences. Snrpn imprinting mutations (paternal methylation imprints in oocytes) were detected in 2 of 32 alleles and in 1 of 30 alleles from IVG and vitrified oocytes, respectively, whereas all 28 Snrpn alleles from IVC oocytes displayed correct methylation imprints. For H19 we did not find imprinting mutations in IVC and vitrified oocytes (24 and 27 alleles studied, respectively), in contrast, 4 of 24 alleles in IVG oocytes were hypermethylated. Our results suggest that (incomplete) IVG may be associated with a slightly increased rate of imprinting mutations. In contrast, vitrification does not seem to increase the rate of methylation reprogramming defects.

W4-02

Microindel Detection in short read sequence data

Krawitz P.[1], Rödelsperger C.[1], Jäger M.[1], Bauer S.[1], Schweiger M.[2], Robinson P.[1]
[1]Institute for Medical Genetics Charite, Berlin, Germany, [2]Max Planck Institute for Molecular Genetics, Berlin, Germany

To date the genetic loci of fewer than half of all monogenic disorders have been discovered. High throughput sequencing via second generation sequencing techniques of large linked regions and even whole exomes of a small number of affected individuals is a promising strategy for indentifying genes underlying these rare Mendelian disorders. Several recent studies have demonstrated the effectiveness of resequencing and single-nucleotide variant (SNV) detection by deep short-read sequencing platforms. While several reliable algorithms are available for automated SNV detection, the automated detection of microindels in deep short-read data presents a new bioinformatics challenge.

We systematically analyzed how the short-read mapping tools MAQ, Bowtie, BWA, Novoalign and RazerS perform on simulated datasets that contain indels and evaluated how indels affect error rates in SNV detection. We implemented a simple algorithm to compute the equivalent indel region (eir), which can be used to process the alignments produced by the mapping tools in order to perform indel calling. Using simulated data that contains indels, we demonstrate that indel detection works well on short-read data:

The detection rate for microindels (<4 bp) is above 90%. Our study provides insights into systematic errors in SNV detection that is based on ungapped short sequence read alignments. Gapped alignments of short sequence reads can be used to reduce this error and to detect microindels in simulated short-read data. A comparison with microindels automatically identified on the ABI Sanger and Roche 454 platform indicates that microindel detection from short sequence reads identifies both overlapping and distinct indels.

We have recently applied this strategy to the analysis of various datasets generated by capture technologies designed to enrich either a genomic interval as well as a dataset generated using the exome enrichment procedure of Agilent. We will present the results of these experiments and discuss the importance of using state-of-the art algorithms for detection of nucleotide substitutions and small indels in mutation detection in human genetics.

W4-03

A novel resequencing array platform for hereditary retinopathies (RetChip v1.0) – initial results with the Stargardt disease module

Fiebig* B.S.[1], Schulz* H.[1], Fritsche* L.[1], Meier K.[1], Roth H.[1], Stempfl T.[2], Weber B.H.F.[1]
[1]Institut für Humangenetik, Regensburg, Germany, [2]Kompetenzzentrum für Fluoreszente Bioanalytik, Regensburg, Germany

Stargardt disease (STGD) is a frequent hereditary macular dystrophy with juvenile onset. It is predominantly caused by biallelic mutations in the ABCA4 gene and to a lesser extent by mutations in ELOVL4 and CNGB3. Routine genetic testing of ABCA4 is mainly hampered by substantial allelic heterogeneity and the large size of the coding sequence. To overcome these limitations and to provide a platform for fast, reliable and economic DNA testing of patients, we developed a microarray-based resequencing assay that enables parallel analysis of the complete coding regions and exon/intron boundaries of ABCA4, ELOVL4 and CNGB3 (Stargardt disease module). This module, which is part of the so-called RetChip v1.0 array, was validated by analyzing seven STGD patients with known disease-associated mutations in the ABCA4 gene identified by standard Sanger sequencing. This confirmed the ability of the array to reliably identify single base-pair changes. So far, data from 20 Stargardt disease patients are available. Two disease-associated alleles in the ABCA4 gene were detected in 11 patients (55%) and one disease allele was detected in 5 patients (25%) either in the ABCA4 or the CNGB3 gene. No disease-associated sequence variation in either gene was detected in 4 patients (20%).
The Stargardt disease module of the RetChip v1.0 microarray is a valuable tool to detect nucleotide changes with accuracy comparable to the gold standard of Sanger sequencing. It is a prototype for a family of next generation diagnostic tools in ophthalmic genetics, bridging clinical and scientific research.
* these authors contributed equally

W4-04

Mutation screening in 86 known X-linked mental retardation genes by droplet-based multiplex PCR and massive parallel sequencing

Hu H.[1], Wrogemann K.[1,2], Kalscheuer V.[1], Tzschach A.[1], EURO MRX Consortium[3], Richard H.[1], Ropers H.H.[1], Chen W.[1]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany, [2]University of Manitoba, Winnipeg, Canada, [3]Berlin Germany, Nijmegen The Netherlands, Tours France, Paris France, Leuven Belgium)

The recent advance in massive parallel sequencing technology has revolutionized the way of detecting genomic variations in the field of human and medical genetics. However, even with the dramatically improved efficiency, resequencing of the complete human genome in a large cohort of patients is still economically formidable and is sometimes also unnecessary. Thus, isolating a subset of the human genome for targeted resequencing is required and several different strategies have been recently developed to meet these needs. In this study we applied a novel droplet based multiplex PCR method to screen 86 known X-linked mental retardation genes for mutations in 24 unrelated patients. The functionally relevant regions of these genes were sequenced with the Illumina/Solexa platform. High specificity and uniformity of enrichment was achieved. On average, 67.9% unambiguously mapped reads were from amplicons and the sequence depth of 80% targeted bases was approximately 26 fold. Out of 24 patients, 3 known pathogenic mutations were found and another 7 potentially disease-causing mutations were identified, which were subsequently confirmed by Sanger sequencing. Whereas the robust performance of our approach showed that its utility can be extended to the study of other monogenic diseases with high genetic heterogeneity, this study also demonstrates the problems encountered in explaining the sequencing results, which need to be carefully evaluated for large scale mutation screening projects, especially those for diagnostic purposes.

W4-05

The important role of maternal weight as a covariate in first trimester screening: a study on 12804 cases

Sancken U.[1], Kitschmann-Prawitt M.[1], Vogt P.[1], Decker J.[1], Decker J.[2], Bolz H.[1], Bolz H.[3], Bergmann C.[1], Bergmann C.[4]
[1]Bioscientia Center of Human Genetics, Ingelheim, Germany, [2]Medical School of Johannes Gutenberg University, Mainz, Germany, [3]Institute of Human Genetics Cologne, Cologne, Germany, [4]Department of Human Genetics RWTH Aachen University, Aachen, Germany

We resume a discussion that came up during the prenatal diagnosis session at the GfH meeting in Aachen last year. Background: The two main calculation programmes for first trimester aneuploidy screening in Germany are those of the Fetal Medicine Foundation (FMF) UK and of the FMF Germany. The English programme takes into consideration that maternal weight is one of the covariates that influence marker concentrations and hence the results of risk calculation; the German programme does not consider this effect of maternal weight. In order to evaluate the effect of the importance of this inequality we compared screening results being assessed by the use of the two programmes during routine practice. There were 7908 evaluable cases assessed by the programme of the FMF UK (cohort A) and 4896 cases assessed by the programme of the FMF Germany (cohort B). In both cohorts the biochemical marker concentrations of PAPP-A and free ß-hCG were significantly influenced by maternal weight. Due to international standards this bias can be avoided by applying appropriate weight-adjustment formulas. Accordingly, the correlation bias disappeared after converting the concentrations into weight-adjusted MoM (multiples of the median) levels by the English programme. The German programme converts marker concentrations into DoE (degrees of extremeness) instead of MoM levels, however does not adjust them for maternal weight and other covariates. This results in a significant correlation ($p<0{,}001$) between increased maternal weight and the frequency of increased risks assessed for both trisomy 21 and trisomy 13/18. In cohort A no such unfavourable effects occur.
This phenomenon is especially important for the small group of women in cohort B who exceed weights of 100 kg: more than 20 % of them are assigned trisomy 13/18 risks higher than 1:300 which is unrealistically high as compared to the average frequencies of elevated risks for the whole group of the cohort B. In comparison there is no such increase of frequencies of elevated risks in the 100-150 kg interval of cohort A.
In conclusion, we feel that first trimester screening algorithms definitely need to be adjusted for maternal weight.

W5 EVOLUTION / FUNCTIONAL ANALYSIS II

W5-01

Promoter methylation at a candidate locus for language abilities has been conserved in adult cortices of human and non-human primates

Schneider E.[1], Mayer S.[2], El Hajj N.[1], Farcas R.[2], Jensen L.R.[3], Kuss A.[3], Zechner U.[2], Haaf T.[4]
[1]Institute of Human Genetics, Wuerzburg, Germany, [2]Institute of Human Genetics, Mainz, Germany, [3]MPI of Molecular Genetics, Berlin, Germany, [4]Institute of Human Genetics, Würzburg, Germany

The accelerated development of human brain functions, in particular language acquisition is unique in mammalian evolution. Apart from the thoroughly studied CNTNAP2 and FOXP2, genes underlying autism spectrum disorders (ASD) are considered as prime candidates for language abilities. Association studies have linked a cluster of imprinted genes on chromosome 7q31–q32 to ASD, which is characterized by impaired social communication and language. The aim of our study was to compare the promoter methylation and expression patterns of three genes at this candidate locus, MEST, COPG2 and TSGA14, in frontal

cortices of humans and non-human primates. MEST is imprinted in humans and mice, whereas COPG2 and TSGA14 are imprinted in the mouse but not in humans. We have established bisulfite pyrosequencing assays to quantify promoter methylation of MEST, COPG2 and TSGA14 in adult male frontal cortices of 13 humans, 3 chimpanzees, 1 rhesus macaque, 3 baboons and 5 marmosets. We found comparable methylation levels in all analyzed species, namely 42-48% mean methylation of MEST, 0.6-3.3% methylation of COPG2, and 1.2-6.3% methylation of TSGA14. These results demonstrate that both the imprinted status of MEST and the non-imprinted status of COPG2 and TSGA14 are not human-specific, but have been conserved during primate evolution for at least 35-40 million years. Gene expression was analyzed using a Sentrix Human-6 Expression Beadchip and subsequently validated by quantitative real time RT PCR. In contrast to the highly conserved promoter methylation patterns, expression of the three studied genes varied considerably between species. Although it is well known that the relationship between promoter methylation and gene expression is not linear for most genes, our results suggest that methylation patterns are more stable and more highly conserved than gene expression patterns. In this light, comparative methylation analyses may provide a powerful tool for pinpointing evolutionary changes in gene regulation that have contributed to human-specific brain development and function.

W5-02

Evolutionary chromosome rearrangements may be triggered by non-random nuclear neighbourhoods

Neusser M.[1], Grasser F.[2], Cremer T.[2], Müller S.[1]
[1]Institut für Humangenetik Klinikum der Ludwig-Maximilians-Universität, Munich, Germany, [2]Department Biologie II Anthropologie und Humangenetik Ludwig-Maximilians Universität, Munich, Germany

We investigated by 3D-FISH whether non-random nuclear neighbourhoods may have been triggered chromosome rearrangements, focussing on the evolutionary fusion of human chromosome 2 and the reciprocal translocation t(5;17) in the gorilla.
Our results revealed that in great ape fibroblast nuclei the territories of human chromosome 5 and 17 homologs and the two ancestral homologs of human chromosome 2 (2a and 2b) show a higher frequency of touching surfaces than predicted by computer modelling. Despite this, breakpoint flanking BAC clones showed no extraordinary close physical proximity in fibroblast nuclei, although all four loci were oriented toward the nuclear centre and were located exposed at the chromosome territory surface. Both findings can be correlated with the rather gene-dense local genomic environment in which these loci reside.
In stark contrast, when analyzing meiotic cells from Rhesus monkey testis cryo-sections, clearly preferential neigborhoods were found for chromosome 2a and 2b: close proximity of flanking loci (<2µm distance) was frequent in spermatogonia (15%) and in spermatocytes (34%). Moreover, both loci were located in the nuclear periphery. For chromosome 5 and 17 breakpoints, "kissing" was rare, but flanking loci showed central nuclear positioning, again resulting in short inter-chromosomal distances.
In conclusion, the fusion of human chromosome 2 may have been caused by specific side-by-side arrangements during meiosis, while the preferentially central localisation of chromosome 5 and 17 loci may have acted as probabilistic trigger for the evolutionary translocation t(5;17). Hence, for an individual the proximity of gene dense genomic regions in the nuclear interior can result in deleterious chromosome rearrangements, but on an evolutionary scale, reshuffling of gene dense genomic regions may be frequent and advantageous, and can promote evolutionary genomic innovation.

W5-03

Direct SNP halpotyping at the LPA gene locus reveals particular haplotype patterns indicative of recent positive selection in Africans

Schmidt K.[1], Khalifa M.[2], Kraft HG.[1], Utermann G.[1]
[1]Department of Medical Genetics & Molecular and Clinical Pharmacology, Innsbruck, Austria, [2]Faculty of Science at the Al-Azhar University, Cairo, Egypt

The LPA gene (LPA, MIM 152200) harbours a copy number variation (CNV) of a 5.5 kb Kringle IV-2 (KIV-2) repeat, with alleles of 1 to > 40 KIV-2 copies. This results in a size variation of the encoded apolipoprotein(a) (apo(a)), which covalently binds to a LDL like particle and thus forms lipoprotein(a) (Lp(a)). LPA is the major gene controlling Lp(a) levels, which are highly heritable (h^2 app. 0.8). An inverse correlation between apo(a) size and Lp(a) plasma concentrations exists, with the size of the KIV-2 CNV explaining up to 75 % of the total variation in Lp(a) plasma concentrations, which range from < 0.1 mg/dl to > 200 mg/dl. Nevertheless, a large variation of Lp(a) concentrations is observed with apo(a) isoforms of the same size. Mean Lp(a) plasma concentrations are markedly different between world populations, with Africans exhibiting on average two- to threefold higher Lp(a) levels compared to Europeans.
While apo(a)/Lp(a) is established as a risk factor for atherosclerotic diseases, its physiological function is still unknown. LPA is an evolutionary young gene which derived about 40 million years ago from a duplication of the neighbouring plasminogen gene.

In order to elucidate whether the marked differences of the LPA trait between different world populations are due to genetic drift or might reflect selection, we have conducted extensive resequencing at and around the LPA gene in 20 individuals each from Africa (Gabonese), Europe (Austrians), and Asia (Chinese). Analyses were extended for selected regions to three more African populations (Egyptians, South African Bantu, and Khoi San). For all populations, data on the KIV-2 CNV, a pentanucleotide repeat polymorphism (5'PNRP) at the promoter region of LPA, and Lp(a) concentrations were available.
The KIV-2 CNV allowed separating the two LPA alleles in individuals heterozygous for LPA alleles of different CNV size using pulsed field gel electrophoresis and thus direct SNP haplotyping became possible for a fragment spanning app. 320 kb upstream to 168 kb downstream of the LPA gene. This allowed extending the direct haplotype analysis at the regions flanking the LPA gene to assess the length of haplotypes for extended haplotype homozygosity (EHH) testing.
In all African populations, a haplotype was identified which is characterized by mostly short KIV-2 CNV alleles (typically 10 repeats), a nearly exclusively African allele at the 5'PNRP (7 repeats), and several distinctive SNPs exclusively found on this haplotype, all of them derived alleles compared to the chimpanzee sequence. Given the short haplotype structure typical for Africans and also found at the LPA locus, this haplotype extended far beyond the LPA gene by comparison. Haplotype network analysis confirmed the relatively young age of this African haplotype, which was seen at comparatively high frequencies (e.g. 7.5 % in the Gabonese, possibly much higher in the Egyptians), being one of the most frequent haplotypes in the African populations. Hence this haplotype bears all hallmarks of recent positive selection. Curiously when looking at the overall higher Lp(a) concentrations in Africans, this haplotype was consistently found with lower than expected Lp(a) levels given its short KIV-2 CNV size. Whether this phenotypic association is due to a non-synonymous SNP close to a site described to be of importance for Lp(a) particle formation, or to two newly identified SNPs at the LPA promoter region remains to be examined.

W5-04

SPOC1 (PHF13) is a stem cell factor in the testis

Bördlein A.[1], Scherthan H.[2], Molter T.[1], Nelkenbrecher C.[1], Meyer B.[1], Bösl M. R.[3], Birke K.[4], Lütjen-Drecoll E.[4], Kinkley S.[5], Staege H.[5], Will H.[5], Winterpacht A.[1]

[1]Institute of Human Genetics, Erlangen, Germany, [2]Bundeswehr Institute of Radiobiology, Munich, Germany, [3]Max-Planck-Institute for Neurobiology, Martinsried, Germany, [4]Institute of Anatomy II, Erlangen, Germany, [5]Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Hamburg, Germany

We recently identified a novel gene, SPOC1 (PHF13), and demonstrated that expression is negatively associated with survival time in a cohort of ovarian cancer patients. We showed that SPOC1 encodes a protein which in cell lines associates dynamically with chromatin and plays a role in chromosome condensation and cell division, probably by functioning as a reader of epigenetic marks. Here, we report the generation of Spoc1 mutant mice from a gene trap embryonic stem cell clone. The only overt phenotypic abnormality in homozygous Spoc1-/- animals is a pronounced testis hypoplasia with a progressive loss of germ cells from an initially normal germ epithelium resulting in tubuli with a Sertoli-cell-only phenotype. Besides a higher rate of apoptosis no difference in proliferation rate, number of primary gonocytes, or in the initiation and progression of meiosis could be identified. The reported phenotype is remarkably reminiscent of the testis phenotype observed in mice lacking PLZF and NANOS2, major factors for spermatogonial stem cell (SSC) renewal. In contrast to the situation in Plzf-/- or conditional Nanos2 mutant mice, however, we observed no overall loss of spermatogonial stem cells but rather an altered distribution in the tubules. Our data strongly indicate that the Spoc1-/- phenotype is caused by a progression failure of PLZF-expressing, undifferentiated type-A spermatogonia. Thus, SPOC1 represents a novel stem cell factor indispensable for sustained spermatogenesis which is involved in the early steps of spermatogonial stem cell differentiation.

W5-05

A functional link between DYT1 and DYT6 dystonia: Regulation of DYT1 gene expression by the transcription factor activity of THAP1 (DYT6)

Osmanovic A.[1], Braunholz D.[2], Orolicki S.[3], Albrecht M.[2], Gillessen-Kaesbach G.[2], Klein C.[3], Lohmann K.[3], Kaiser F.J.[1]

[1]Institute of Human Genetics,Lübeck,Germany, [2]Institute of Human Genetics,Lübeck, Germany, [3]Section of Clinical and Molecular Neurogenetics,Lübeck, Germany

While dystonia is clinically characterized by involuntary twisting, repetitive movements and abnormal postures, its molecular pathophysiology is not well understood. Mutations in two genes have been associated with monogenic forms of primary (isolated) dystonia: i) mutations in the AAA protein TorsinA cause DYT1 dystonia; ii) mutations in the recently identified THAP1 gene lead to the phenotypically similar DYT6 dystonia. The THAP1-encoded THAP1 protein consists of 213 amino acids and belongs to the family of sequence-specific DNA-binding factors. Apart from the family-designating THAP (Thanatos-associated protein) domain at the N-terminus, responsible for the interaction with DNA, THAP1 contains a proline-rich region, and a putative nuclear localization signal (NLS) towards the C-terminus. Recent studies demonstrated that THAP1 is involved in transcriptional regulation.

To gain insights into the molecular mechanisms explaining the phenotypic overlap of DYT1 and DYT6 dystonia, we characterized the role of THAP1 in transcriptional regulation of DYT1, i. e. we tested whether the transcription factor THAP1 directly regulates DYT1 promoter activity. For this, a fragment of 1 kb representing the predicted DYT1 promoter was amplified and inserted into the pGL4 luciferase reporter plasmid. Co-transfection with full-length wild-type THAP1 expression plasmids strongly increased the DYT1 promoter activity by up to 200% compared to cells transfected with empty expression vector indicating direct regulatory control of DYT1 by THAP1. By analyzing a set of different truncated DYT1 promoter constructs in reporter gene assays, we narrowed the most critical region for promoter activity down to a stretch of about 220 bps 5´ of exon 1.

To confirm that THAP1 binds to the DYT1 promoter with an alternative method, we performed chromatin immuno-precipitation assays (ChIPs) with purified nuclear fractions of human neuroblastoma cells (SH-SY5Y) transfected with THAP1-FLAG. By subsequent PCR, we confirmed that THAP1 specifically binds within the DYT1 promoter region.

Furthermore, site-directed in-vitro mutagenesis was used to generate THAP1 expression constructs with different missense mutations identified in patients with DYT6. Functional analysis of these mutant THAP1 proteins revealed that substitutions of specific amino acids within the THAP domain significantly reduce THAP1 activity on the DYT1 promoter.

Our data clearly show that THAP1 directly regulates the activity of the DYT1 promoter, providing experimental evidence linking the molecular pathways underlying DYT1 and DYT6 dystonia. The function of THAP1 in regulation of gene expression establishes transcriptional dysregulation as a cause of dystonia. Identification of additional target genes will further elucidate the pathophysiology of dystonia.

W6 EPIGENETICS

W6-01

Identification and characterization of differentially methylated regions in three bovine imprinted genes

Hansmann T.[1], Zechner U.[2], Heinzmann J.[3], Niemann H.[3], Haaf T.[1]

[1]Institute of Human Genetics, Würzburg, Germany, [2]Institute of Human Genetics, Mainz, Germany, [3]Institute of Farm Animal Genetics, Mariensee, Germany

Correct imprinting is crucial for normal fetal and placental development in mammals. Accumulating experimental evidence in animal models indicates that different assisted reproductive technologies (ART) can affect the methylation and expression of imprinted genes. Because of legal and ethical restrictions ART-induced epigenetic effects are difficult to study in humans. Bos taurus is an agriculturally important species in which ART is commonly employed. It exhibits a similar preimplantation development and a similar gestational length as humans and therefore becomes increasingly interesting as a model organism for human oocyte and embryo development. Previous expression studies have suggested imprinting of the bovine orthologues of the human H19, SNRPN, and PEG3 genes. In order to study the effects of specific in vitro maturation (IVM) conditions on bovine oocytes and the resulting embryos at the epigenetic level, we have characterized the differentially methylated regions (DMRs) of three imprinted bovine genes, bH19, bSNRPN, bPEG3. First, we performed a thorough in silico sequence analysis of the evolutionary conservation of known human/mouse DMRs in the bovine genome. For bH19 we identified a differentially methylated 300 bp region approximately 6 kb upstream of the bH19 promoter, containing a CpG-island with high similarity to the human sequence. Other DMRs with lower sequence conservation were identified in the adjacent 3 kb. These additional CpG islands lie 6 kb to 3 kp upstream of the promoter and constitute the putative imprinting control region (ICR). In general, the genomic structure of the bH19 ICR was found to be less conserved in cattle than that of the known bPEG3 and bSNRPN DMRs. Both classical bisulfite sequencing and bisulfite pyrosequencing were applied to analyze the methylation patterns of the identified potential DMRs in bovine sperm, parthenogenetic embryos, placenta and three somatic tissues (heart, liver, and kidney). All observed DMR methylation patterns were consistent with imprinting of the three studied bovine genes. In summary, we identified a DMR in the bH19 ICR and confirmed the

imprinted status of the bPEG3 and bSNRPN DMRs. This will allow us to determine the stages in oocyte maturation and embryo development at which critical methylation marks are set. Finally, we will expose bovine oocytes to different IVM systems, fertilize them in vitro, culture these to the blastocyst stage, and determine the effects of these treatments on DNA methylation marks.

W6-02

Alterations in the DNA Methylation Pattern of DSD-Patients with 46,XY Karyotype Caused by Mutations in the Androgen Receptor Gene

Ammerpohl O.[1], Bens S.[1], Richter J.[1], Holterhus P.M.[2], Hiort O.[3], Siebert R.[1]
[1]Institute of Human Genetics, Kiel, Germany, [2]Clinic of Pediatrics UK S-H, Kiel, Germany, [3]Department of Pediatrics UK S-H, Luebeck, Germany

Disorders of sexual development (DSD) are rare syndromes and depending on the specific molecular cause, patients can develop a primarily female, male or intersex phenotype.
Mutations in the androgen receptor gene (AR) might lead, despites a XY constitution of the gonosomes, to either complete androgen insensitivity (CAIS) resulting in a female phenotype or partial androgen insensitivity (PAIS) resulting in a milder phenotype. The normal activation of the androgen receptor establishes a sex specific gene expression pattern causing a male phenotype, which is also reflected by a sex specific DNA methylation pattern. Mutations in the AR would provide the unique opportunity to identify genes which DNA methylation status depend on AR activity.
Using the HumanMethylation27 Bead Chip we have investigated the DNA-methylation status of more than 27,000 CpG loci in 28 primary genital fibroblast cell lines originated from the labia majora of 11 XY-individuals with PAIS and 17 XY-individuals with CAIS. Scrotal fibroblasts, which reflect the corresponding homologous tissue, from 10 XY-males without AR mutation acted as control.
By this approach we identified 49 loci differentially methylated between XY-individuals with AR mutation and XY-males without AR mutation. Additionally, we detected 65 loci showing high variability in their DNA methylation pattern in samples of DSD patients in contrast to a homogenous methylation pattern in controls.
A further comparison of the DNA methylation pattern in whole blood samples derived from 26 XY-AIS individuals with those one of 25 normal donors (XY-males and XX-females) resulted in 199 loci significantly differentially methylated in AIS-individuals and controls. Interestingly, these loci were enriched for genes involved in development and differentiation.
Therefore, we could show for the first time that mutations in a sex hormone receptor interfering with its activity and having a significant impact on the phenotype are also reflected by epigenetic alterations in the methylome of XY-individuals.

W6-03

Ovarian stimulation leads to downregulation of developmentally important reprogramming genes and aberrant imprinted gene methylation in mouse preimplantation embryos

Linke M.[1], May A.[1], ElHajj N.[2], Daser A.[1], Reifenberg K.[3], Haaf T.[2], Zechner U.[1]
[1]Institute of Human Genetics, Mainz, Germany, [2]Institute of Human Genetics, Würzburg, Germany, [3]Central Laboratory Animal Facility, Mainz, Germany

Ovarian stimulation with high dosage of gonadotrophins is a key tool of assisted reproductive technology (ART) to treat human subfertility/infertility. Recent findings have associated ovarian stimulation with the increased incidence of imprinting disorders in humans as well as defects in genome-wide methylation reprogramming and, in particular, imprinting in mice. Here, we set out to determine the impact of ovarian stimulation on the expression of developmentally important reprogramming genes (Apex1, Lig1, Lig3, Mbd2, Mbd3, Mbd4, and Polb) and imprinted gene (Snrpn, H19) methylation in single early mouse morula embryos (16-cell stage). Using absolute quantification of mRNA by real-time RT-PCR, we demonstrate that ovarian stimulation leads to downregulation of mRNAs encoding the base excision repair proteins APEX1 and POLB as well as the 5-methyl-CpG-binding domain protein MBD3 in individual morula embryos. Whole mount immunofluorescence staining of late morula embryos (32-cell stage) with an antibody against APEX1 followed by semiquantitative analysis of the staining intensities also revealed individual embryos with lower protein expression levels after ovarian stimulation and a correlation of mRNA expression with gene activity. Using methylation analysis by multiplex limiting dilution bisulphite genomic pyrosequencing to determine methylation patterns at single allele resolution, we further observed an association of ovarian stimulation with loss of methylation at the maternal Snrpn and the paternal H19 alleles. Our data argue for a negative impact of ovarian stimulation during female gametogenesis as well as early embryo development affecting prezygotic establishment as well as postzygotic maintenance of imprints and mediated by misexpression of reprogramming factors.

W6-04

Promoter methylation of the candidate tumour suppressor gene EFS in uveal melanomas with poor prognosis

Neumann LC.[1], Maßhöfer L.[1], Weinhäusel A.[2], Thomas S.[3], Horsthemke B.[1], Zeschnigk M.[1]
[1]Institut für Humangenetik,Essen,Germany, [2]Austrian Research Centers,Seibersdorf,Austria, [3]Augenklinik der Universität Essen,Essen,Germany

In mammalian genomes cytosine methylation plays an important role in the regulation of gene activity. DNA methylation patterns are established during early embryonic development and maintained throughout adult life. Aberrant methylation patterns are associated with various diseases including cancer. Particularly, hypermethylation of promoter CpG islands can lead to the inactivation of tumour suppressor genes (TSG). In uveal melanoma, which is assumed to develop from melanocytes located in the uveal tract, we identified methylation of the candidate tumour suppressor gene Embryonal Fyn-associated Substrate (EFS). In this tumour loss of an entire chromosome 3 (monosomy 3) is strongly associated with metastatic disease, whereas tumours with disomy 3 rarely give rise to metastases. To evaluate the role of EFS in uveal melanoma initiation and progression we analysed the methylation states of 11 CpG dinucleotides in the promoter CpG island of the EFS in primary uveal melanomas, cultured melanocytes, blood cells and different human tissues. In uveal melanomas with monosomy 3 the EFS CpG island was almost completely methylated. In tumours with disomy 3 and in melanocytes EFS was predominantly unmethylated suggesting that EFS is hypermethylated in tumours with poor prognosis. In most normal human tissues analysed (liver, fibroblasts, buccal swab, kidney, brain and muscle) EFS showed no or only a weak methylation signal whereas almost complete methylation was observed in whole blood. We found a negative correlation between EFS expression and EFS methylation in the tumour samples. Furthermore, reactivation of expression could be induced by 5-aza-deoxycytidine treatment of a uveal melanoma cell line indicating that EFS promoter methylation regulates EFS expression.

W6-05

Functional significance of differential RB1 methylation

Kanber D.[1], Berulava T.[1], Horsthemke B.[1], Lohmann D.[1], Buiting K.[1]
[1]Institut für Humangenetik, Essen, Germany

Recently we have identified a differentially methylated CpG island (CpG 85) in a 5'-truncated, processed pseudogene in intron 2 of the retinoblastoma 1 (RB1) gene. The CpG island is methylated on the maternal chromosome and serves as a weak promoter for an alternative transcript on

the paternal chromosome. Furthermore we observed an allelic expression imbalance of RB1 in favour of the maternal allele. These findings suggest that lack of methylation of CpG 85 and possibly the expression of the alternative transcript interferes with the expression of the regular transcript from the same allele, i.e. paternal allele. To test this hypothesis lymphoblastoid cells (LCs) were treated with 5-aza-2'-deoxycytidine (AzadC), which inhibits the DNA methyltransferase DNMT1. After the treatment methylation of CpG 85 and RB1 expression was analysed. The methylation analysis by methylation-specific PCR (MS-PCR) revealed that all AzadC treated LCs show hypomethylation as expected. The expression analysis by fluorescence-tagged primer extension analysis showed that in all hypomethylated LCs the degree of skewing is reduced. This could be explained by the loss of methylation of the maternal allele which then resembles the paternal allele. We also detected that in two hypermethylated LCs with the highest degree of methylation the degree of skewing is also reduced. This could be due to the gain of methylation of the paternal allele which then resembles the maternal allele. Our findings show that there is a link between allele specific methylation of CpG 85 and allelic expression imbalance of RB1. Methylation analysis of CpG 85 in retinoblastoma tumor samples revealed that in 21 samples with loss of heterozygosity almost all samples were almost or completely methylated, irrespective of the parental origin of the remaining allele. Of 10 samples without loss of heterozygosity all samples were completely methylated except of two samples which showed a slight hypomethylation and differential methylation, respectively. The clinical relevance of aberrant RB1 methylation found in the tumor samples is yet unknown.

W7 COMPLEX DISEASES

W7-01

Genome-wide association study identifies new susceptibility loci for non-syndromic cleft lip with or without cleft palate

Mangold E.[1], Ludwig K.U.[2], Birnbaum S.[1], Baluardo C.[3], Ferrian M.[3], Herms S.[2], Reutter H.[1], de Assis N.A.[1], Al Chawa T.[1], Mattheisen M.[1], Steffens M.[4], Barth S.[2], Kluck N.[2], Paul A.[5], Becker J.[2], Lauster C.[6], Schmidt G.[6], Braumann B.[7], Scheer M.[8], Reich R.H.[9], Hemprich A.[10], Pötzsch S.[11], Blaumeiser B.[12], Moebus S.[13], Krawczak M.[14], Schreiber S.[14], Meitinger T.[15], Wichmann H.-E.[16], Steegers-Theunissen R.P.[17], Kramer F.-J.[18], Cichon S.[2], Propping P.[1], Wienker [1]Institute of Human Genetics – University of Bonn, Bonn, Germany, [2]Department of Genomics – University of Bonn, Bonn, Germany, [3]Medical Genetics Unit – University of Ferrara, Ferrara, Italy, [4]Institute of Medical Biometry / Informatics and Epidemiology – University of Bonn, Bonn, Germany, [5]Department of Otolaryngology – Asklepios Klinik Altona, Hamburg, Germany, [6]Department of Cleft Lip and Cleft Palate Surgery – Humboldt University of Berlin, Berlin, Germany, [7]Department of Orthodontics – University of Cologne, Cologne, Germany, [8]Department of Oral and Maxillo-Facial Surgery – University of Cologne, Cologne, Germany, [9]Department of Oral and Maxillo-Facial-Plastic Surgery – University of Bonn, Bonn, Germany, [10]Department of Oral and Maxillo-Facial Surgery – University of Leipzig, Leipzig, Germany, [11]Monitoring of Congenital Malformations Saxony Anhalt – University of Magdeburg, Magdeburg, Germany, [12]Department of Medical Genetics – University of Antwerp, Antwerp, Belgium, [13]Institute of Medical Informatics / Biometry and Epidemiology – University Duisburg-Essen, Essen, Germany, [14]PopGen Biobank – Christian-Albrechts University Kiel, Kiel, Germany, [15]Department of Human Genetics – Technische Universität München, Munich, Germany, [16]Institute of Epidemiology – Helmholtz Zentrum München, Neuherberg, Germany, [17]University Medical Center, Rotterdam, The Netherlands, [18]Department of Oral and Maxillofacial Surgery – University of Göttingen, Göttingen, Germany, [19]Dental Hospital & School – University of Dundee, Dundee, UK

Non-syndromic orofacial clefts are considered to have a multifactorial etiology with a strong genetic background. The most frequent form is the non-syndromic cleft lip with or without cleft palate (NSCL/P).

We recently reported a key susceptibility locus for NSCL/P at chromosome 8q24.21 that was identified using a genome-wide association (GWA) approach in a relatively small sample of 224 NSCL/P cases and 383 controls (Birnbaum et al. Nature Genet 2009). This locus was only the second susceptibility locus to have been unequivocally identified for NSCL/P to date, the other being the IRF6 locus.

In order to identify further cleft susceptibility loci, we enlarged our sample by genotyping an additional set of 177 NSCL/P cases and adding the genotypes of 940 population-based controls. This resulted in an overall sample of 401 cases and 1,323 controls of Central European origin. Genotyping was performed using Illumina BeadChips (Human610-Quad; HumanHap550k). After excluding markers from the previously described 8q24.21-locus, 20 SNPs with P values below 10-5 remained. For replication in an independent NSCl/P triad sample we selected the 20 top SNPs and added 38 SNPs (back-up markers and SNPs with P values <10-4). Genotyping using MALDI-TOF mass spectrometry was successful for 45 markers in 665 NSCL/P triads of European origin. After combining the GWA and replication samples, genome-wide significant evidence of association was found for three SNPs at two loci (17q22: rs227731, Pcomb = 1.07 ×10-8, and 10q25.3: rs7078160, Pcomb = 1.92 ×10-8, and rs4752028 Pcomb = 2.48 ×10-8). Noggin (NOG) and ventral anterior homeobox 1 (VAX1) are promising candidate genes within these regions. Two further loci (13q31.1, 15q13.3) and a single marker (rs7590268 on 2p21) were replicated, although they fell short of achieving genome-wide significance. The joint population attributable risk (PAR) for the two new loci at 17q22 and 10q25.3, the key susceptibility locus at 8q24.21, and the IRF6 locus estimated from the combined sample was 54.6%.

In summary, we have identified two novel NSCL/P susceptibility loci with genome-wide significance on 17q22 and 10q25.3, and three further loci (13q31.1, 15q13.3 and 2p21) for which there is suggestive evidence (Mangold et al. Nature Genet, in press).

The study was supported by the DFG.

W7-02

Genome wide association study identifies three loci associated with psoriatic arthritis

Hüffmeier U.[1], Uebe S.[1], Ekici A. B.[1], Apel M.[1], Bowes J.[2], Giardina E.[3], Korendowych E.[4], Juneblad K.[5], Behrens F.[6], Traupe H.[7], Wichmann H. E.[8], Lascorz J.[1], Klareskog L.[9], Lohmann J.[10], Fitzgerald O.[11], Alenius G. M.[5], McHugh N J.[4], Novelli G.[3], Burkhardt H.[6], Barton A.[12], Reis A.[1]
[1]Institute of Human Genetics, University of Erlangen-Nuremberg, Germany, [2]Department of Epidemiology, University of Manchester, Great Britain, [3]Department of Biopathology, University of Rome Tor Vergata and Fondazione PTV Policlinico Tor Vergata, Italy, [4]Royal National Hospital for Rheumatic Diseases and Department of Pharmacy and Pharmacology, University of Bath, Great Britain, [5]Department of Public Health and Clinical Medicine/ Rheumatology, University Hospital Umeå, Sweden, [6]Division of Rheumatology at Department of Internal Medicine II, Johann Wolfgang Goethe University Frankfurt/ Main, Germany, [7]Department of Dermatology, University of Münster, Germany, [8]Institute of Epidemiology, Helmholtz Center Munich, Germany, [9]Karolinska Institute, Stockholm, Sweden, [10]Psoriasis rehabilitation hospital, Bad Bentheim, Germany, [11]St. Vincents University Hospital and UCD School of Medicine and Medical Science and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Ireland, [12]Departments of Rheumatology and Epidemiology, University of Manchester, Great Britain

Psoriatic arthritis (PsA) is an inflammatory arthritis mostly accompanied by psoriasis vulgaris and seronegativity for rheumatoid factor. In order to identify further susceptibility genes, we performed a SNP array based genome wide association study in 609 German patients and 990 control individuals. Analysis of 1,585,307 confidentially imputed SNPs revealed three loci of genome-wide significance (p<5*10-8) that were replicated in independent cohorts of ~1800 patients and more than 7000 control individuals of European origin. We confirmed HLA-

C (p = 6.64*10-24, OR = 2.46 [2.06-2.94]) and IL12B (p = 4.27*10-13, OR = 2.51 [1.94-3.24]) as PsA susceptibility genes, and observed several independent signals within the HLA region. The best IL12B variant - located 72kb upstream - was independent from previously reported IL12B-SNPs. Association to several intragenic variants including a missense-variant (1.62*10-9<p<9.91*10-9) in a previously not associated gene revealed this component of the IL-17 receptor signalling pathway as a new susceptibility gene. Interestingly, association to psoriasis vulgaris without joint manifestation was weaker than to PsA indicating that this risk factor plays a greater role in the pathogenesis of the arthritic component. Our study adds new susceptibility variants at known susceptibility loci and identifies a novel pathway for PsA.

W7-03

Dissecting the MHC locus in psoriatic arthritis

Uebe S.[1], Hüffmeier U.[1], Burkhardt H.[2], Apel M.[1], Ekici A.B.[1], Wichmann H.E.[3], Gieger C.[3], Becker T.[4], Steffens M.[4], Herold C.[4], Wienker T.F.[4], Reis A.[1]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute for Clinical Immunology and Rheumatology, Erlangen, Germany, [3]Helmholtz Center, Munich, Germany, [4]IMBIE, Bonn, Germany

Both in psoriasis and psoriatic arthritis (PsA), many studies have shown strong evidence for association within the major histocompatibility complex (MHC) region on chromosome 6q. For type I psoriasis, this is in fact the foremost known risk factor. It has, however, thus far proven difficult to interpret these data because of the complex LD structure and high degree of genetic heterogeneity within this region. In a genome wide association analysis using 603 German PsA patients and 899 population based controls (KORA), we were able to confirm this locus. We observed strong evidence for association with many SNPs within the region with best allelic chi-square p = 6.79E-20 for rs13191343. In order to clarify whether this is due to one or several loci within the same region, we performed a stepwise logistic regression analysis using rs13191343 as covariate in the first step. The most strongly associated SNP from every step was then used as an additional covariate in the next one, until logistic p values for all SNPs on chromosome 6q were above 5.0E-6. Thus, we were able to identify 5 distinct SNPs which, all together, account for most of the observed association in the MHC region. Using a log-linear model as implemented in the program INTERSNP, we then checked for possible interaction within all 10 possible SNP pairs. After correcting for the number of tests performed (n = 10), no evidence for interaction between any of the 5 SNPs could be found. As they are also in no or negligible LD, we conclude that at least five different loci are responsible for the strong association signals for PsA within the MHC region. Interestingly, only one of these overlaps one of 3 recently identified MHC loci for psoriasis vulgaris, suggesting that the remainder are specific to PsA. This adds to the growing body of evidence for an overlapping but distinct genetic basis for skin and joint manifestations of the disease.

W7-04

Coronary Artery Disease Genomewide Replication and Metaanalysis (CARDIoGRAM) Design of a prospective metaanalysis of 14 GWAS

Erdmann J. (Universität zu Lübeck, Lübeck, Germany)

CAD is the leading cause of death in U.S. and Europe. GWAS have uncovered at least 13 common alleles associated with CAD. However, each variant confers a modest effect, and together, the variants explain a small fraction of heritability. These observations suggest that additional loci harboring CAD-associated variants might be discoverable with larger samples and improved statistical power.
To accomplish this, we assembled CARDIoGRAM that pools GWAS data from ADVANCE, CADomics, CHARGE, deCode, GerMIFS I, II and III (KORA), LURIC/AtheroRemo, MedStar/PennCath, MIGen, OHGS, and the WTCCC. In total, the consortium comprises more than 22,000 well-characterized cases with CAD and more than 60,000 controls. In each individual study, genome-wide genotyping was carried out on either Affymetrix or Illumina platforms, and imputation was conducted to generate genotypes for 2.2 million SNPs in each study.
With the aim of conducting a type II meta-analysis using GWA results from the single studies and consortia, SOPs were generated in order to harmonize the QC and data analyses.
Extensive QC was performed both study-wise and centrally so as to provide standardized data formats of high quality. With the assembled sample size, the estimated power to detect modest effects is substantially increased. For instance, even for genome-wide significance, the power is about 80% for an odds ratio of 1.1, provided that the minor allele frequency is at least 10%. Meta-analyses for CAD phenotype as well as for important subgroups including myocardial infarction and early-onset CAD will be carried out. Following the initial evaluations, wet lab replication genotyping of top results will be sought in more than 15,000 additional cases and
15,000 controls. CARDIoGRAM brings together an enormous wealth of GWA studies data on CAD and myocardial infarction, thus representing the largest study to date to uncover the inherited basis for the leading public health problem in the industrialized world. Results of the meta-analysis will be presented during the meeting.

W7-05

Genome-wide survey implicates CNVs in early-onset bipolar disorder

Priebe L.[1,8], Degenhardt F.[1,8], Herms S.[1,8], Mattheisen M.[1,8,9], Nieratschker V.[2], Weingarten M.[1,8], Witt S.[2], Breuer R.[2], Paul T.[2], Alblas M.[3,8], Moebus S.[4], Lathrop M.[5], Leboyer M.[6], Schreiber S.[7], Rietschel M.[2], Nöthen MM.[1,8], Mühleisen TW.[1,8], Cichon S.[1,8]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Central Institute for Mental Health - Division Genetic Epidemiology in Psychiatry, Mannheim, Germany, [3]Human Genetics, Bonn, Germany, [4]Institute for Medical Informatics, Biometry and Epidemiology, Essen, Germany, [5]Centre National de Génotypage, Evry Cedex, France, [6]INSERM U-513 - Faculté de Médecine AP-HP - Albert Chenevier and Henri Mondor Hospitals - Department of Psychiatry, Créteil, France, [7]Institute for Clinical Molecular Biology, Kiel and Department of General Internal Medicine, Kiel, Germany, [8]Department of Genomics at the Life and Brain Center, University of Bonn, Germany, [9]Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany

We used genome-wide single-nucleotide polymorphism (SNP) data to screen for the presence of copy number variants (CNVs) in 882 patients with bipolar disorder (BD) and 872 controls; 290 (33%) of the patients had an early age-at-onset of <=21 years. We systematically filtered for CNVs covering >30 markers and directly affecting at least one RefSeq gene. We tested whether a) the genome-wide burden of CNVs differed between patients and controls and b) the frequency of specific CNVs was different between patients and controls. Burden analysis revealed that the frequency and size of CNVs did not differ significantly between all patients and controls. Separate burden analyses of patients with an age-at-onset <=21 years (AO<=21y) and age-at-onset >21 years (AO>21y), however, showed that the frequency of microduplications was higher in patients with an AO<=21y compared to controls (P=0.00022) and that the average size of singleton microdeletions was larger in AO<=21y patients than in controls (P=0.0074). A search for specific CNVs identified two common variants: a 248 kb microduplication on chromosome 6q27, overrepresented in the AO<=21y subgroup (5.86%) compared to controls (2.52%, P=0.0079), and a 160 kb microduplication on chromosome 10q11, overrepresented in all BD patients (6.01%) compared to controls (3.67%; P=0.035). Moreover, rare microdeletions and –duplications of a shared 224 kb region on 9q34 were associated with BD (0.6% in patients, 0% in controls; P=0.031). Our data suggest that CNVs have an influence on the development of early-onset (<=21 years) BD. Our study adds further support to previous hypotheses that there may be differences in etiology between early-onset and later onset BD patients.

W7-06

Genome-wide association study of PR (PQ) interval in 28,517 individuals identifies nine association signals in ion channels and developmental genes, five of which are also associated with atrial fibrillation

Pfeufer A.[1], van Noord C.[2], Marciante K.D.[3], Arking D.E.[4], Larson M.G.[5], Smith A.V.[6], Tarasov K.V.[7], Müller M.[8], Perz S.[9], Wichmann H.E.[8], Meitinger T.[1], Levy D.[5], Gudnason V.[6], Ellinor P.T.[10], Sanna S.[11], Kääb S.[12], Witteman J.C.M.[2], Alonso A.[13], Benjamin E.J.[5], Heckbert S.R.[3]
[1]TU and HMGU Munich, Munich, D, [2]Erasmus Medical Center, Rotterdam, NL, [3]University of Washington, Seattle, USA, [4]Johns Hopkins University, Baltimore, USA, [5]Boston University, Boston, USA, [6]Icelandic Heart Association, Kopavogur, IS, [7]National Institute on Aging, Baltimore, USA, [8]LMU and HMGU Munich, Munich, D, [9]HMGU Munich, Munich, D, [10]Harvard Medical School, Boston, USA, [11]Istituto Neurphisiologico e Neurofarmacologico, Cagliari, IT, [12]LMU Munich, Munich, D, [13]University of Minnesota, Minneapolis, USA

The authors present this work on behalf of the AGES, ARIC, CHS, Framingham, KORA, Rotterdam and SardiNIA studies and the CHARGE consortium. +++

The electrocardiographic PR interval reflects atrial and atrioventricular nodal conduction, disturbances of which increase risk of atrial fibrillation (AF). To identify underlying common genetic variation, we meta-analyzed genome-wide association results for PR interval from seven community-based studies of European-ancestry individuals in the CHARGE consortium: AGES, ARIC, CHS, FHS, KORA, Rotterdam Study, and SardiNIA (N=28,517). Statistically significant loci (P<5x10-8) were tested for association with AF (N=5,741 cases). We identified nine loci associated with PR interval. At chromosome 3p22.2, we observed two independent associations in voltage gated sodium channel genes SCN10A and SCN5A, while six loci were near cardiac developmental genes CAV1/CAV2, NKX2-5 (CSX1), SOX5, WNT11, MEIS1, and TBX5/TBX3. Another signal was at ARHGAP24, a locus without known relevance to the heart. Five of the nine loci, SCN5A, SCN10A, NKX2-5, CAV1/CAV2, and SOX5, were also associated with AF (P<0.0056). Common genetic variation, particularly in ion channel and developmental genes, contributes significantly to atrial and atrioventricular conduction and to AF risk.

W8 MENTAL REDARDATION

W8-01

CNTNAP2 and NRXN1 are mutated in recessive, severe mental retardation resembling Pitt-Hopkins syndrome and target a common synaptic protein in Drosophila

Zweier C.[1], de Jong E.[2], Zweier M.[1], Orrico A.[3], Ousager L.[4], Collins A.[5], Bijlsma E.[6], Oortveld M.[2], Ekici A.[1], Reis A.[1], Schenck A.[2], Rauch A.[7]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Department of Human Genetics, Nijmegen, The Netherlands, [3]Unita Operativa Medicina Molecolare, Siena, Italy, [4]Department of Clinical Genetics, Odense, Denmark, [5]Wessex Clinical Genetics Service, Southampton, UK, [6]Department of Clinical Genetics, Leiden, The Netherlands, [7]Institute of Medical Genetics, Zurich-Schwerzenbach, Switzerland

Heterozygous copy number variations and single nucleotide polymorphisms of CNTNAP2 and NRXN1, two distantly related members of the neurexin superfamily, have been repeatedly reported in association with developmental language disorders, autism spectrum disorder, epilepsy, and schizophrenia, thus pointing to a shared molecular basis underlying different neuropsychiatric disorders. We now identified homozygous and compound heterozygous deletions and mutations via molecular karyotyping and mutational screening in CNTNAP2 and NRXN1 in four patients with severe mental retardation and variable features such as epilepsy and breathing anomalies, phenotypically overlapping with Pitt-Hopkins syndrome. With a frequency of at least 1% in our cohort of 179 patients, recessive defects in CNTNAP2 appear to significantly contribute to severe mental retardation. In contrast to the presynaptic adhesion protein NRXN1, evidence for a synaptic function of CNTNAP2 was lacking so far. Using Drosophila as a model we now demonstrate that, as was known for Nrx-I, also the CNTNAP2 ortholog Nrx-IV localizes to synapses and can reorganize them by influencing density of active zones, the synaptic domains of neurotransmitter release. Moreover, we show that Nrx-I and Nrx-IV converge on a common molecular target, the presynaptic protein bruchpilot. Thus, similar phenotypical aspects resulting from defects in both genes in humans may result from an analogous shared synaptic mechanism.

W8-02

The Golgi matrix protein COH1 regulates endosomal trafficking

Seifert W.[1], Kühnisch J.[2], Maritzen T.[3], Haucke V.[3], Horn D.[2], Hennies H.C.[1]
[1]Cologne Center for Genomics, Universität zu Köln, Germany, [2]Institut für Medizinische Genetik, Charité - Universitätsmedizin Berlin, Germany, [3]Institut für Chemie und Biochemie, Freie Universität Berlin, Germany

So far, COH1 is a protein of unknown function that is assumed to play a role in regulating TGN to endosome sorting of membrane proteins because of its homology to yeast Vps13p. Mutations in COH1 cause the autosomal recessive Cohen syndrome, mainly characterized by mental retardation, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. For functional analysis of COH1 we cloned the ubiquitously expressed COH1 transcript, encoding a large protein of 3997 residues. Immunofluorescence analysis revealed that both overexpressed and endogenous COH1 localized to the Golgi apparatus. Upon Brefeldin A treatment, COH1 acted as Golgi matrix protein, showing a high degree of co-localization with cis-Golgi markers at ER exit sites. Consistently, depletion of COH1 using RNAi induced fragmentation of the Golgi ribbon into swollen mini-stacks. In addition, the microtubule and actin cytoskeleton appeared disorganized. Regarding its homology to yeast Vps13p, we found that depletion of COH1 in mammalian cells alters endosomal trafficking of epidermal growth factor receptor, resulting in prolonged activation of a downstream signaling cascade. Moreover, we found a protein-protein interaction supporting the hypothesis that COH1 serves as scaffold at the Golgi-to-endosome boundary. Although a phenotype/genotype correlation has not been established, it is very likely that observations made upon knock-down of COH1 resemble the situation in Cohen syndrome patients as most COH1 mutations represent a loss of function due to premature protein truncation and/or mRNA non-sense mediated decay. Further work is required to elucidate the pathomechanism of Cohen syndrome.

W8-03

Deep sequencing leads to the identification of 3 independent mutations affecting the ST3GAL3 gene in patients with autosomal recessive mental retardation from 3 consanguineous Iranian families

Kuss A. W.[1], Hu H.[1], Garshasbi M.[1], Kahrizi K.[2], Bahman I.[2], Mohseni M.[2], Motazacker M. M.[1], Abbasi-Moheb L.[1], Esmaeeli-Nieh S.[1], Wrogemann K.[3], Ghadami S.[2], Puettmann L.[1], Soltani-Banavandi M. J.[2], Darvish H.[2], Jamali P.[4], Nikoui P.[5], Soleimani F.[2], Tzschach A.[1], Chen W.[1], Ropers H. H.[1], Najmabadi H.[2]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany, [2]Genetics Research Center of the University of Social Welfare and Rehabilitation Sc, Tehran, Iran, [3]Department of Biochemistry & Medical Genetics of the University of Manitoba, Winnipeg, Canada, [4]Shahroud Welfare Institution, Shahroud, Iran, [5]Bandar Abbas Welfare Institution, Bandar Abbas, Iran

The large majority of mutations causing mental retardation (MR) is thought to affect autosomal genes, yet to date, only six genes including the recently described TRAPPC9 gene (Mir et al., Am J Hum Genet. 2009, in press), are known to be involved in non-syndromic autosomal recessive MR (NS-ARMR). By autozygosity mapping in 78 families

NS-ARMR we have shown that this disorder is extremely heterogeneous (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8). Indeed, allelic NS-ARMR causing mutations have so far only been found in a single gene (Garshasbi et al., 2008, Am. J. Hum. Genet. 82(5):1158-64; Molinari et al., 2008, Am J Hum Genet. 82(5):1158-64). In the course of an ongoing large-scale investigation into the molecular causes of ARMR, we have identified four consanguineous families, three with non-syndromic and one with syndromic ARMR, where autozygosity mapping yielded single overlapping linkage intervals on chromosome 1p34. It was thus tempting to speculate that the underlying gene defect involves the same locus, MRT4 (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8), which might then potentially account for several percent of the ARMR causing mutations in the Iranian population. We therefore used Chromosome sorting to enrich Chr1 from patient lymphoblastoid cell lines followed by next generation sequencing for searching the underlying gene defect in two of the four families. This revealed two different missense mutations affecting the ST3GAL3 gene. Subsequent screening of this gene in the two remaining families revealed an additional sequence change with a putatively damaging splicing effect. All three changes cosegregate with the disease and were not found in more than 300 control chromosomes. This supports our original assumption that ST3GAL3 might be the first gene with a considerably increased mutation frequency in ARMR. ST3GAL3 encodes a sialyl transferase, which catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates. We are presently investigating the impact of the mutations on ST3GAL3 protein function in order to better understand the pathogenesis of MR in these patients.

W8-04

Two independent mutations in the ZC3H14 gene are associated with non-syndromic autosomal recessive mental retardation

Garshasbi M.[1], Kahrizi K.[2], Tzschach A.[1], Leung S.[3], Behjati F.[2], Falah M.[4], Ghasemi-Firouzabadi S.[2], Esmaeeli-Nieh S.[1], Goswami C.[1], Corbett A.[3], Ropers H. H.[1], Najmabadi H.[2], Kuss A. W.[1]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany, [2]Genetics Research Center University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, [3]Emory Universtiy, Atlanta GA, USA, [4]Tehran Welfare Institution, Tehran, Iran

Autosomal recessive mental retardation (ARMR) is extremely heterogeneous, and there is reason to believe that the number of underlying gene defects goes into the thousands. To date, however, only six genes have been implicated in non-syndromic ARMR (NS-ARMR): PRSS12 (neurotrypsin), CRBN (cereblon), CC2D1A, GRIK2, TUSC3 and the recently found TRAPPC9 (Mir et al. Am J Hum Genet. 2009, in press). As part of an ongoing systematic study aiming to identify ARMR genes, we investigated a consanguineous family comprising three patients with NS-ARMR. Genome-wide SNP typing enabled us to map the relevant genetic defect to a 4.56 Mbp interval on chromosome 14. This interval encompasses a total of 26 genes, which were screened by Sanger sequencing for exonic and splice site mutations. A R154X nonsense mutation was found in ZC3H14, a recently described CCCH-type zinc finger gene (Leung et al., Gene 439:71, 2009). This sequence variant was not seen in 188 Iranian controls. Mutation screening in another NS-ARMR family with three affected sibs and a linkage interval encompassing ZC3H14 revealed a homozygous 25 bp deletion 16 bp downstream of exon 16. Again, this variant was absent in healthy Iranian controls (n=188). Database screening revealed that the genomic segment harbouring this deletion is transcribed. This was confirmed by RT-PCR, also indicating that this transcript is part of the ZC3H14 gene and suggesting that the 25 bp deletion is a functionally relevant second mutation.
ZC3H14 has four protein isoforms that bind specifically to polyadenosine RNA via their common Cys3His zinc fingers, and it is thought to modulate post-transcriptional gene expression. By Western blotting and immunostaining we have shown that in cell lines of patients with the R154X mutation, which affects isoforms with the nuclear localization signal, three large ZC3H14 isoforms are indeed absent from the nucleus. Thus, ZC3H14 defects may give rise to NS-ARMR by compromising mRNA binding and processing. Gene expression profiling and CLIP (cross-linking and immunoprecipitation) followed by next generation sequencing are underway to identify specific polyA-RNA targets of ZC3H14 and to shed more light on its function.

W8-05

Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a relatively frequent cause of severe mental retardation and diminish MECP2 expression

Zweier M.[1], Gregor A.[1], Zweier C.[1], Engels H.[2], Sticht H.[3], Wohlleber E.[2], Bijlsma E. K.[4], Holder S. E.[5], Zenker M.[1], Rossier E.[6], Grasshoff U.[6], Johnson D. S.[7], Robertson L.[7], Firth H. V.[8], Ekici A. B.[1], Reis A.[1], Rauch A.[9]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute of Human Genetics, Bonn, Germany, [3]Institute of Biochemistry, Erlangen, Germany, [4]Department of Clinical Genetics, Leiden, The Netherlands, [5]North West Thames Regional Genetics Service, Harrow, UK, [6]Institute of Human Genetics, Tuebingen, Germany, [7]Sheffield Children's Hospital, Sheffield, UK, [8]Department of Medical Genetics, Cambridge, UK, [9]Institute of Medical Genetics, Zurich-Schwerzenbach, Switzerland

The etiology of mental retardation remains elusive in the majority of cases. Microdeletions within chromosomal bands 5q14.3q15 were recently identified as a recurrent cause of severe mental retardation, epilepsy, muscular hypotonia, variable brain and minor other anomalies. By molecular karyotyping we identified two novel 2.4 and 1.5 Mb microdeletions of this region in patients with a similar phenotype. Both deletions contained the MEF2C gene, which is located proximally to the previously defined smallest region of overlap. Nevertheless, due to its known role in neurogenesis, we considered MEF2C as a phenocritical candidate gene for the phenotype associated with 5q14.3q15 microdeletions. We therefore performed mutational analysis in 362 patients with severe mental retardation of unknown etiology. Within this cohort we found 2 truncating and 2 missense de novo mutations in MEF2C which establishes defects in this transcription factor as a novel relatively frequent autosomal dominant cause of severe mental retardation accounting for as much as 1.1% of patients. By using a transcriptional reporter assay we show that MEF2C mutations diminish synergistic transactivation of E-box promotors including that of MECP2, for which we found diminished expression by RT-PCR in vivo. We therefore hypothesize that the phenotypic overlap of patients with MEF2C mutations and atypical Rett syndrome is due to the involvement of a common pathway.

W8-06

CSMD3 – a candidate gene for autosomal dominant mental retardation and epilepsy

Fritsch A.[1], van Maldergem L.[2], Tönnies H.[3], Kutsche K.[1], Rosenberger G.[1]
[1]Institute for Human Genetics, Hamburg, Germany, [2]Centre de Génétique Humaine, Liège, Belgium, [3]Institute for Human Genetics, Kiel, Germany

Epilepsy is one of the most common secondary disabilities in patients with mental retardation, the prevalence increasing with the severity of the intellectual disability. We report on a female patient with severe mental retardation and seizures carrying an apparently balanced chromosome translocation [46,XX,t(2;8)(q13;q23.3)]. Array CGH analysis did not reveal any genetic imbalance. Mapping of the breakpoint regions by fluorescence in situ hybridization identified BAC clones overlapping the two breakpoints. The breakpoint in 2q13 directly disrupts the ACOXL gene encoding a protein similar to acyl-Coenzyme A oxidase with a proposed function in fatty acid metabolism. The breakpoint in 8q23.3 interrupts the CSMD3 gene (CUB and Sushi multiple domain 1), a giant gene composed of 73 exons and encoding a putative transmembrane protein

which is predominantly expressed in adult and fetal brain. Recently, a female patient with tricho-rhino-phalangeal syndrome type II, short stature, mental retardation, and an interstitial deletion in 8q23 covering the genes TRPS1 and CSMD3 has been described [1]. More importantly, in two unrelated male patients with de novo translocations and a common breakpoint in 8q23 of ~5 Mb one breakpoint was found to map downstream and the other upstream of CSMD3. Both patients had an autistic disorder and showed developmental delay. In addition, one patient presented with epilepsy. Thus, removal of cis-regulatory elements required for spatially, temporally, and quantitatively correct activity of CSMD3 might have caused the phenotype in the two translocation patients [2]. Taken together, these data suggest that heterozygous mutations affecting long-range regulatory elements of CSMD3 or the gene itself might be associated with mental retardation and epilepsy.

[1] Riedl S. et al., Am J Med Genet 131 (2), 200-203 (2004).
[2] Floris C. et al., Eur J Hum Genet 16 (6), 696-704 (2008).

W9 NEUROGENETICS

W9-01
SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy

Riessland M.[1], Ackermann B.[1], Förster A.[1], Jakubik M.[1], Hauke J.[1], Garbes L.[1], Fritzsche I.[2], Mende Y.[1], Blümcke I.[2], Hahnen E.[1], Wirth B.[1]
[1]Institute of Human Genetics and Center for Molecular Medicine Cologne, Cologne, Germany, [2]Department of Neuropathology University of Erlangen, Erlangen, Germany

Proximal spinal muscular atrophy (SMA) is a common autosomal recessively inherited human disease characterized by degeneration of alpha-motor neurons within the spinal cord that results in muscle weakness and atrophy. SMA is caused by functional loss of the survival motor neuron gene 1 (SMN1), whereas disease severity is mainly influenced by the number of SMN2 copies. SMN2, which produces only low levels of full-length mRNA/protein, can be modulated by small molecules and drugs, thus offering a unique possibility for SMA therapy.
Here, we analysed suberoylanilide hydroxamic acid (SAHA), a FDA-approved histone deacetylase inhibitor, as a potential drug in two severe SMA mouse models each carrying two SMN2 transgenes: US-SMA mice with one SMN2 per allele (Smn-/-;SMN2tg/tg) and Taiwanese-SMA mice with two SMN2 per allele (Smn-/-;SMN2tg/wt), both on pure FVB/N background. The US-SMA mice were embryonically lethal with heterozygous males showing significantly reduced fertility. SAHA-treatment of pregnant mothers rescued the embryonic lethality giving rise to SMA offspring. Crossbreeding of Smn-/-;SMN2tg/tg x Smn-/+ mice produced 50% Taiwanese-SMA offspring with a mean age of survival of 9.9 days. Treatment with 25 mg/kg/2x/day SAHA increased lifespan of SMA mice by 30%, significantly improved motor function abilities, reduced degeneration of motor neurons within the spinal cord and increased the size of neuromuscular junctions and muscle fibers compared to vehicle-treated SMA mice. SMN RNA and protein levels were significantly elevated in various tissues including spinal cord and muscle. Hence, SAHA, which lessens the progression of SMA, might be suitable for SMA therapy.

W9-02
Cellular mechanisms resulting in hereditary spastic paraplegia type SPG31

Beetz C.[1], Pamminger T.[1], Khundadze M.[1], Kurth I.[2], Mumtaz R.[1], Hübner A.K.[1], Hennings J.C.[1], Irintchev A.[3], Deufel T.[1], Hübner C.A.[1]
[1]Institute of Clinical Chemistry, Jena, Germany, [2]Inst. f. Humangenetik; Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany, [3]Clinic of otorhinolaryngology, Jena, Germany

Hereditary spastic paraplegia (HSP) is characterized by a progressive spastic gait disorder that is caused by degeneration of upper motor neuron axons. HSP is a genetically very heterogeneous disorder following either autosomal dominant, autosomal recessive, or X-linked inheritance. Among the autosomal dominant subtypes, SPG31 is a more common finding and is caused by loss-of-function mutations in REEP1, a gene of largely unknown function so far. To characterize the role of REEP1 in the pathogenesis of HSP, we generated a Reep1-knockout mouse model. Knockout mice show a progressive, dose-dependent gait disorder which is consistent with the presence of spastic paraplegia. In situ hybridisation of mouse sections of various developmental stages revealed early expression of Reep1, which was restricted to the nervous system. For subcellular localization studies we subsequently established an antibody specific for Reep1. By subcellular fractionation and immunohistochemistry we could show that endogenous Reep1 co-localizes with PDI, a marker of the endosplasmic reticulum (ER). As atlastin, another protein involved in the pathogenesis of HSP, has recently been shown to localize to the ER, we tested whether both proteins interact. Here we show that Reep1 indeed co-immunoprecipitates and co-localizes with atlastin. We hence propose that Reep1 like atlastin contributes to the biogenesis of the ER. This hypothesis is currently tested by ultra-structural analysis of brain tissue and primary neuronal cultures from wildtype and Reep1-knockout mice.

W9-03
Massively parallel sequencing of ataxia genes after array-based enrichment

Hoischen A.[1], Gilissen C.[1], Neveling K.[1], Arts P.[1], Wieskamp N.[1], van der Vliet W.[1], Vermeer S.[1], Steehouwer M.[1], de Vries P.[1], Meijer R.[1], Seiqueros J.[2], Knoers N.V.A.M.[1], Buckley M.F.[1], Scheffer H.[1], Veltman J.A.[1]
[1]Department of Human Genetics Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, [2]UnIGENe CGPP IBMC ICBAS University Porto, Porto, Portugal

Massively parallel sequencing has tremendous diagnostic potential for the simultaneous analysis of many disease genes but requires new ‚front-end' methods to enrich templates prior to sequencing. Here we report on the validation of an array-based sequence capture method for medical resequencing approaches in heterogeneous genetic disorders. As a model disease we chose autosomal recessive ataxia and selected 5 subjects with 7 known pathogenic mutations as well as 2 unaffected controls. Genomic sequences of seven disease genes, including intronic sequences, 5kb of genomic sequence up- and downstream of each gene, as well as two control loci were targeted on a single oligonucleotide array, comprising 2Mb of genomic sequence in total. After enrichment each of the patients' DNA samples was analyzed by one quarter of a Roche GS FLX Titanium sequencing run, resulting in an average of 65 Mb sequence data per patient. This was sufficient to reach an average 25-fold coverage per base in all targeted regions. Enrichment showed high specificity, on average 80% of all uniquely mapped reads located within or adjacent to the targeted regions. Importantly, this approach enabled detection of known deletions and hetero- and homozygous point mutations in 6 out of 7 mutant alleles, as well as more than 99% accuracy for known SNP variants. Our results also clearly show reduced coverage for sequences in repeat rich regions, which significantly impacts reliable detection of genomic variants. We conclude that massive parallel sequencing of enriched samples enables tailor-made diagnosis of

heterogeneous genetic disorders and qualifies for rapid implementation. To facilitate this diagnostic potential, we're now implementing a multiplex approach, in order to allow analysis of 100 samples for up to 1Mb targeted sequences.

W9-04

Genome-wide association- and replication study suggests HOMER1 in the aetiology of major depressive disorder

Degenhardt F.[1,11], Mattheisen M.[1,3,11], Frank J.[2], Treutlein J.[2], Breuer R.[2], Steffens M.[3], Herms S.[1,11], Wichmann HE.[4], Schreiber S.[5], Jöckel KH.[6], Strohmaier J.[2], Roeske D.[7], Gross M.[8], Hoefels S.[8], Lucae S.[7], Binder EB.[7], Wienker TF.[3], Schulze TG.[9], Schmäl C.[2], Zimmer A.[10], Bettecken T.[7], Müller-Myhsok B.[7], Maier W.[8], Nöthen MM.[1,11], Rietschel M.[2], Cichon S.[1,11]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Department of Genetic Epidemiology in Psychiatry at the Central Institute of Mental Health, Mannheim, Germany, [3]IMBIE, Bonn, Germany, [4]Institute of Epidemiology at the Helmholtz Center Munich, German, Research Center for Environmental Health, Neuherberg, Germany, Institute of Medical Informatics, Biometry, and Epidemiology, Munich, Germany, Klinikum Grosshadern, Munich, Germany, [5]Institute for Clinical Molecular Biology, Kiel and Department of General Internal Medicine, Kiel, Germany, [6]IMBIE, Essen, Germany, [7]Max Planck Institute of Psychiatry, Munich, Germany, [8]Department of Psychiatry, Bonn, Germany, [9]NIMH, Bethesda, USA, [10]Institute of Molecular Psychiatry, Bonn, Germany, [11]Department of Genomics at the Life and Brain Center, University of Bonn, Germany,

Major depressive disorder (MDD) is a common neuropsychiatric disorder with considerable heritability. In order to identify common genetic risk factors for MDD, we preformed a genome-wide association study (GWAS) in 604 patients with a DSM-IV diagnosis of MDD and 1364 population based controls. All individuals were of self reported German ancestry and were either genotyped on Illumina's Human Hap550 or human610-quad arrays, sharing a consensus set of ~550,000 SNPs. Following statistical analysis for single marker association with MDD, the top hundred SNPs were followed up in a replication sample of 409 patients and 541 controls. One SNP, located in the CD5L gene which is connected to the regulation of the immune system, achieved genome-wide significant association (p=6.18E-8) in the GWAS but this finding was not replicated. Two SNPs showed nominally significant association in both the GWAS and the replication samples: (i) SNPA (p-combined=4.66E-6), located in an intergenic region, and (ii) SNPB (p-combined=7.28E-7), located in a putative regulatory region of the gene HOMER1, which showed gene-wide association in both the GWAS (p<8.94E-4) and the replication (p<4.57E-4) study. Variation in this gene has previously been related to schizophrenia and Homer1 knock-out mice show behavioural traits that are characteristic of depression and schizophrenia. Furthermore, transcriptional variants of Homer1 result in dysregulation of cortical-limbic circuitry. Our results, combined with evidence from animal studies, provide evidence for an involvement of HOMER1 in the aetiology of major depression, and give a hint that genetic variation impacts on depression via dysregulation of cognitive and motivational processes.

W9-05

Common and rare variants in the schizophrenia-associated microdeletion region on 1q21.1

Forstner AJ.[1,6], Basmanav FB.[1,6], Priebe L.[1,6], Mattheisen M.[1,5,6], Freudenberg J.[2], Nieratschker V.[3], Breuer R.[3], Moebus S.[4], Rietschel M.[3], Nöthen MM.[1,6], Mühleisen TW.[1,6], Cichon S.[1,6]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Center for Genomics and Human Genetics at Feinstein Institute for Medical Research, New York, USA, [3]Department of Genetic Epidemiology in Psychiatry at Central Institute of Mental Health, Mannheim, Germany, [4]Institute for Medical Informatics Biometry and Epidemiology, Essen, Germany, [5]Institute of Medical Biometry Informatics and Epidemiology, Bonn, Germany, [6]Department of Genomics at Life and Brain Center, Bonn, Germany

We and others have recently identified rare microdeletions on chromosome 1q21.1 as the strongest genetic risk factor for schizophrenia known to date (odds ratio ~15). In the present study, we aimed to explore to what extent common and rare susceptibility variants in the region spanned by the microdeletion might contribute to the disease allele spectrum.
In a larger sample of 1,169 patients and 3,714 controls we analyzed common single nucleotide polymorphisms (SNPs) using Illumina HH550 data. The most strongly associated SNP (p = 0.008899) was located within an intergenic region and did not withstand a permutation-based correction for multiple testing.
To identify potential rare coding variants, we applied a systematic exon-based re-sequencing approach covering seven RefSeq genes. Approximately 25 kb of sequence information per individual were generated from 96 DSMIV-diagnosed schizophrenia patients (derived from parent-offspring trios) and 96 sex-matched controls. Among the patients, two 1q21 microdeletion carriers were included in order to investigate a potential unmasking of recessive alleles. Re-sequencing has been completed and provides some evidence for an over-representation of previously unknown exonic single base exchanges in patients (n= 17 in 96 patients and n= 8 in 96 controls, MAF = 1 %). Twelve of these rare variants detected in patients represent non-synonymous amino acid changes with a predicted effect on protein structure, one is a stop mutation. Statistical evaluation is underway and will be presented.
We hypothesize that such rare and potentially functional alterations may contribute to the schizophrenia disease allele spectrum at this particular locus. All rare variants identified in the patients are currently being tested for Mendelian inheritance in order to investigate the existence of de novo events. An extension of the re-sequencing samples to increase power to detect such rare variants is envisaged for the future.

AJ.Forstner and FB.Basmanav contributed equally to this work.

W9-06

Analysis of common and rare variants in the schizophrenia susceptibility gene NRXN1

Basmanav FB.[1,4], Forstner AJ.[1,4], Priebe L.[1,4], Mattheisen M.[1,4,5], Nieratschker V.[2], Breuer R.[2], Moebus S.[3], Rietschel M.[2], Nöthen MM.[1,4], Mühleisen TW.[1,4], Cichon S.[1,4]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Department of Genetic Epidemiology in Psychiatry at Central Institute of Mental Health, Mannheim, Germany, [3]Institute for Medical Informatics, Biometry and Epidemiology, Essen, Germany, [4]Department of Genomics at Life and Brain Center, University of Bonn, Germany, [5]Institute for Medical Biometry, Informatics and Epidemiology, Bonn, Germany

Schizophrenia is a genetically complex neuropsychiatric disorder with a lifetime prevalence of approximately 1%. A recent study reported a statistically significant over-representation of exon-disrupting copy number variants (CNVs) of neurexin 1 (NRXN1) on chromosome 2p16.3 in schizophrenia patients. This result strongly supported previous observations of NRXN1 microdeletions in a small number schizophrenia

patients, reported by other two studies. Apart from these genetic findings, neurexins are particularly interesting functional candidate genes. They represent alternatively spliced cell-adhesion molecules located at presynapses which interact with postsynaptic neuroligins. This receptor-ligand interaction probably helps to organize protein networks at excitatory and inhibitory synapses.
In the current study, we aimed to investigate to what extent genetic variation other than structural variants, namely common and rare single base exchanges in NRXN1 might also confer susceptibility to schizophrenia. To analyze the possible contribution of common single nucleotide polymorphisms (SNPs), we analyzed a large German-Dutch sample of 1,169 patients and 3,714 controls using data from Illumina's HumanHap550 BeadArrays. Overall, 291 SNPs with a minor allele frequency (MAF) >3% were tested. After permutation-based correction for multiple testing, there was no evidence for association of any of these common SNPs with schizophrenia.
In order to investigate the possible contribution of rare coding single-base substitutions, we performed an exon- and splice site-targeted re-sequencing of the NRXN1 isoforms alpha1, alpha2 and beta using the Sanger Method. We re-sequenced target fragments that covered 11.5 kb per individual in 96 schizophrenia patients (most of them selected for early age-at-onset and positive family history of schizophrenia or other psychiatric disorders) and 96 sex-matched controls.
We filtered for rare variants with minor allele frequencies up to 3%. We observed an over-representation of rare variants in patients of 16 : 9, most of which were novel, i.e. not known to databases of genetic variation. When we subsequently filtered for novel non-synonymous rare variants only, we observed a slight over-representation in patients of 3 : 1. The observed over-representations of rare variants in patients did not reach statistical significance, probably due to limited power of the small sample. There is a clear need to extend re-sequencing to larger numbers of patients and controls and to test the possible functional consequences of the identified rare non-synonymous variants.
In summary, our results do not provide evidence for an involvement of common SNPs at the NRXN1 locus to schizophrenia. We do observe a statistically non-significant over-representation of rare coding variants in schizophrenia patients compared to controls. Although the power of our study is still limited, our results tentatively suggest that besides the rare exon-disrupting CNVs that have been reported to be associated with schizophrenia, other rare coding variants may contribute to the spectrum of susceptibility variants at the NRXN1 locus.
FB.Basmanav and AJ.Forstner contributed equally to this work.

W10 MOLECULAR BASIS OF DISEASE

W10-01
Mutations in MBTPS2 (S2P) – a protease indispensable for cholesterol homeostasis and ER stress response – cause IFAP syndrome

Oeffner F.[1], Happle R.[2], König A.[2], Betz RC.[3], Bornholdt D.[1], Neidel U.[1], Redler S.[3], Grzeschik KH.[1]
[1]Zentrum für Humangenetik, Marburg, Germany, [2]Klinik für Dermatologie, Marburg, Germany, [3]Institut für Humangenetik, Bonn, Germany

IFAP syndrome (Ichthyosis Follicularis with Atrichia and Photophobia) is a rare X-linked oculocutaneous disease characterized by the clinical triad of follicular ichthyosis, photophobia and –most striking – total or subtotal atrichia in patients. Secondary features include neurological symptoms such as psychomotor retardation and brain anomalies, nail dystrophy, atopic reactions, herniation, aganglionic megacolon and renal, vertebral or testicular disturbancies. Only males show the full phenotype, whereas female carriers may present with linear skin lesions following the lines of Blaschko.
We present evidence that IFAP syndrome is caused by functional deficiency of MBTPS2 (Membrane Bound Transcription Factor Protease, Site 2) – a Golgi membrane embedded zinc metalloprotease that activates signaling proteins involved in cholesterol homeostasis and ER stress response. Functional studies enable us to characterize missense mutations as well as splice site anomalies and other intron mutations. These include histochemical analyses and luciferase reporter assays of cells transiently transfected with MBTPS2 wildtype and mutant expression plasmids, as well as complementation experiments to analyze the growth of stably transfected M19 cells in sterol deficient media. The experiments presented suggest that the severity of the clinical phenotype is correlated to the functional activity of the enzyme, and that at least a residual activity of MBTPS2 is essential for survival, which might explain the lack of knockout mouse models for this enzyme. This is the first distinct human phenotype that has been successfully associated with functional deficiency of MBTPS2. It provides new prospects for studying the role of this important protease essential for development and survival.

W10-02
New insights into pathomechanisms in the chromosome instability disorder Nijmegen breakage syndrome

Krenzlin H.[1], Salewsky B.[1], Melchers A.[1], Demuth I.[1], Digweed M.[1]
[1]Institut für Humangenetik Charité, Berlin, Germany

As an integral member of the MRE11/ RAD50/ NBN (MRN) complex, nibrin plays an essential role in processing DNA double strand breaks (DSB). Hypomorphic mutations of the NBN gene result in truncated nibrin fragments which give rise to the autosomal- recessive Nijmegen breakage syndrome (NBS). In contrast, null mutant mice are not viable. NBS is mainly characterized by chromosomal instability, immunodeficiency, growth retardation and increased cancer susceptibility.
Recent studies indicated that disturbances in redox homeostasis due to impaired DSB processing might result in elevated levels of reactive oxygen species. Clearly this could contribute to DNA damage, chromosomal instability and cancer occurrence. In order to test this hypothesis we exploited murine fibroblasts previously isolated from conditional null mutant mice. Deletion of the loxP- flanked exon 6 in these cells (Nbnins-6/lox-6) can be achieved via incubation with Cre recombinase fusion protein. Furthermore we have examined several fibroblast and lymphoblastoid NBS patient cell lines in order to determine the impact of truncated p70 nibrin on maintaining cellular redox homeostasis. We used the radiomimetic drug Bleomycin to generate DSB and determined the cellular ROS burden at different time points after induction via specific in vitro fluorescence staining and FACS analysis.
In these experiments, null mutant cells demonstrated highly elevated intracellular ROS levels in comparison to their wild type counterparts 12 hours post DSB-induction. Furthermore we have been able to demonstrate that fibroblast- and lymphoblastoid cell lines derived from patients carrying the hypomorphic p70 nibrin fragment have similarly disturbed redox homeostasis. DSB repair involves poly- ADP- ribosylation (PAR) at the break site via PAR- polymerase utilizing NAD as a substrate. Western blots have indicated that the same patient cells show increased and prolonged poly adenosine diphosphate ribosylation compared to their wild type counter parts. Thus NAD depletion could be one of the possible mechanisms leading to decreased ROS scavenging.
In conclusion we have been able to detect elevated levels of reactive oxygen species in murine conditional knock out cells as well as in human patient cells carrying a hypomorphic NBN mutation. We suggest that the DSB repair deficiency in NBS patient cells leads to extreme poly-ADP ribosylation and depletion of the NAD pool. Loss of the anti-oxidant NAD(P)H then results in increased cellular ROS levels. This secondary mutagenic consequence of a primary DSB repair failure probably contributes to the extremely high cancer incidence in NBS.

W10-03

Heterozygous NTF4 mutations impairing neurotrophin-4 signaling in patients with primary open angle glaucoma

Pasutto F.[1], Matsumoto T.[2], Mardin C.Y.[3], Sticht H.[4], Brandstätter J.H.[5], Michels-Rautenstrauss K.[6], Weisschuh N.[7], Gramer E.[8], Ramdas W.D.[9], van Koolwijk L.M.E.[10], Klaver C.C.W.[9], Vingerling J.R.[9], Weber B.H.F.[11], Kruse F.E.[3], Rautenstrauss B.[12], Barde Y.A.[2], Reis A.[1]

[1]Institute of Human Genetics, Erlangen, Germany, [2]Biozentrum, Basel, Switzerland, [3]Department of Ophthalmology, Erlangen, Germany, [4]Institute of Biochemistry, Erlangen, Germany, [5]Animal Physiology, Erlangen, Germany, [6]Institute of Human Genetics, Erlangen, Germany, [7]Molecular Genetics Laboratory, Tübingen, Germany, [8]University Eye Hospital, Würzburg, Germany, [9]Department of Ophthalmology, Rotterdam, The Netherlands, [10]Department of Epidemiology, Rotterdam, The Netherlands, [11]Institute of Human Genetics, Regensburg, Germany, [12]MGZ-Medical Genetics Centre, Munich, Germany

Glaucoma, a main cause of blindness in the developed world, is characterized by progressive degeneration of retinal ganglion cells (RGCs) resulting in irreversible loss of vision. While members of the neurotrophin gene family in various species are known to support the survival of numerous neuronal populations, including RGCs, it is less clear whether they are also required for survival and maintenance of adult neurons in humans. Here we report seven different heterozygous mutations in Neurotrophin-4 (NTF4) gene accounting for about 1,7% of primary open angle glaucoma patients of European origin. Molecular modeling predicted a decreased affinity of neurotrophin 4 protein (NT-4) mutants with its specific tyrosine kinase receptor B (TrkB). Expression of recombinant NT-4 carrying the most frequent mutation was demonstrated to lead to a decreased activation of TrkB. These findings suggest a novel pathway in the pathophysiology of glaucoma trough loss of neurotrophic function and may eventually open the possibility to use ligands activating TrkB to prevent the progression of the disease.

W10-04

Mutations in PDE6C encoding the catalytic subunit of the cone phosphodiesterase in patients with Rod Monochromacy and in the murine cpfl1 mutant

Wissinger B.[1], Chang B.[2], Grau T.[1], Dangel S.[1], Hurd R.[2], Jurklies B.[3], Sener C.[4], Andreasson S.[5], Dollfus H.[6], Kohl S.[1], Heckenlively J.[7]

[1]Centre for Ophthalmology-University Clinics, Tübingen, Germany, [2]The Jackson Laboratories, Bar Harbor, USA, [3]University Eye Hospital, Essen, Germany, [4]University Eye Hospital, Ankara, Turkey, [5]University Eye Hospital, Lund, Sweden, [6]Hopitaux Universitaires de Strasbourg, Strasbourg, France, [7]Kellogg Eye Center, Ann Arbor, USA

The cpfl1 mouse mutant was discovered during a screening study for visual function phenotypes at Jackson Lab. It is characterized by the absence of light-adapted cone response while the dark-adapted rod response is essentially normal. Histological investigations revealed a rapid and selective loss of cone photoreceptors in the first few weeks of life. We found that cpfl1 is inherited as an autosomal recessive trait and performed a genome-wide linkage analysis that eventually localized cpfl1 to an 0.7 cM interval on mouse chromosome 19, including the Pde6c locus encoding the catalytic subunit of the cone photoreceptor cGMP phosphodiesterase. Comparative cDNA analysis of the Pde6c gene revealed a 116bp-insertion between exons 4 and 5, and in addition an 1bp-deletion in exon 7 in the cpfl1 mutant. Both mutations result in frame-shifts introducing premature termination codons. Comparative genomic sequencing of intron 4 of the Pde6c gene revealed that the 116bp cDNA-insertion in cpfl1 is part of a larger 1522bp insertion at the genomic level including sequences of the murine diaphanous gene locus.

The lack of photopic ERG responses in the cpfl1 mutant is reminiscent of achromatopsia in humans. Using marker segregation analysis we found that 2 of 4 yet unsolved achromatopsia families showed concordant segregation with the PDE6C locus. Screening of the PDE6C gene by sequencing all coding exons in these two families revealed compound heterozygous mutations in both families: c.481-12T>A and c.1483-2A>G in one family, and c.1805A>T p.His602Leu and c.2368G>A p.Glu790Lys in the other. Among 24 additional singleton patients, we identified one further patient with two heterozygous nonsense mutations, c.826C>T p.Arg276X and c.2457T>A p.Tyr819X, and another patient with a homozygous 1bp insertion, c.1682_83insA p.Tyr561X in PDE6C. All mutations showed consistent segregation in the respective families. Applying minigene constructs in a heterologous expression system we could demonstrate that c.1483-2A>G as well as the c.481-12T>A mutations in fact induce splicing defects. We also tested whether the p.Glu790Lys as well as the p.His602Leu substitution affects the catalytic activity of the enzyme. We expressed both mutants as PDE5/PDE6 chimeric proteins in Sf9 cells and assayed purified proteins for cGMP hydrolyses activity. While the p.His602Leu mutant display only baseline activity not significantly different from the untransfected control, we found that the p.Glu790Lys mutant still had residual catalytic activity that reaches about 40% of wildtype enzyme activity.

In summary, our study provides convincing evidence that mutations in PDE6C cause autosomal recessively inherited achromatopsia. In addition we showed that the murine cpfl1 mutant is a homologous animal model for PDE6C-associated achromatopsia featuring complete lack of cone function and rapid and selective degeneration cone photoreceptors.

This study was funded in parts by grants of the Deutsche Forschungsgemeinschaft (Wi1189/6-3 and KFO134-Ko2176/1-1)

W10-05

Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11

Hennies HC.[1], Rohr J.[2], Koch F.[3], Seifert W.[1], Grieve S.[4], Pagel J.[3], Strauß J.[3], Kasper B.[5], Nürnberg G.[1], Maul-Pavicic A.[2], Beutel K.[3], Janka G.[3], Griffiths G.[4], Ehl S.[2], Zur Stadt U.[3]

[1]Cologne Center for Genomics, Köln, Germany, [2]Centre of Chronic Immunodeficiency, Freiburg, Germany, [3]Research Institute Children's Cancer Center, Hamburg, Germany, [4]Cambridge Institute for Medical Research, Cambridge, UK, [5]Department of Immunology and Cell Biology, Borstel, Germany

Rapid intracellular transport and secretion of cytotoxic granules through the immunological synapse require a balanced interaction of several proteins. Disturbance of this highly regulated process underlies familial hemophagocytic lymphohistiocytosis (FHL), a genetically heterogeneous autosomal recessive disorder characterized by a severe hyperinflammatory phenotype. Here we have assigned a novel FHL type, FHL-5, to a 1 Mb region on chromosome 19p using high resolution SNP genotyping in eight unrelated FHL patients from consanguineous families. Subsequently we found nine different mutations, either truncating or missense, in STXBP2 in twelve patients from Turkey, Saudi Arabia, and Central Europe. STXBP2 encodes syntaxin binding protein 2 (Munc18-2) involved in the regulation of vesicle transport to the plasma membrane. We have identified syntaxin 11, a SNARE protein mutated in FHL-4, as an interaction partner of STXBP2. This interaction is eliminated by the missense mutations found in our FHL-5 patients, which leads to a decreased stability of both proteins as shown in patient lymphocytes. Activity of natural killer and cytotoxic T cells was markedly reduced or absent as determined by CD107 degranulation. Our findings thus identify a key role for STXBP2 in lytic granule exocytosis.

W10-06

Mutations in PYCR1 Cause Cutis Laxa with Progeroid Features due to Reduced Mitochondrial Stress Resistance

Reversade B.[1], Escande-Beillard N.[1], Dimopoulou A.[2], Fischer B.[2], Li Y.[3], Kayserili H.[4], Al-Gazali L.[5], Brancati F.[6], Lee H.[7], O'Connor B.[7], Schmidt-von Kegler M.[8], Merriman B.[7], Masri A.[9], Guerra D.[10], Ferrari P.[11], Nanda A.[12], Rajab A.[13], Grix A.[14], Sommer A.[15], Svarirayan R.[16], Janecke A.[17], Steichen E.[18], Sillence D.[19], Haußer I.[20], Budde B.[21], Nürnberg P.[21], Seemann P.[22], Zambruno G.[23], Dallapiccola B.[6], Schülke M.[24], Robertson S.[25], Hamamy H.[26], Wollnik B.[3], Van Maldergem L.[27], Mundlos S.[2], Kornak U.[2]
[1]Institute of Medical Biology, Singapore, Singapore, [2]Institut für Medizinische Genetik, Berlin, Germany, [3]Center for Molecular Medicine Cologne, Cologne, Germany, [4]Medical Genetics Department Istanbul Medical Faculty, Istanbul, Turkey, [5]Department of Paediatrics Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates, [6]CSS-Mendel Institute, Rome, Italy, [7]Department of Human Genetics David Geffen School of Medicine, Los Angeles, USA, [8]Max-Planck-Institut für Molekulare Genetik, Berlin, Germany, [9]Departments of Pediatrics Obstetrics and Gynecology Faculty of Medicine, Amman, Jordan, [10]Department of Biomedical Sciences, Modena, Italy, [11]Department of Pediatrics, Modena, Italy, [12]As'ad Al-Hamad Dermatology Center, Al-Sabah Hospital, Kuwait, [13]Genetic Unit DGHA, Muscat, Sultanate of Oman, [14]The Permanente Medical Group, Sacramento, USA, [15]The Ohio State University College of Medicine and Nationwide Children's Hospital, Columbus, USA, [16]Royal Children's Hospital and Genetic Health Services Victoria, Parkville, Australia, [17]Division of Clinical Genetics Innsbruck Medical University, Innsbruck, Austria, [18]Klinik für Kinder- und Jugendheilkunde, Innsbruck, Austria, [19]Department of Clinical Genetics The Children's Hospital at Westmead, Sydney, Australia, [20]Universitaets-Hautklinik Heidelberg, Heidelberg, Germany, [21]Cologne Center for Genomics, Köln, Germany, [22]Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany, [23]Laboratory of Molecular and Cell Biology IDI-IRCCS, Rome, Italy, [24]Department of Neuropediatrics and NeuroCure Clinical Research Center, Berlin, Germany, [25]Department of Paediatrics and Child Health, Dunedin, New Zealand, [26]National Center for Diabetes Endocrinology and Genetics, Amman, Jordan, [27]Centre de génétique humaine CHU du Sart-Tilman, Liège, Belgium

Autosomal recessive cutis laxa (ARCL) describes a group of syndromal disorders that are often associated with a progeroid appearance, lax and wrinkled skin, osteopenia, and mental retardation. Homozygosity mapping in several kindreds with ARCL identified a candidate region on chromosome 17q25. By high-throuput sequencing of the entire candidate region we detected disease-causing mutations in the gene PYCR1 encoding pyrroline-5-carboxylate reductase, an enzyme involved in proline biosynthesis. Subsequent screening revealed mutations in patients with cutis laxa which were categorized clinically as wrinkly skin syndrome (WSS; MIM 278250), gerodermia osteodysplastica (GO; MIM 231070), or de Barsy syndrome (DBS; MIM 219150). Serum proline levels were not significantly reduced in patients. All mutations entailed reduction of protein expression to a variable degree. We found that the gene product localizes to mitochondria. Altered mitochondrial morphology, membrane potential and increased apoptosis rate upon oxidative stress were evident in patient fibroblasts. Knockdown of the orthologous gene and a highly identical paralog in zebrafish led to epidermal abnormalities that were accompanied by a massive increase of apoptosis. Thus, local production of proline in mitochondria seems to be important for cellular stress resistance, especially in connective tissues, resulting in premature cell loss and progeroid changes.

W11 DEVELOPMENTAL GENETICS

W11-01

More than patterning - HOXD13 defines bone types of the distal limb skeleton

Villavicencio-Lorini P.[1], Kuss P.[2], Friedrich J.[2], Haupt J.[3], Farooq M.[4], Duboule D.[5], Hecht J.[3], Mundlos S.[1]
[1]Institute of Medical Genetics, Charité Berlin, Germany, [2]Max Planck Institute for Molecular Genetics, Berlin, Germany, [3]Berlin-Brandenburg Center for Regenerative Therapies, BCRT Charité Berlin, Germany, [4]National Institute for Biotechnology & Genetic Engineering NIBGE, Faisalabad, Pakistan, [5]National Research Centre Frontiers in Genetics, University of Geneva, Switzerland

HOX genes play an important role during embryonic body patterning. Assembled in clusters they determine the morphological characteristics of each body segment along craniocaudal axis according to their activation in 3´ to 5´ chromosomal direction. Posterior Hox proteins have a dominant effect over the function of anterior paralogues in common areas of expression, meaning e.g. that there is homeotic transformation of the lumbar vertebral bodies into thoracic vertebral bodies with ribs if there is a loss of function of the posterior HOX gene or in reverse manner if there is a gain of function. Homeotic transformation has been postulated in case of limb malformations caused by mutated 5´ HOXA or HOXD-genes but has not been proven so far.
The skeleton of the hand is a highly ordered composition of multiple bone elements, each well defined in size and shape according to its functional specification. The phalanges and metacarpals are longitudinal in shape and show two types of ossification: the primary ossification leading to cortical bone along the diaphysis and the secondary ossification at the epiphysis, typically without cortical bone but covered with joint cartilage. In contrast, the round carpal bones show only secondary ossification.
We investigated the role of HOXD13 in bone differentiation of the distal limb skeleton analyzing the ossification in the mouse mutant synpolydactly homolog (spdh), which carries a poly-alanine expansion in Hoxd13 similar to patients with synpolydactyly. We show that the phalanges and metacarpals of the spdh mutant adopt features of carpal bones by exhibiting only secondary ossification and developing joint cartilage in areas where cortical bone is normally located. Phenotype comparisons with other Hox knock out models reveal an antimorphic effect of the poly-alanine Hoxd13 mutant which seems to be the consequence of poly-alanine expansion mutations leading to interactions with other poly-alanine containing proteins. Additional we show that Hoxd13 is directly involved in the osteogenic programming of mesenchymal cells necessary for primary ossification.
Our results explain how the bone type in the distal hand skeleton is defined by HOXD13 and how poly-alanine expansion mutations can override the buffering effect of other distally expressed HOX-genes leading to proximo-distal shift of bone characteristics. These findings are new for the appendicular skeleton and of importance for the understanding of skeletal organogenesis beyond the skeletal patterning, meaning that each body region harbours its own osteogenic program by a defined HOX-code.

W11-02

Cranioectodermal dysplasia is a ciliary disorder caused by mutations in the IFT122 gene

Walczak-Sztulpa J.[1], Eggenschwiler J.[2], Osborn D.[3], Brown D. A.[2], Emma F.[4], Klingenberg C.[5], Hennekam R. C.[6], Torre G.[4], Garshasbi M.[1], Tzschach A.[1], Szczepanska M.[7], Krawczynski M.[8], Zachwieja J.[9], Zwolinska D.[10], Beales P. L.[3], Ropers H. H.[1], Latos-Bielenska A.[11], Kuss A. W.[1]

[1]Max Planck Institute for Molecular Genetics, Berlin, Germany, [2]Department of Molecular Biology of Princeton University, Princeton, USA, [3]Molecular Medicine Unit of the University College London Institute of Child Health, London, UK, [4]Children´s Hospital and Research Institute Bambino Gesu´, Rome, Italy, [5]Department of Paediatrics of the University Hospital of North-Norway and University of Tromsø, Tromsø, Norway, [6]Institute of Child Health of the University College London, London, UK, [7]Department of Gynecology and Obstetrics of Poznan University of Medical Sciences, Poznan, Poland, [8]Department of Gastroenterology and Metabolism of Poznan University of Medical Sciences, Poznan, Poland, [9]Department of Paediatric Cardiology and Nephrology of Poznan University of Medical Sciences, Poznan, Poland, [10]Department and Clinic of Paediatric Nephrology of Wroclaw Medical University, Wroclaw, Poland, [11]Department of Medical Genetics of Poznan University of Medical Sciences, Poznan, Poland

Cranioectodermal dysplasia (CED, Sensenbrenner syndrome, OMIM 218330) is a rare disorder characterized by craniofacial, skeletal, and ectodermal abnormalities. Most of the 20 cases reported to date are sporadic, but a few familial cases support an autosomal recessive inheritance pattern.

In this study we collected 13 patients with CED symptoms from 12 independent families. In two families the patients had consanguineous parents, and in one of these, two siblings were affected, permitting us to perform linkage analysis and homozygosity mapping. These analyses revealed a single region of homozygosity with a significant LOD score (3.57) on chromosome 3q21-3q24. The investigation of candidate genes from this interval revealed a homozygous missense mutation in the IFT122 (WDR10) gene that co-segregated with the disease. We then examined IFT122 in our patient cohort and identified one additional homozygous missense change in the patient from the second consanguineous family. In addition, we found compound heterozygosity for two different IFT122 mutations in a sporadic patient: a donor splice site mutation in combination with a third missense change. None of the changes were found in 340 chromosomes from ethnically matched controls. The IFT122 gene procuct is a component of the retrograde intraflagellar transport and plays an important role in the assembly and maintenance of eukaryotic cilia and flagella. We therefore investigated the abundance and morphology of primary cilia in fibroblasts from one male patient. These experiments showed significantly reduced cilia frequency and length in patient fibroblasts as compared to controls. To corroborate our assumption that the IFT122 mutations we identified are functionally relevant and therefore responsible for the patient phenotype we knocked down ift122 in zebrafish embryos. In keeping with our findings in patient cells, these experiments revealed a characteristic ciliary phenotype. As only 4 out of 13 CED patients in our cohort harboured mutations in IFT122, it must be assumed that this disorder is genetically heterogeneous. Still, by identifying CED as a ciliopathy our study suggests that the causative mutations in the unresolved cases are most likely to affect primary cilia function as well.

W11-03

Disturbed Wnt signaling due to mutations in CCDC88C causes an unclassified autosomal recessive brain malformation

Ekici A.B.[1], Hilfinger D.[1], Jatzwauk M.[1], Hehr U.[2], Wenzel D.[3], Lorenz I.[3], Staatz G.[3], Reis A.[1], Rauch A.[4]

[1]Institute of Human Genetics, University Erlangen-Nuremberg, Germany, [2]Department of Human Genetics, University of Regensburg, Germany, [3]Department of Pediatrics, University Erlangen-Nuremberg, Germany, [4]Institute of Medical Genetics, University Zurich, Switzerland

We performed positional cloning in a consanguineous family of North African origin with an unclassified severe autosomal recessive brain malformation. Three of five affected individuals died in early childhood but two could be analyzed, one surviving child and one fetus from a terminated pregnancy after ultrasound detection. MR and US images of the affected individuals showed enlarged ventricles, unilateral diverticle, hydrocephalus, hypoplastic cerebellum and ventrally dislocated cerebellum, mesencephalon and brain stem. Initially a phenotypic overlap with schizencephaly was suspected. Therefore mutations in Emx2 where excluded in affected family members.

A genome-wide linkage analysis was performed using Affymetrix250K arrays. This homozygosity mapping resulted in a single 3.4Mb interval encompassing 33 positional candidate genes. After prioritizing for expression in brain and Emx2 involved pathways we screened the family for mutations in the CCDC88C gene (HkRP2), encoding a Hook-related protein with a binding domain for the Wnt signaling pathway protein Dishevelled. This revealed a homozygous substitution in the donor splice site of intron 29 in affected individuals. RT-PCR studies showed a reduced length of amplified product and expected exclusion of exon 29 was confirmed by sequencing. This position is highly conserved throughout evolution and sequencing of 224 controls showed no alteration at this position. Western-blot analysis showed absent protein expression in lymphoblastoid cell lines from affected individuals and reduced expression in the mother, as compared to controls. CCDC88C was ubiquitously expressed in humans, but most prominently in the fetal brain, especially in pons and cerebellum, while only cortex and medulla oblongata were expressed in adult brain. Expression profiling of 84 genes from the Wnt-signaling pathway in peripheral blood from the index patient revealed an overall reduced expression of genes from this pathway with a specific altered expression pattern consistent with a negative feed-back loop via increased beta-catenin expression. We therefore conclude that this is a bona-fide loss-of-function mutation. Finally, mutation screening in 58 other patients with schizencephaly revealed no mutation in this gene, supporting the notion that this is a novel, rare brain malformation disorder caused by mutations in CCDC88C. We thus identified a further essential component of the Wnt-signaling pathway in brain development in humans.

W11-04

Brachydactyly type E (BDE) due to deletion and point mutations of PTHLH (parathyroid hormone-like hormone)

Klopocki E.[1], Hennig B.P.[1], Dathe K.[1], Koll R.[1], de Ravel T.[2], Baten E.[3], Weigel J.F.W.[4], Krüger G.[5], Hiort O.[6], Seemann P.[7], Mundlos S.[1]

[1]Institut für Medizinische Genetik Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Centre for Human Genetics University Hospital Leuven, Leuven, Belgium, [3]AZ Saint Lucas, Brugge, Belgium, [4]University Children's Hospital, Leipzig, Germany, [5]Abteilung Medizinische Genetik Universitätsklinikum Rostock, Rostock, Germany, [6]Departments of Pediatrics University of Lübeck, Lübeck, Germany, [7]Berlin-Brandenburg Centre for Regenerative Therapies, Berlin, Germany

Brachydactylies are a family of limb malformations characterized by short hands/feet due to aplastic or hypoplastic skeletal elements. Autosomal dominant brachydactyly type E (BDE, MIM 113300) is characterized by a general shortening of metacarpals/metatarsals and/or phalanges. Here we describe a novel disease gene for BDE in five unrelated families.

Initially, a microdeletion of ~900 kb was detected by array-CGH in a large pedigree with BDE, short stature and learning disabilities. This deletion encompasses PTHLH, the gene coding for parathyroid hormone-related protein (PTHRP). PTHRP is known to regulate the balance between chondrocyte proliferation and the onset of hypertrophic differentiation during endochondral bone development. Inactivation of Pthrp in mice results in short limbed dwarfism due to premature differentiation of chondrocyte. Based on our initial finding we screened a cohort of individuals with BDE and short stature for mutations in PTHLH. We identified two missense (p.L44P and p.L60P), a nonstop (p.X178WextX*54), and a nonsense (p.K120X) mutation. Functional testing of the L60P missense mutation in chicken micromass culture using the RCAS retroviral expression system showed an earlier differentiation of the chondrocytes compared to the wildtype which indicates that the missense mutation results in a loss of function.
Since mutations of the primary mediator of PTHRP/PTH receptor signaling, GNAS1, are associated with Albright Hereditary Osteodystrophy (AHO) which includes a skeletal phenotype strikingly similar to the BDE phenotype we conclude that PTHRP and the downstream pathway is particularly important in the growth of bones of the hands/feet. In summary, we describe PTHLH as a novel disease gene for BDE.

W11-05

Microduplications including a long-range enhancer of SHH (ZRS) cause a spectrum of limb malformations including Laurin-Sandrow syndrome

Lohan S.[1], Koll R.[1], Dölken S.[1], Farooq M.[2], Baig S.M.[2], Wajid M.[2], Habenicht R.[3], Kjaer K.W.[4], Mundlos S.[1], Klopocki E.[1]
[1]Institute for Medical Genetics Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Human Molecular Genetics Laboratory National Institute for Biotechnology & Genetic Engineering, Faisalabad, Pakistan, [3]Handchirurgie Kinderkrankenhaus Wilhelmstift, Hamburg, Germany, [4]Wilhelm Johannsen Centre for Functional Genome Research, University of Copenhagen, Denmark

Sonic hedgehog (SHH) is an important morphogen during early embryogenesis and regulates limb development. 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 (ZPA regulating sequence, ZRS). Point mutations in the ZRS cause an ectopic SHH expression on the anterior side of the limb bud and thus lead to preaxial extra digits. In humans ZRS point mutations and microduplications are associated with the triphalangeal thumb and polysyndactyly (TPT-PS, OMIM #174500) phenotype.
We have examined three families presenting with different limb anomalies including polysyndactyly of hands and feet (family 1), syndactyly type IV (SD4) combined with a mirror image polydactyly of the feet (family 2), and Laurin-Sandrow syndrome (family 3). Laurin-Sandrow syndrome (LSS) is characterized by duplication of ulna and fibula with absence of radius and tibia and preaxial polysyndactyly of hands and feet, frequently in a mirror-like configuration. The underlying genetic cause for LSS is currently unknown. By microarray-based CGH using whole genome 244K Agilent arrays as well as custom arrays for the SHH locus we detected microduplications of non-coding sequence 5' of the SHH gene on 7q36.3 which include the ZRS in all cases. These aberrations range in size from 16.6 to 165 kb and are arranged in direct tandem orientation as we could show by a direct sequencing strategy.
As shown for point mutations in the ZRS these limb malformations are presumably due to an ectopic SHH expression on the anterior side as well as an enhanced SHH expression on the posterior side of the limb bud.
In summary we demonstrated that microduplications including the ZRS region in 7q36.3 result in various limb anomalies i.e. syndactylies, pre- and postaxial polydactylies. Interestingly there is an inverse correlation between aberration size and phenotype severity. The smallest duplication of ~16kb is associated with the most severe phenotype - Laurin-Sandrow syndrome. These cases are further examples for malformations caused by genomic aberrations in conserved non-regulatory elements.

W11-06

Establishment of a Mouse Model with Misregulated Chromosome Condensation due to Defective Mcph1 Function

Trimborn M.[1], Ghani Kakhki M.[2], Walther D.[3], Dopatka M.[3], Dutrannoy V.[2], Busche A.[1], Meyer F.[1], Nowak S.[2], Nowak J.[2], Zabel C.[2], Klose J.[2], Esquitino V.[2], Garshasbi M.[3], Dopatka M.[3], Kuss A.[3], Dopatka M.[3], Ropers H.[3], Dopatka M.[3], Mueller S.[4], Gavvovidis I.[5], Schindler D.[5], Sperling K.[2], Neitzel N.[2]
[1]Institute of Medical Genetics, Berlin, Germany, [2]Institute of Human Genetics, Berlin, Germany, [3]MPI for Molecular Genetics, Berlin, Germany, [4]Center for Stroke Research, Berlin, Germany, [5]Institute of Human Genetics, Wuerzburg, Germany

Mutations in the human gene MCPH1 cause primary microcephaly associated with a unique cellular phenotype with premature chromosome condensation (PCC) in early G2 phase and delayed decondensation post-mitosis (PCC syndrome). The gene encodes the BRCT-domain containing protein microcephalin/BRIT1. Apart from its role in the regulation of chromosome condensation, the protein is involved in the cellular response to DNA damage. We report here on the first mouse model of impaired Mcph1-function. The model was established based on an embryonic stem cell line from BayGenomics (RR0608) containing a gene trap in intron 12 of the Mcph1 gene deleting the C-terminal BRCT-domain of the protein. Although residual wild type allele can be detected by quantitative real-time PCR cell cultures generated from mouse tissues bearing the homozygous gene trap mutation display the cellular phenotype of misregulated chromosome condensation that is characteristic for the human disorder, confirming defective Mcph1 function due to the gene trap mutation. While surprisingly the DNA damage response (formation of repair foci, chromosomal breakage, and G2/M checkpoint function after irradiation) appears to be largely normal in cell cultures derived from Mcph1gt/gt mice, the overall survival rates of the Mcph1gt/gt animals are significantly reduced compared to wild type and heterozygous mice. However, we could not detect clear signs of premature malignant disease development due to the perturbed Mcph1 function. Moreover, the animals show no obvious physical phenotype and no reduced fertility. Body and brain size are within the range of wild type controls. Gene expression on RNA and protein level did not reveal any specific pattern of differentially regulated genes. To the best of our knowledge this represents the first mammalian transgenic model displaying a defect in mitotic chromosome condensation and is also the first mouse model for impaired Mcph1-function.

EDUCATIONAL SESSIONS

EDU 1 Genetische Prädiktion bei multifaktoriellen Krankheiten
Moderation: Arne Pfeufer (München), Wolf Rogowski (München)

Teil I - Arne Pfeufer - Genomweite Assoziationsstudien, häufige und seltene Risikoallele - Möglichkeiten und Grenzen der prädiktiven Humangenetik

Teil II - Wolf Rogowski - Ökonomische Evaluation genetischer Tests auf häufige Risikoallele am Beispiel der hereditären Hämochromatose

Die wissenschaftlichen und technologischen Fortschritte bei genomweiten Assoziationsstudien (GWAS) und bei der Hochdurchsatz-Sequenzierung (Next-Generation Sequencing, NGS) zur Genotypisierung häufiger („Polymorphismen", SNPs) und seltener Genomvarianten („Mutationen") haben in den letzten 5 Jahren die Möglichkeiten, die Bedeutung und den Einfluss der Humangenetik in der wissenschaftlichen und der kurativen Medizin nachhaltig gesteigert. Es ist zu erwarten, dass sich dieser Trend weiter fortsetzen wird. Das gilt auch und insbesondere für ihre nicht-klassischen Tätigkeitsbereiche wie z.B. für die Erforschung und die Therapie häufiger sogenannter Volkskrankheiten und anderer multifaktorieller Erkrankungen.
Im ersten Teil der Fortbildung werden die wissenschaftlichen Grundlagen dieser technologischen und konzeptuellen Innovationen erläutert. Dabei wird neben dem Bereich der Genidentifikation („gene mapping") insbesondere der Bereich der prädiktiven Humangenetik („risk prediction", „individualized medicine") wissenschaftlich erläutert und das entsprechende Hintergrundwissen vermittelt. Dabei werden unter anderem Begriffe aus der Humangenetik (penetrance and expressivity, allelic and overall heritability, a priori and a posteriori probability), genetischen Epidemiologie (relative risk (RR), population attributable risk (PAR), odds ratio (OR), hazard ratio (HR)) und der Testtheorie (positive and negative predictive value (PPW, NPW)) behandelt. Die Anwendung prädiktiver Humangenetik zur Primärprävention (z.B. Genetisches ab initio Screening), zur Sekundärprävention (z.B. Priorisierung für klassische Vorsorgeuntersuchungen) und Tertiärprävention sowie Therapieoptimierung (z.B. individualisierte Medizin) und zur familienbasierten Prävention („family risk counseling") wird behandelt. Ein besonderer Schwerpunkt des Vortrages liegt in der Integration humangenetischer und genetisch epidemiologischer Sichtweisen und Konzepte.
Im zweiten Teil der Fortbildung werden die gesundheitsökonomischen Implikationen von Tests auf häufige Genvarianten mit kleinen Effektstärken erläutert. Dem Zuwachs unserer medizinischen Möglichkeiten steht aktuell ein abnehmendes Potenzial zur Finanzierung dieser Leistungen durch die Solidargemeinschaft sowie eine alternde Bevölkerung mit höheren Erkrankungsprävalenzen gegenüber. Aus diesem Grunde gewinnt die Kosteneffektivität als Bewertungskriterium neuer medizinischer Leistungen zunehmend an Bedeutung. Der aktuelle Stand der Methodik und Verwendung ökonomischer Evaluationen zur Beurteilung neuer genetischer Tests wird erläutert. In einem abschließenden Bespiel werden anhand des Bevölkerungsscreenings auf hereditäre Hämochromatose (HFE, HLA-H; MIM 235200) Methoden der entscheidungsanalytischen Modellierung von Kosten pro gewonnenem Lebensjahr dargestellt.
Abstract: Genomweite Assoziationsstudien (GWAS) und Next-Generation Sequencing (NGS) haben in den letzten 5 Jahren die Möglichkeiten und den Einfluss der Humangenetik insbesondere in ihren nicht-klassischen Tätigkeitsbereichen deutlich gesteigert wie z.B. in der Erforschung und Therapie häufiger Volkskrankheiten. Im ersten Teil der Fortbildung wird der Bereich der prädiktiven Humangenetik (u Screening, Prävention und individualisierter Therapie) wissenschaftlich erläutert und das entsprechende Hintergrundwissen vermittelt. Ein besonderer Schwerpunkt des Vortrages liegt in der Integration humangenetischer und genetisch epidemiologischer Sichtweisen und Konzepte. Im zweiten Teil der Fortbildung wird das Problem von Erstattungsentscheidungen zu genetischen Tests vor dem Hintergrund knapper Ressourcen im Gesundheitswesen erörtert. Die Methodik der ökonomischen Evaluationen zur Beurteilung neuer genetischer Tests wird erläutert. Sie wird beispielhaft dargestellt am Bevölkerungsscreening auf hereditäre Hämochromatose (HFE, HLA-H; MIM 235200).

EDU 2 Mikrodeletionssyndrome
Moderation: Anita Rauch (Zürich), Dagmar Wieczorek (Essen)

Mikrodeletionen und -duplikationen – ein Update

Die Identifizierung der ersten Mikrodeletionen, wie z.B. der Mikrodeletion 22q11.2 und des Williams-Beuren-Syndroms, liegt fast 20 Jahre zurück. Seitdem haben Array-Technologien die Möglichkeiten der zytogenetischen Untersuchungen revolutioniert. Das Screening großer Kohorten von Patienten mit mentaler Retardierung hat in den letzten Jahren zur Charakterisierung von zahlreichen neuen Mikrodeletions- und Mikroduplikationssyndromen geführt. Neben einigen schon länger bekannten Entitäten sollen auch neuere Mikrodeletionssyndrome exemplarisch dargestellt werden.

EDU 3 Familiäre Tumorerkrankungen
Moderation: Elke Holinski-Feder (München), Walther Vogel (Ulm)

Erblicher kolorektale Karzinome: Genetische Entitäten – Erkrankungsrisiken – Vorsorge
Elke Holinski-Feder (München)

Erbliche gastrointestinale Tumorerkrankungen lassen sich in unterschiedliche Entitäten differenzieren, die jeweils unterschiedliche Erkrankungsrisiken für kolorektale Karzinome aber auch für assoziierte Tumorerkrankungen aufweisen. Die genetischen Grundlagen und die Differenzierung dieser Entitäten sowie die sich hieraus ergebenden Aspekte für die Patientenversorgung sollen dargestellt werden.
(Hereditary colorectal cancer can be subdivided into different genetic entities with variable cancer risks for colorectal and associated tumors. Disease causing genetic mechanisms, the molecular differentiation of these entities and consecutive surveillance aspects will be presented.)

Familiäres Prostata-Karzinom – Genetik und Beratung
Walther Vogel (Ulm)

Familiäre Häufung wird beim Prostata-Karzinom ebenso regelmäßig beobachtet, wie beim Mamma-Karzinom, signalisiert hier aber ein manchmal beträchtliches Relatives Risiko für männliche Verwandte. Dies sollte Anlass zur Genetischen Beratung sein und ist relevant für die Interpretation urologischer Befunde (z.B. PSA-Wert). Da bislang wohl wegen der ungewöhnlich hohen Heterogenität außer BRCA2 keine Hochrisikogene identifiziert werden konnten, ist das Risiko nur ausnahmsweise durch genetische Untersuchungen zu präzisieren. Die Rolle von niedrig penetranten Varianten für die Risikovorhersage wird derzeit noch untersucht; erste Ergebnisse lassen auf eine baldige Verfügbarkeit von Tests hoffen.

EDU 4 Neuromuskuläre Erkrankungen
Sabine Rudnik-Schöneborn (Aachen), Tiemo Grimm (Würzburg)

Muskeldystrophien – Algorithmen der molekulargenetischen Diagnostik
Tiemo Grimm (Würzburg)

Zu den häufigsten Muskeldystrophien gehört die Muskeldystrophie Duchenne/Becker (DMD/BMD), die X-chromosomal rezessiv vererbt werden. Etwa 65 % der Mutationen sind Deletionen, ca. 7 % Duplikationen und ca. 28 % Duplikationen. Die hohe Neumutationsrate und das relativ häufige Auftreten von Keimzellmosaiken, bereitet in der genetischen Beratung und Diagnostik Probleme. Gliedergürtelmuskeldystrophien, die selten autosomal dominant und häufiger autosomal rezessiv vererbt werden, lassen sich klinisch in der Regel nur schwer von DMD/BMD unterscheiden. Ein sinnvoller Algorithmus für die molekulargenetische Diagnostik, der klinische Daten, Familienanamnese und molekulargenetische Befunde berücksichtigt, wird vorgestellt.

Myotone Dystrophien – Phänotypen und aktuelle Pathogenese
Benedikt Schoser (München)

Myotone Dystrophien zeichnen sich durch sehr variable klinische Symptome in nahezu allen Organsystemen des Menschen aus. Multisystemische Symptome betreffen Skelettmuskulatur, Gehirn, Auge, Herz und Endokrinium.
Bis dato sind 2 Formen der myotonen Dystrophien bekannt: die klassische 1909 von Steinert beschriebene DM1, und die 1994 von Ricker beschrieben DM2. Genetisch ursächlich für DM1 ist ein abnorm expandiertes CTG (Cytosin-Thymin-Guanin)-Triplett-Repeat im 3'-UTR des Dystrophia-myotonica-Proteinkinase-Gens (DMPK-Gen) auf Chromosom 19, während DM2 auf ein abnorm expandiertes Tetranukleotid-CCTG-Repeat im Intron 1 des Zinkfinger-9-Gens (ZNF-9) auf Chromosom 3q zurückzuführen ist. Der heterogenen Ätiologie mit 2 genetischen Loci liegt pathogenetisch eine RNA-Prozessierungsstörung mit Fehlregulation und alternativem Spleißen von organspezifisch exprimierter Genen zugrunde. Zusätzliche Störungen des RNA-Metabolismus sind zusätzlich vorhanden. Im Vortrag werden Phänotypen und aktuelle Daten zur Pathogenese vorgestellt.

Hereditäre neurogene Muskelatrophien – Genotyp-Phänotyp-Beziehungen und Algorithmen der molekulargenetischen Diagnostik
Sabine Rudnik-Schöneborn (Aachen)

Neurogene Muskelatrophien stellen häufige neurologische Erkrankungen dar, die durch eine unzureichende Innervation der Skelettmuskeln charakterisiert sind. In dieser Übersicht wird der aktuelle Kenntnisstand der klinischen und genetischen Diagnostik bei den spinalen Muskelatrophien (SMA) und den hereditären motorisch-sensiblen Neuropathien (HMSN) dargestellt. Der Algorithmus der molekulargenetischen Diagnostik richtet sich nach dem Alter des Patienten, dem Verteilungsmuster der betroffenen Muskeln, eventueller Zusatzsymptomatik, Laborbefunden, elektrophysiologischen und histologischen Untersuchungsergebnissen und nach dem Familienbefund. Bei mehr als 90% der SMA-Patienten, aber nur bei 30-50% der HMSN-Formen steht eine molekulargenetische Einordnung zur Verfügung.

POSTER

P-Cancer genetics

P-CancG-001
DNA bank for Polish patients with a predisposition for intestinal polyposis
Plawski A.[1], Podralska M.[1], Krokowicz P.[2], Drews M.[2], Slomski R.[1], Paszkowski J.[3], Lipinski D.[4], Cichy W.[5]
[1]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland, [2]Karol Marcinkowski University of Medical Sciences, Poznan, Poland, [3]Karol Marcinkowski University of Medical Sciences, Pozna, Poland, [4]Institute of Human Genetics Polish Academy of Sciences, Poznan Poland, [5]Karol Marcinkowski University of Medical Sciences, Poznan PolandIntroduction: Intestinal polyposis syndromes include a group of diseases conditioned by the occurrence of hereditary mutations. Here we present a collection of DNA samples derived from persons from families with a diagnosed adenomatous polyposes which comprise: familial polyposis coli together with its recessive form, Turcot's syndrome, inherited mixed polyposis as well as persons with recognised hamartomatous polyposes: juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome and Proteus syndrome. The objective of this study was to present current achievements associated with the establishment of the DNA Bank for intestinal polyposis.

Materials and methods: Investigations were conducted on DNA isolated from cells of the peripheral blood. The search for mutations in APC, MUTYH, PTEN, BMPR1A, SMAD4 and STK11 genes preconditioning the occurrence of individual diseases was performed employing SSCP, HA, DHPLC as well as RFLP techniques and DNA sequencing.
Results: At the present time, the DNA Bank comprises the total of 1097 DNA samples derived from 449 families with intestinal polyposis of which 945 samples come from persons in whose families Familial Adenomatous Polyposis (FAP) occurred. In addition, the collected data also contain material for analyses derived from 25 families with Peutz-Jeghers syndrome and 20 families with juvenile polyposis as well as single cases with the Cowden syndrome, Proteus syndrome and desmoid tumors. The performed molecular investigations allowed identification of mutations ranging from 44 to 50%.
Conclusions: With regard to the quantity of the material collected for analyses and the efficacy level of the employed molecular methods, the obtained results are in keeping with the results found in the literature from the field of genetics and medicine and do not differ from world standards. The collection of data and materials for investigations in the case of rare diseases allows qualitative, organisational and economic optimisation of the performed investigations.
The study was supported by the Polish Ministry of Science and Higher Education projects no N401 331936, N401 331936,N402 481537

P-CancG-002
The transcriptional activity of a cancer susceptibility region on 8q.24 is higher in prostatic carcinoma than in surrounding prostatic tissue
Assum G.[1], Gulic M.[1], Böhm C.[1], Kastler S.[1], Lüdeke M.[1], Wieland-Lange S.[1], Schmoll B.[1], Högel J.[1], Möller P.[2], Küfer R.[3], Vogel W.[1], Maier C.[1]
[1]Institute of Human Genetics, Ulm, Germany, [2]Department of Pathology, Ulm, Germany, [3]Department of Urology, Ulm, Germany

There are several regions on 8q24 which are independently associated with an increased prostate cancer risk. The responsible variants are not yet known. The only expressed coding sequence in this 1,2 mb long so-called "gene-desert" is the retrogene POU5F1B (see accompanied abstract). Additionally, there are no small regulatory RNAs annotated in this area which could be affected by variants located in this region. For this reason, it is assumed that risk alleles may affect potential regulatory sequences which might have an influence on the expression of the

neighboured c-MYC gene. One putative c-MYC enhancer was identified recently. But the high number of associated variants contradicts the suggestion of individual regulatory elements. Our recent results show that the region is transcribed from numerous promoters resulting in various transcripts, overexpressed in prostatic carcinoma compared to surrounding prostatic tissue. We hypothesize that variants on 8q24 may have an influence on the chromatin structure of this genomic region which in turn may affect the transcriptional activity and hence the activity of potential enhancer structures located there. If this is true, there should be an allelic discrepancy of RNA-expression in cells of heterozygous persons.
To confirm this hypothesis, we performed quantitative RT-PCR in prostatic carcinoma tissue compared to surrounding prostatic tissue to measure the expression of non-coding RNAs located in the 8q "gene desert" and of the c-MYC gene which is bordering this genomic region downstream.
Our results show a weak expression not only of the retrogene POU5F1B as described earlier but also from several non-coding RNAs in prostatic tissue. We could show an overexpression of these sequences in prostatic carcinoma compared to surrounding prostatic tissue. In contrast, c-MYC is expressed at high levels in the prostate but was shown not to be overexpressed in prostatic carcinoma tissue compared to surrounding prostatic tissue. Furthermore, there are first hints of differences in the allelic expression of RNAs on 8q in prostatic tissue of heterozygous people.
Thus, the function of variants on 8q may well be exerted through an effect on chromatin modification in cis. Allele-specific methylation assays are in progress to investigate possible differences of DNA-modifications in heterozygous people which should further confirm this hypothesis.

P-CancG-003

Rs606555 in close vicinity to the CCND1 gene as a potential modifier SNP for breast cancer risk in BRCA2 mutation carriers

Becker A.[1], Engel C.[2], Wappenschmidt B.[1], Köhler J.[1], Meindl A.[3], Arnold N.[4], Fiebig B.[5], Weber B.[6], Varon-Mateeva R.[7], Sutter C.[8], Kast K.[9], Schäfer D.[10], Deißler H.[11], Preisler-Adams S.[12], Gadziki D.[13], Niederacher D.[14], Froster U.[15], Schmutzler R.[1]
[1]Department of Obstetrics and Gynaecology, Cologne, Germany, [2]Institute for Medical Informatics Statistics and Epidemiology, Leipzig, Germany, [3]Department of Obstetrics and Gynaecology, Munich, Germany, [4]Department of Obstetrics and Gynaecology, Kiel, Germany, [5]Institute of Human Genetics, Regensburg, Germany, [6]Institute of Human Genetics, Würzburg, Germany, [7]Institute of Human Genetics, Berlin, Germany, [8]Institute of Human Genetics, Heidelberg, Germany, [9]Department of Obstetrics and Gynaecology, Dresden, Germany, [10]Institute of Human Genetics, Frankfurt am Main, Germany, [11]Department of Obstetrics and Gynaecology, Ulm, Germany, [12]Institute of Human Genetics, Münster, Germany, [13]Institute of Cell and Molecular Pathology, Hannover, Germany, [14]Department of Obstetrics and Gynaecology, Düsseldorf, Germany, [15]Institute of Human Genetics, Leipzig, Germany

Introduction: Germline mutations in BRCA1 and BRCA2 confer high risks of breast and ovarian cancer. However, evidence suggests that these risks are modified by other genetic or environmental factors. A recent genome-wide association study performed by the German consortium of hereditary breast and ovarian cancer (GC-HBOC) has shown that the homozygous rare alleles at SNP rs606555, close to CCND1, are associated with increased risk in BRCA1/2 negative breast cancer cases (Overall OR= 0.796 , 95% CI 0.718 – 0.883, p= 1.44x10E-5, n= 3049 cases/3813 controls). In this study we evaluated the minor allele of rs606555 as a potential risk modifier in BRCA1/2 mutation carriers.
Material/Methods: A total amount of 1297 samples collected in 12 centres of the GC-HBOC was genotyped for rs606555 by iQTM5 Multicolor Real Time PCR detection system Version 2.0 (BIORAD). Statistical analysis was performed by Cox-Regression analysis (endpoint BC and OC, 2 df model, additive model by per-allele effect) including stratification by year of birth.
Results: In BRCA2 associated breast cancer, the rare allele of rs606555 was significantly associated with an increased risk. The per allele effect of rs606555 revealed a hazard ratio (HR) of 1.33 (95% CI 1.03 – 1.71) and a p-value of 0.028. Rare homozygotes even reached a HR of 4.55 (95% CI 2.67 - 7.77) and a p-value of 2.6x10E-8. In contrast, no significance was seen in BRCA1 associated breast cancer or BRCA1/2 associated ovarian cancer.
Discussion: We found a significantly increased risk for breast cancer in BRCA2 mutation carriers for the rare allele of rs606555. The SNP investigated is located on chromosome 11q13, 71022 bp upstream of the cell cycle progression protein gene CCND1. Its product, Cyclin D1, is one of the most frequently overexpressed proteins and commonly amplified genes in breast cancer. Therefore, further investigations will not only include verification but also protein expression experiments to understand the nature of rs606555 and its modifier role in BRCA2 breast cancer.

P-CancG-004

Rs1175332 in close vicinity to the TLE1 gene as a potential modifier SNP for breast cancer risk in BRCA1 mutation carriers

Bossung V.[1], Engel C.[2], Wappenschmidt B.[1], Köhler J.[1], Meindl A.[3], Arnold N.[4], Fiebig B.[5], Weber B.[6], Varon-Mateeva R.[7], Sutter C.[8], Kast K.[9], Schäfer D.[10], Deißler H.[11], Preisler-Adams S.[12], Gadziki D.[13], Niederacher D.[14], Froster U.[15], Schmutzler R.K.[1]
[1]Department of Obstetrics and Gynaecology, Cologne, Germany, [2]Institute for Medical Informatics Statistics and Epidemiology, Leipzig, Germany, [3]Department of Obstetrics and Gynaecology, Munich, Germany, [4]Department of Obstetrics and Gynaecology, Kiel, Germany, [5]Institute of Human Genetics, Regensburg, Germany, [6]Institute of Human Genetics, Würzburg, Germany, [7]Institute of Human Genetics, Berlin, Germany, [8]Institute of Human Genetics, Heidelberg, Germany, [9]Department of Obstetrics and Gynaecology, Dresden, Germany, [10]Institute of Human Genetics, Frankfurt am Main, Germany, [11]Department of Obstetrics and Gynaecology, Ulm, Germany, [12]Institute of Human Genetics, Münster, Germany, [13]Institute of Cell and Molecular Pathology, Hannover, Germany, [14]Department of Obstetrics and Gynaecology, Düsseldorf, Germany, [15]Institute of Human Genetics, Leipzig, Germany

Introduction: Germline mutations in BRCA1 and BRCA2 confer high risks of breast and ovarian cancer. However, evidence suggests that these risks are modified by other genetic or environmental factors. A recent genome-wide association study performed by the German consortium of hereditary breast and ovarian cancer (GC-HBOC) has shown that the homozygous rare alleles at SNP rs1175332, close to TLE1, are associated with increased risk in BRCA1/2 negative breast cancer cases (Overall OR= 0.841, 95% CI 0.777 - 0.910, p= 1.89*10-5, n= 3003 cases/3057 controls). In this study we evaluated the minor allele of rs1175332 as a potential risk modifier in BRCA1/2 mutation carriers.
Material/Methods: A total amount of 1277 samples collected in 12 centres of the GC-HBOC was genotyped for rs1175332 by iQTM5 Multicolor Real Time PCR detection system Version 2.0 (BIORAD). Statistical analysis was performed by Cox-Regression analysis (endpoint BC and OC, 2 df model, additive model by per-allele effect) including stratification by year of birth.
Results: The per allele effect of the minor allele of rs1175332 revealed a protective effect for breast cancer in BRCA1 mutation carriers with a hazard ratio (HR) of 0.85 (95% CI 0.74 – 0.97) and p-value of 0.020. In contrast, no significant association was seen for BRCA1 associated ovarian cancer or BRCA2 associated breast and ovarian cancer.
Discussion: The investigated SNP rs1175332 is located on chromosome 9q, 562974 bp upstream of the Groucho family gene TLE1. The protein TLE1 is part of a repressor complex, including 13 polypeptides, among them PARP1 and RAD50. Both proteins play a role in homologous recombination mediated double strand DNA repair, which could explain

the protective effect of a SNP associated with TLE1. Further validation experiments are underway.

P-CancG-005

Karyotype Evolution in Patients with Myelodysplstic Syndromes Detected by Sequential FISH Analyses of Circulating CD34+ Cells from Peripheral Blood

Braulke F.[1], Schanz J.[1], Shirneshan K.[1], Seraphin J.[2], Detken S.[2], Metz M.[3], Brümmendorf T.H.[4], Götze K.[5], Trümper L.[1], Haase D.[1]
[1]Department of Hematology and Oncology, University of Goettingen, Germany, [2]Gemeinschaftspraxis for Hematology and Oncology, Northeim, Germany, [3]Gemeinschaftspraxis for Hematology and Oncology, Goettingen, Germany, [4]Department of Hematology and Oncology, University of Aachen, Germany, [5]Department of Hematology and Oncology, Tech. University of Munich, Germany

Introduction: In myelodysplastic syndromes (MDS) chromosomal aberrations occur in 50-80% of patients (pts) and play a major part in pathogenesis, prognosis, diagnosis and treatment allocation. The acquisition of clonal abnormalities in pts with initially normal karyotype, the expansion of an aberrant cell clone with a given anomaly or the occurrence of new, secondary abnormalities are called karyotype evolution (KE). A model of stepwise cytogenetic changes is proposed, but only a few systematic studies had focused on this phenomenon. In MDS most chromosomal anomalies detected by cytogenetic banding analyses of bone marrow (bm) metaphases are provable by fluorescence in situ hybridisation (FISH) of bm as well as by enriched CD34+ stem cells from peripheral blood (pb). In a multicentric German diagnostic study we prospectively analyse circulating CD34+ cells in MDS pts by FISH to detect chromosomal aberrations in pb and follow the clone size during the clinical course and under different therapies.
Methods: CD34+ stem cells from pb are enriched by immunomagnetic cell sorting (MACS®) and analysed by FISH afterward using a "Superpanel" (D7/CEP7, EGR1, CEP8, CEP XY, D20, TP53, IGH/BCL2, TEL/AML1, RB1, MLL, 1p36/1q25, CSF1R) for initial screening, after 12 and 24 months and in every case of suspected disease progression and a "Standardpanel" (EGR1, D7/CEP7, CEP8, TP53, D20, TEL/AML1, CEP X/Y) every 2 months during the 1. year and every 3 months during the 2. year. The results of pb and bm-FISH analyses and chromosome banding analyses of bm metaphases will be compared with each other and related to pb counts, therapy modalities and clinical courses.
Results: As yet, 105 pts from 8 German centers of haematology/oncology are included in our study. With regard to age, gender distribution and MDS subtypes according to WHO classification the study cohort is representative for the disease. The median follow-up time is 4.5 months (1-13 months). In 51 pts (49%) chromosomal aberrations were detected by FISH of circulating CD34+ cells, including 20 pts with del(5q), 17 pts with anomalies of chromosome 7 and 8 pts with del(12p). So far, we observed a KE in 9 cases (18%): del(5q) plus acquired monosomy 21; del(11q) plus acquired del(7q) and del(17p) and later complete monosomy 7; del(20q), trisomy 8 plus acquired loss of Y-chromosome; del(7q) plus acquired del(17p); del(5q-), del(7q), del(17p) plus acquired del(12p); trisomy 13, del(12q), del(17p) plus acquired monosmy 7; initially normal karyotype plus acquired del(21q); initially normal karyotype plus acquired loss of X-chromosome; and initially normal karyotype developing towards a tetraploid karyotype with del(7q) and trisomy 11. In 3 cases the KE was accompanied or followed by cytomorphological progression to higher stages of MDS or secondary AML. Discussion: The frequency of KE in our cohort is comparable to other studies published in literature. But with regard to our median observation time of only 4.5 months as yet, the number of diagnosed cases with KE is huge. Our results show that close-meshed sequential FISH analyses of circulating CD34+ cells are a feasible and sensitive method to monitor MDS pts, and it is less invasive than a bm biopsy. KE can be diagnosed early in the course of disease, so it might help to learn more about rare abnormalities, clonal evolution and genetic mechanisms of progression in MDS.

P-CancG-006

Mutation analysis of TMC6 and TMC8 in patients with epidermodysplasia verruciformis

Burger B.[1], Mertz K.[2], Kempf W.[2], Itin P.[1]
[1]University Hospital Basel, Basel, Switzerland, [2]Labor Kempf und Pfalz, Zürich, Switzerland

Epidermodysplasia verruciformis (EV) is a rare autosomal recessive dermatosis, characterized by a combination of gene mutation and HPV infection. Patients with EV suffer on the development of planar warts, which may develop to squamous cell cancer (SCC). In 2002 Ramoz et al discovered that patients with EV reveal mutations in the genes TMC6 and TMC8. (both on chromosome 17q25.3). TMC6 and TMC8 are members of the transmembrane channel-like proteins which are localized in the endoplasmic reticulum. The function of TMCs is unknown and their influence to development of EV is not well understood. Mutations in TMC6 or TMC8 could be found in 75% of all patients with EV. Further responsible genes are supposed on chromosome 2p and a X-chromosomal inheritance is also described.
We examined two mal patients presenting the typical signs of EV. One patient developed first planar warts during childhood, the second patient as an adult. We analysed the TMC6 and TMC8 genes by direct sequencing on an ABI prism 3100.
No mutation in TMC6 or TMC8 could be found by sequence analysis. Both patients reveal a lot of polymorphisms as described in the databases. Some polymorphisms are silent mutations and their influence to EV is unknown. Polymorphism rs7208422 (Asn->Ile), which might be the reason of EV when occurring homozygous, is shown in heterozygous status in our patients. The influence of further polymorphisms is still unknown. We will analyse their occurrence more in detail and discuss a possible connection to EV.

P-CancG-007

Wilms tumor cells with WT1 mutations have characteristic features of mesenchymal stem cells

Busch M.[1], Duhme C.[1], Brandt A.[1], Beier M.[1], de Torres C.[2], Mora J.[2], Royer H.-D.[3], Royer-Pokora B.[1]
[1]Institute of Human Genetics and Anthropology, Düsseldorf, Germany, [2]Department of Oncology, Barcelona, Spain, [3]Center of Advanced Studies and Research CAESAR, Bonn, Germany

Wilms tumor (WT) is a genetically heterogeneous, embryonic kidney tumor resulting from abnormalities in the mesenchymal to epithelial transition (MET) during kidney development. The cells of origin for WT are still unknown. About 10-15% of the Wilms tumors habour mutations of the tumor suppressor gene WT1 and show loss of the corresponding wildtype allele (LOH). These tumors are often correlated with stromal histology and ectopic mesenchymal elements like striated and smooth muscle cells, chondrocytes, osteocytes and adipocytes. This tumor subgroup also frequently has associated mutations in the CTNNB1 gene. Here we describe the differentiation potential of five primary Wilms tumor cell lines, established from five individual stromal-predominant Wilms Tumors with WT1 mutations. In in vitro differentiation experiments we show that the cells have a limited capacity for myogenic, chondrogenic, osteogenic and adipogenic differentiation. The differentiation potential varies between the WT cell lines with different genetic backgrounds, depending on WT1 and CTNNB1 mutations and their LOH status. In addition the Wilms tumor cell lines show high similarity to human mesenchymal stem cells (hMSC) in gene expression profiles, which confirm the stem cell like nature of the Wilms tumor cells. In conclusion we could show that the Wilms tumor cell lines resemble human mesenchymal stem cells.

P-CancG-008

Downregulation of tumor suppressor genes induced by histone deacetylation in hepatocellular carcinoma

Buurman R.[1], Steinemann D.[1], Kreipe H.[2], Wilkens L.[3], Schlegelberger B.[1], Skawran B.[1]
[1]Institute of Cell and Molecular Pathology, Hannover Medical School, Germany, [2]Institute of Pathology, Hannover Medical School, Germany, [3]Institute of Pathology KRH - Klinikum Nordstadt, Hannover, Germany

Background: Hepatocellular carcinoma (HCC) is the fourth most common carcinoma in the world. Alteration of histone acetylation is a common hallmark of human tumour cells and strongly alters transcription of many genes involved in the control of proliferation, cell survival, differentiation and genetic stability. In previous studies, we have shown that histone deacetylases (HDAC1-3) are consistently upregulated in dedifferentiated HCC.
Objectives: We therefore aim to understand the effects of altered mRNA expression induced by histone deacetylation in the development and progression of HCC.
Methods: Accordingly, we investigated the influence of histone deacetylation in extensively characterized HCC cell lines (Huh7, HepG2, HLE, HLF) and normal liver cell lines (THLE-2, THLE-3) by downregulation of HDAC1, HDAC2 and HDAC3 using siRNA and histone deacetylase inhibitor trichostatin A (TSA).
Results: Our data showed that treatment of HCC cell lines with the histone deacetylase inhibitor trichostatin A leads to increased apoptosis, decreased proliferation and increased histone acetylation of Lys 5, 8, 12 and 16 of histone H4. Using mRNA profiling we have already identified three genes (CDKN1A, APC2 and GADD45G) that were significantly upregulated during HDAC inhibitor treatment.
Conclusion: CDKN1A is a known tumor suppressor gene in HCC and almost all HDAC inhibitors are known to induce cyclin-dependent kinase (CDK) inhibitors, such as p21 (CDKN1A), which are responsible for cell-cycle arrest in G1 and G2 phases and subsequent cell differentiation (1). APC2 and GADD45G were described as tumor suppressor genes in other solid tumors like colorectal cancer and nasopharyngeal carcinoma (2, 3). Thus, during HCC development and progression chromatin remodelling seems to induce downregulation of tumor suppressor genes, resulting in increased proliferation and survival.

P-CancG-009

Gene expression study by microarray in c-kit mutant mice

Cardos G.[1], Savi G.[1], Hinescu M.E.[1]
[1]„Victor Babes" National Institute of Pathology, Bucharest, Romania

The c-kit gene codifies for a tyrosine-kinase receptor with essential role in differentiation and maintain of interstitial Cajal cells (ICC) from gastro-intestinal tract, involved in digestive motility, but also in GIST (gastro-intestinal tumors) pathogenesis. Other extra-digestive organs have been shown to contain interstitial Cajal-like cells (ICLC), whose functions are yet unknown.
We have performed comparative investigation of gene expression in normal and mutant mice by DNA microarray in order to contribute in understanding the physiology of ICC and ICLC. Different digestive and extra-digestive organs were sampled from control and mutant mice (WBB6F1/J-KitW/KitW-v/J strain). Total RNA was extracted by AllPrep DNA/RNA Mini Kit (Qiagen) and analyzed by Bioanalyzer and RNA 6000 Nano assay Kit (Agilent Technologies). DNA microarray from Whole Mouse Genome Microarray Kit (Agilent Technologies) were hybridized and scanned by Agilent DNA Microarray Scanner. The DNA microarray data analysis was carried out by Feature Extraction 5.1.1. and GeneSpring GX 10.Expression Analysis Software (Agilent Technologies). More than 3000 genes demonstrated differential expression by >2 fold in mutant versus control mice. Some genes involved in transcription regulation (such as: Dmbx1, Eif4a1, Zfp593 and Zfp69), cellular transport and cell junctions (Abi2, CaCng8, Hbb-b1), in signal transduction and metabolic process regulation (such as Calcr, Cyp3a44, Mcpt1 genes) were up-regulated. Other genes involved in apoptosis, intra-cellular transport and inter-cellular communication (such as Wnk1, Sema5a, Nf1, Tnfrsf21 genes) were down-regulated in mutant versus control mice. After validation by RT-qPCR, some of the identified genes may become candidate biomarkers for studying ICC and associated pathology in humans.
Acknowledgments: Mrs Sorina Mihaela Chirieac and Leontina Dita for technical assistance.
Financial support: PN 06.26 – 02.18 and PN 09.33.02.09 Projects.

P-CancG-010

Comparison of column-based and column-free methods for combined immunomagnetic cell separation and chromosome banding analyses in multiple myeloma

Giesen V.[1], Bachmann I.[1], Rieder H.[1]
[1]Institut für Humangenetik und Anthropologie, Düsseldorf, Germany

Introduction: Multiple Myeloma (MM) is a malignant monoclonal plasma cell proliferation, clinically classified as B-Cell Non-Hodgkins-Lymphoma. In MM, clonal metaphase chromosome aberrations are detected in only 30-50%, whereas molecular cytogenetic analyses on immunologically characterized MM cells show 70-90% chromosomal aberrations. A combination of immunomagnetic cell separation and chromosome preparation has successfully been used to enrich target cell metaphases in acute lymphoblastic leukemia. We therefore tested three different immunomagnetic separation techniques in combination with chromosome preparation for the applicability in MM chromosome analysis.
Methods: Two column-free (DYNAL, method 1; Robosep, method 2) and one column-based (MACS, method 3) immunomagnetic methods were tested. Three multiple myloma cell lines expressing CD138 surface antigen (U266, OPM-2, RPMI-8226) were used as target cells. The target cells were cultivated together with 50% K562 cells as contaminating cells. Combined immunomagnetic cell separation and chromosome preparation was carried out by adding colcemide, incubation with CD138 conjugated immunobeads, immunmagnetic cell separation, chromosome preparation and G-banding. The analysis of a total of 50 metaphases was attempted in each CD138-positive fraction to assess the sensitivity and specificity of the respective method. The quality of the chromosome preparations of the CD138-positve fractions was determined by evaluating the proportions of identifiable chromosomes/metaphase. The metaphases were grouped in cells with 100-75%, 75-50%, or less than 50% identifiable chromosomes. Each experiment was performed in triplicate.
Results: Cell line U266 revealed an average number of 28, 27, and 6 metaphases in methods 1, 2 and 3, repectively. OPM-2 showed 50 metaphases in methods 1 and 2 and 41 metaphases in method 3. Whereas RPMI-8226 had a median number of 48 metaphases in 1, 33 in 2 and 10 in 3. In sensitivity analysis U266 showed 38% correctly detected metaphases in the first and the automated method and 20% in the colum based procedure. The OPM-2 on the other hand revealed 75% sensitivity in 1, 58% in 2 and 40% in 3. RPMI-8226 had 63% correctly separated MM cells in 1, 51% in 2 and 0% in 3. With respect of metaphase quality, method 1 revealed the most metaphases in the first group, method 2 in the second group, and method 3 in the last group.
Conclusion: Of the three immunmagnetic cell separation systems the column-free procedures revealed a higher metaphase yield, a better sensitivity and a superior metaphase chromosome quality than the column based system. Thus, combined immunomagnetic cell separation and chromosome banding analysis with a column-free system may be recommended in the e

P-CancG-011

Chromosomal aberrations in Squamous Cell Carcinoma detected by Comparative Genomic Hybridization

Granzow M.[1], Böhm-Steuer B.[1], Popp S.[2], Holtgreve-Grez H.[1], Schoell B.[1], Boukamp P.[3], Jauch A.[1]
[1]Institute of Human Genetics, Heidelberg, Germany, [2]Institute of Human Genetics and German Cancer Research Center, Heidelberg, Germany, [3]German Cancer Research Center, Heidelberg, Germany

Cutaneous squamous cell carcinomas (SCC) are keratinocyte-derived tumours arising from cells of the interfollicular epidermis. Evidentially, these lesions develop predominantly in sun-exposed regions of the body and account for approximately 20% of cutaneous malignancies. Although knowledge of genetic changes during carcinogenesis has deepened in recent years, very little is known about the extent of genomic heterogeneity. Comparative genomic hybridization of multiple SCC biopsies provides an excellent tool to gain more detailed insight into genomic differences. Therefore, 54 specimens corresponding to 44 different patients were analysed, where multiple biopsies from a single patient were obtained from different tumour sides.
Abrrations were found in more than 80% of biopsies, recurrent gains were mapped to chromosome 17q (54%), 17p (49%), Xq (45%) and 20q (44%). The most frequent losses involved chromosome 3p (29%), 9p (29%), and 4q (22%). Furthermore, we observed a distinct pattern of chromosomal imbalances which allowed the division into a group with gains only and another with gains and losses/exclusive losses.
The first group represented 39% of all analysed biopsies and is characterized by prevalent gains of chromosome 17p/q (72%), 20q (68%), 20p (58%) and Xp/q (57%). The second group included 43% of the specimens, where recurrent gains of chromosomal material were 3q (50%), 17q (36%), Xq (36%) and 17p (27%). The most frequent losses affected chromosome 3p (55%), 9p (54%), 4q (44%), 12p (28%) and 11 q(27%).
Our results indicate, that SCCs do not necessarily represent a class of tumours with homogenous chromosomal aberrations but can be considered as an entity including two groups of imbalances. It is tempting to speculate that different mechanisms in tumour development cause the observed phenomenon. The amplification of oncogenes might be primarily responsible for the group of SCCs with chromosomal gains only, whereas additional loss of tumour suppressor genes presumably counts for the manifestation of the second group.

P-CancG-012

Hyperdiploidy is a rare genetic category in patients with AL amyloidosis and inversely associated with translocation t(11;14)

Jauch A.[1], Bochtler T.[2], Schönland S.[2], Hegenbart U.[2], Brough M.[1], Pschowski-Zuck S.[1], Kirn D.[1], Heiss C.[3], Benner A.[3], Neben K.[2], Moos M.[2], Hose D.[2], Ho A.D.[2], Goldschmidt H.[2], Bartram C.R.[1]
[1]Institute of Human Genetics, Heidelberg, Germany, [2]Internal Medicine V, Heidelberg, Germany, [3]German Cancer Research Center, Heidelberg, Germany

In multiple myeloma hyperdiploidy and non-hyperdiploidy have cytogenetically been described as two major pathogenetic pathways. In this study we assessed 363 patients with early monoclonal plasma cell disorders (184 AL amyloidosis (AL) patients and 179 patients with monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma stage I (MM I)) for their ploidy status by interphase fluorescence in situ hybridization (FISH). Hyperdiploidy was defined by the Wuilleme score for extra copies of chromosomes 5, 9, and 15. In the clinical correlation we found hyperdiploidy associated with older age, presence of an intact immunoglobulin, kappa light chain isotype and higher bone marrow plasmocytosis. In the oncogenetic tree model, the dichotomy concept of hyperdiploidy versus non-hyperdiploidy could be confirmed also in AL. However, the hyperdiploidy frequency was low in AL with 10% as compared to MGUS with 29% ($p<0.001$). Markedly, in AL the progression of monoclonal gammopathy into MM was paralleled by an increased hyperdiploidy frequency (10% versus 43%, $p<0.001$). As for the cytogenetic aberration pattern, t(11;14) and hyperdiploidy were inversely associated in both AL+/- MM I and MGUS / MMI. Whereas gain of 17p13 and 19q13 were closely associated with hyperdiploidy as determined by the Wuilleme score, gain of 11q23 reflected hyperdiploidy poorly in AL due to its frequent association with t(11;14).

P-CancG-013

RET protooncogene mutations in Polish patient with medullary thyroid cancer

Kaczmarek-Rys M.[1], Hoppe-Golebiewska J.[1], Ziemnicka K.[2], Wrotkowska E.[2], Sowinski J.[2], Slomski R.[1]
[1]Institute of Human Genetic Polish Academy of Science, Poznan, POLAND, [2]Department of Endocrinology and Internal Diseases University School of Medical Sciences, Poznan, POLAND

Medullary thyroid carcinoma (MTC) is uncommon tumor of thyroid gland, descended from C type follicular cells and constitutes about 5-8% of all types of thyroid cancer. Considerate malignancy and course of a disease it takes a place between high and low differentiated thyroid cancers. MTC may appear in incidental (80%) or familial form (FMTC) but also as a major feature of the multiple endocrine neoplasia type 2 syndromes (MEN 2). Two different syndromes of MEN 2 can be distinguished: more common, associated with phaeochromocytomas and hyperparathyroidism - MEN 2A and with mucosal neuromas, ganglioneuromatosis of the gastrointestinal tract - MEN 2B. 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 protoncogene RET. The most of affected with MEN2A have change in one of the six cystein's codons located within exon 10 and 11 of RET protooncogene. The most frequent mutation in patients affected with MEN2B syndrome is single nucleotide substitution in codon 918. In FMTC individuals' mutations in exons 8, 10, 11, 13, 14 and 15 equally are found.
Performed analysis concerned eleven codons of protooncogene RET, where mutations appears most frequently. As the most appropriate assay to identification of point mutation, with single nucleotide substitution character, pyrosequencing was chosen. In some cases we used PCR-SSCP technique and sequencing. We examined group of 350 individuals including affected with diagnosed medullary thyroid cancer, MEN2A and MEN2B syndromes and also relatives with first or second-degree consanguinity. We found 53 mutations in this group of patient. The most frequent were different variant of changes in codon 634.

P-CancG-014

POU5F1B, an OCT4 retrogene, might play a role in prostate cancer development

Kastler S.[1], Banerjee J.[1], Schmoll B.[1], Wieland-Lange S.[1], Högel J.[1], Möller P.[2], Küfer R.[3], Vogel W.[1], Maier C.[1], Assum G.[1]
[1]Institute of Human Genetics, Ulm, Germany, [2]Department of Pathology, Ulm, Germany, [3]Department of Urology, Ulm, Germany

There are several regions on 8q24 which are independently associated with an increased prostate cancer risk. The responsible variants are not yet known. Neither known is the function of the risk alleles because they are located in a so-called "gene desert" spanning an area of 1,2MB. The only expressed coding sequence within one of these associated regions corresponds to the retrogene POU5F1B. Its mother gene OCT4 encodes a transcription factor which is important for the pluripotency of embryonic stem cells. An activation of OCT4 by translocations between the OCT4 gene locus and the EWSR1 locus has been demonstrated in several human carcinomas. Ectopically expressed in mice, OCT4 leads to the development of neoplasia and subsequently to death. It is thinkable that its retrogene, which shows a homology of

97 % in the DNA sequence and 95 % in the amino acid sequence, might have a similar function and may play a role in prostate cancer development. Previously, we reported an overexpression of POU5F1B-RNA in prostatic carcinoma compared to surrounding prostate tissue. This renders POU5F1B a good candidate gene which might explain the risk on 8q24 for prostate cancer development.
To further investigate POU5F1B as a possible susceptibility gene for prostate cancer development, we analyzed whether a specific variant of the POU5F1B protein is associated with cancer risk and whether the POU5F1B protein is expressed in prostatic tissue and cell-lines.
The 4 coding SNPs within the POU5F1B retrogene which were analyzed in the association study with 505 unrelated prostate carcinoma cases and 213 unrelated controls could be related to 5 haplotypes. Two haplotypes were shown to be more frequent in patients than in controls. One of these carry at all SNP loci the nucleotide also present in the original OCT4 sequence, the other one carries just about one variant allele at one SNP locus. Since the risk haplotypes of POU5F1B are quite homologous to the original OCT4 gene sequence, they may bear a similar function as OCT4 does. We have inconsistent results of the POU5F1B protein expression in vivo. Western Blot assays suggest that the prostatic cancer cell line LNCaP expressing the mRNA of POU5F1B probably does not express its protein. In contrast to that, immunohistochemistry experiments on prostatic tissue resulted in a positive staining of epithelial compartments of the prostate suggesting a POU5F1B expression in prostatic carcinoma and surrounding prostatic tissue.
It is still unclear if the POU5F1B protein bears any functional relevance but it is worth to further investigate if this retrogene contributes to the development of prostate cancer.

P-CancG-015

Suv39h1 deficiency cooperates with c-Myc overexpression in the development of myeloid leukemias

Kuhlmann B.[1], Modlich U.[2], Wolf S.[1], Schienke A.[1], Baum C.[2], Schlegelberger B.[1], Rudolph C.[1]
[1]Institute of Cell and Molecular Pathology, Hannover Medical School, Germany, [2]Department of Experimental Hematology, Hannover Medical School, Germany

Introduction: Epigenetic events play an important role in tumorigenesis. For example, Suv39h1 is required for the epigenetic repression of pericentric heterochromatin by methylation of lysine 9 on histone H3. Therefore, deletion of Suv39h1 may induce chromosomal instability. We aim to investigate the influence of ectopic c-Myc expression in combination with Suv39h1 deficiency on induction of chromosomal instability, incidence and latency of leukemia in a murine retroviral transplantation model.
Methods: Lineage-negative hematopoietic stem cells isolated from C57B16J wild-type and Suv39h1-heterozygous mice were transduced with a retroviral vector encoding c-Myc and GFP, respectively, and transplanted into lethally irradiated mice. Flow cytometry and histological analysis were performed to determine the phenotype of leukemias. Cytospins were stained with May-Grünwald/Giemsa. Chromosomal instability was investigated by spectral karyotyping (SKY).
Results: In five independent experiments, all mice overexpressing c-Myc died within 59 days after transplantation due to the development of a myeloid leukemia. All animals showed a severe hepatosplenomegaly. Flow cytometric analyses of spleen cells showed a myeloid subpopulation (CD11b+/Gr1+) within the GFP+ fraction. Interestingly, leukemia latency of mice with an overexpression of c-Myc and an additional heterozygous deletion in the Suv39h1 gene was significantly longer. Histology showed massive infiltrations of leukemia cells in the liver, spleen and bone marrow, whereas cells with decreased Suv39h1 expression also infiltrated lung, kidney and gut. Myeloid blasts dominated in wild-type mice overexpressing c-Myc. In contrast, we observed fully differentiated granulocytes in Suv39h1-heterozygous mice overexpressing c-Myc. SKY analyses of leukemic clones from 5 of 13 wild-type mice overexpressing c-Myc demonstrated clonal chromosome aberrations. Leukemic cells from Suv39h1-deficient mice overexpressing c-Myc had a normal karyotype (11/11 cases).
Conclusion: Retroviral overexpression of c-Myc caused myeloid leukemia in a bone marrow transplantation mouse model both with an Suv39h1+/- and in a wild-type background. However, the additional epigenetic defect implicated a more severe phenotype. In addition, the Suv39h1 deficiency seems to protect chromosomal stability. The loss of Suv39h1 combined with c-Myc overexpression had an obvious effect on the granulocytic development of leukemia cells. To investigate which pathways were differentially regulated in leukemias associated with c-Myc overexpression in a wild-type background in contrast to leukemias with an additional Suv39h1 deficiency, we performed microarray expression analysis. In conclusion, we showed that the histone methylation defect had a strong influence on c-Myc-induced myeloid leukemia and on the regulation of genomic stability.

P-CancG-016

Role of truncating P53AIP1 variants in a German prostate cancer cohort

Luedeke M.[1], Linnert C.M.[1], Rinckleb A.E.[1], Hoegel J.[1], Vogel W.[1], Maier C.[1]
[1]Institute of Human Genetics, Ulm, Germany

Prostate cancer (PCa) is the most frequent tumour disease and the third leading cause of cancer related death in men in the western world. PCa has the highest heritability among common carcinomas, but its genetics is still elusive. Although common low-risk variants reproducibly reveal association with PCa, they do not account for the majority of familial clustering. On the other hand studies which were designed to identify rare high risk variants have been hampered by the strong disease heterogeneity of PCa.
Recently the P53AIP1 gene has been found mutated in prostate tumour tissue. Two thereby identified truncating alleles (R21insG and S32X) were reported to be recurrent germline variants and were associated with sporadic prostate cancer in the United States. However a validation of these findings, as well as the question if the truncating P53AIP1 variants accumulate further in familial PCa, have not been addressed so far. We genotyped these variants in a cohort of 512 controls, 329 sporadic cases and 379 familial index patients. The nonsense mutation S32X was completely absent in our cohort. The R21insG allele was present in 6 (1.2 %) controls, 7 (2.1 %) sporadic cases and 6 (1.6 %) familial index patients, resulting in a p-value of $p = 0.28$ when correlating controls with sporadic cases, and $p = 0.60$ with familial patients respectively. PCa patients with the R21insG variant showed no significant differences in age of diagnosis, tumour stage, Gleason score or grading compared to all PCa cases.
Due to the small frequency and absence of a strong genotype-disease relation P53AIP1 mutations play a limited role in our German cohort. The S32X variant might be population specific and, contrary to our expectations, mutations do not accumulate in PCa familial cases. Larger samples would be needed in order to refine the exact risk effect size of P53AIP1 variants in PCa.

P-CancG-017

BRCA2 mutation analysis in familial and early onset prostate cancer

Maier C.[1], Wiest I.[1], Luedeke M.[1], Surowy H.[1], Rinckleb A.[1], Herkommer K.[2], Kuefer R.[3], Vogel W.[1]
[1]Institut für Humangenetik, Ulm, Germany, [2]Urologische Klinik rechts der Isar, Munich, Germany, [3]Urologische Universitätsklinik, Ulm, Germany

Although prostate cancer (PCa) is known for the highest heritability (42%) among common cancers, its genetic background is the least clarified and in consequence, the practice in genetic screening is the most backward. While the recent identification of common risk variants seems promising concerning the characterization of multifacto-

rial susceptibility, little is known of high risk prostate cancer genes. To date, the strongest known PCa risk is caused by the BRCA2 gene, as described for male mutation carriers within breast and ovarian cancer families. Studies in prostate cancer cohorts have further demonstrated more aggressive forms of PCa with an earlier onset and shorter survival. In order to assess the role of BRCA2 in PCa cases from families without the strong hereditary breast and ovarian cancer history, we have started mutation analysis on the cohort of the familial prostate cancer project in Ulm. These probands were recruited solely on the criterium of PCa familial clustering, and cancer histories for other sites than prostate were ascertained at the time of enrolment. Presently, 198 probands have been screened, each as the youngest diseased case of 198 pedigrees, which overall comprise 590 affected men (3,0 per family; range 2 - 6). The mean age of onset was 60.2 years (range: 40 - 80) in the sequenced probands. Family history of breast cancer was observed in 34 of the pedigrees (17 %), where prostate and breast cancer cases were present in first-degree relationships. As an early onset group, 94 sporadic PCa cases were included with an age of 60 years or younger at the time of diagnosis (mean: 55.7; range: 29 – 60 years). In the total number of 292 cases sequenced for mutations, two (0.7 %) were found to carry truncating BRCA2 variants, in detail, one frameshift (V1283KX, exon 11) and one nonsense (Q2499X, exon 15) mutation. The families of the two carriers both showed a negative breast cancer history, obviously because of under-representation of indicative female relatives. Out of 23 rare alleles, each of which were observed in not more than two subjects, the majority were classified as neutral after database consulting (BIC, beast cancer information core), literature search or because of co-occurrence with truncating variants in our probands set. Six alleles remain as variants of unknown significance, which could influence splicing (variant IVS2-7T>A) or functionally alter the peptide sequence (variants P655R, K1025E, S2697N, E2981K and I3412V). In summary, the frequency of BRCA2 mutations in families with clustering of PCa appeared to be little higher than on the population level. In order to facilitate subgroup analyses concerning age of onset, tumour aggressiveness or breast cancer family history, further 182 families will be screened. The current data demonstrate a very limited contribution to familial PCa risk in general.

P-CancG-018

Putative „hepitype" in the ATM gene associated with CLL risk

Martín-Guerrero I.[1], Richter J.[2], Ammerpohl O.[2], Enjuanes A.[3], Colomer D.[3], Bosch F.[3], Villamor N.[3], Ardanaz M.[4], Jares P.[3], Campo E.[3], Siebert R.[2], García-Orad A.[1]

[1]University of the Basque Country, Leioa, Spain, [2]Christian-Albrechts-University, Kiel, Germany, [3]Hospital Clinic, Barcelona, Spain, [4]Hospital Txagorritxu, Vitoria, Spain

B-cell chronic lymphocytic leukemia (CLL) is the most common form of lymphoid malignancy in Western countries. CLL samples are characterized by the occurrence of major chromosomal lesions resulting from double strand breaks (DSBs) that have not been correctly repaired. The ATM (ataxia telangiectasia-mutated) protein is the principal integrator of cellular responses to DSBs, playing a crucial role in the detection of DNA damage, as well as in the initiation of DNA repair signalling. Due to the important role of ATM on the control of genomic stability, small variations in functional activity of the ATM gene, even with modest effects in its expression, may modulate the individual susceptibility to develop cancer. These alterations in the functional activity can be the result from several factors, including common single nucleotide polymorphisms and/or epigenetic changes.

In the present study, we evaluated if common genetic and/or epigenetic variants in the ATM gene could modulate CLL risk. The study included a large Spanish population (1503 individuals), 742 patients with CLL and 761 controls. Sixteen SNPs were selected that tag almost all the known common polymorphisms in ATM. Genotyping was performed by using the MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA). DNA methylation studies were carried out in CpG island of the promoter and in gene-body by using methylation specific PCR and pyrosequencing technology.

Following the case-control association analysis, we identified one haplotype within the ATM gene that confers an increased risk of CLL development. Two polymorphisms of this ATM haplotype eliminated one methylated CpG site each at the intronic level, causing changes in the methylation pattern. These changes could explain the mechanism by which the polymorphisms in the haplotype confer susceptibility to CLL. Therefore, the combination of effects of genetic variations with epigenetic changes has led us to identify one putative "hepitype" in the ATM gene associated with CLL risk.

P-CancG-019

Multiple meningioma with different grades of malignancy: case report with genetic analysis applying single-nucleotide polymorphism array and classical cytogenetics

Mocker K.[1], Holland H.[2], Ahnert P.[3], Schober R.[4], Bauer M.[4], Kirsten H.[5], Koschny R.[6], Meixensberger J.[1], Krupp W.[1]

[1]Clinic of Neurosurgery at University of Leipzig, Leipzig, Germany, [2]Translational Centre for Regenerative Medicine, Leipzig, Germany, [3]Institute for Medical Informatics Statistics and Epidemiology at University of Leipzig, Leipzig, Germany, [4]Dept. of Neuropathology at University of Leipzig, Leipzig, Germany, [5]Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany, [6]Dept. of Internal Medicine at University of Heidelberg, Heidelberg, Germany

Meningiomas arise from arachnoidal cap cells and represent one of the most common intracranial primary neoplasm in adults. According to the World Health Organization (WHO), they are classified into benign (WHO grade I), atypical (WHO grade II), and anaplastic (WHO grade III) meningiomas. The majority of meningiomas present as benign tumours with low recurrence rates. Yet, 8-22% are classified as atypical or anaplastic meningiomas with high rates of recurrence, high morbidity, and mortality. Multiple meningiomas with synchronous tumour lesions represent only 1-9% of all meningiomas and usually show a uniform histology. The simultaneous occurrence of different histological grades in these nodules is observed in only one third of multiple meningiomas. Here we report a case of a sporadic multiple meningioma presenting with different histopathological grades of differentiation of the two simultaneous tumour nodules (WHO I and II). The tumour genome of both nodules was analyzed by high-density single nucleotide polymorphism array (SNP-A), GTG-banding, spectral karyotyping (SKY), and locus-specific FISH. GTG-banding and SKY revealed 25 structural and 33 numerical aberrations with a slightly increased aberration frequency in the WHO grade II meningioma. We could confirm terminal deletions on chromosomes 1p, partial deletions on 22q, and/or monosomy 22 as the most frequent aberrations in meningioma. Using locus-specific FISH analysis [LSI TUPLE1/LSI ARSA (ABBOTT/VYSIS)] we found monosomy 22 in 14% of tumour cells of the WHO grade I lesion, and in 34% of tumour cells of the WHO grade II lesion, respectively. Terminal del(22)(q13) was only detectable in meningioma grade II in 10% of neoplastic cells. Interestingly, del(1)(p36.1) could be identified in both meningioma nodules. Furthermore, chromosomal instability with chromatid break chtb(1)(p11) and, as described here for the first time for meningiomas, dicentric chromosomes 4 could be identified. In the present case we detected previously published segmental uniparental disomy (UPD) regions 1p31.1, 6q14.1, 10q21.1, and 14q23.3 in normal control DNA of the patient and in both tumour nodules. Taken together, we describe a very rare case of multiple meningioma with overlapping but also distinct genetic aberration patterns in two nodules of different WHO grades of malignancy.

P-CancG-020

Fine-mapping of the 14q24 breakpoints of the recurrent deletion del(14)(q24q32) in B-cell-Non-Hodgkin lymphoma by tiling array-CGH

Nagel I.[1], Vater I.[1], Tönnies H.[1], Callet-Bauchu E.[2], Calasanz M.J.[3], Sole F.[4], Gesk S.[1], Salido M.[4], Martin-Subero JI.[1], Schroers E.[5], Harder L.[1], Majid A.[6], Dyer MJS.[6], Siebert R.[1]

[1]Institute of Human Genetics, Kiel, Germany, [2]Service d'Hématologie Biologique, Lyon, France, [3]Department of Genetics, Pamplona, Spain, [4]Laboratori de Citogenètica i Biologia Molecular, Barcelona, Spain, [5]MVZ Dortmund Dr. Eberhard und Partner, Dortmund, Germany, [6]MRC Toxicology Unit, Leicester University, UK

Interstitial deletions del(14)(q24q32) involving the ZFP36L1-locus in 14q24 and the IGH-locus in 14q32 are recurrently observed in chronic lymphocytic leukemia (CLL) and other B-cell lymphomas. We previously described breakpoint cloning of del(14)(q24q32) in 17 cases by long distance (LD)-PCR. In 14q24 all of the breakpoints were located within the ZFP36L1-gene. Nevertheless, in additional 17 cases with available DNA and FISH-proven del(14)(q24q32) we failed to clone the breakpoints using LD-PCR. To map the breakpoints in 14q24 in these cases, we designed oligonucleotide-based custom tiling arrays (Agilent) allowing a breakpoint fine-mapping in the region around ZFP36L1. By this approach, we could determine the breakpoints of the deletions del(14)(q24q32) in 14q24 with an accuracy of 63-450 bp (mean 168 bp) in the 16 analyzed cases with sufficient tumor cell content (> 60 %). All breakpoints were located within the ZFP36L1 gene, 14 in the single intron and 2 in the second exon of ZFP36L1, which is comparable to the results of the breakpoint cloning by LD-PCR (12 in the intron and 5 in exon 2 of ZFP36L1). Together with our previous finding of IGH-ZFP36L1 fusion transcripts in del(14)(q24q32)-positive B-cell lymphomas, these results indicate that deregulation of the ZFP36L1 gene might play a pathogenetic role in the development of these lymphomas.

P-CancG-021

Detection of hidden aberrations in karyotypically normal MDS/AML using oligonucleotide array CGH

Pölitz A.[1], Beier M.[1], Hildebrandt B.[1], Servan K.[1], Ingenhag D.[1], Moeller V.[1], Evers C.[2], Germing U.[3], Royer-Pokora B.[1]

[1]Institute of Human Genetics, Duesseldorf, Germany, [2]Institute of Human Genetics, Heidelberg, Germany, [3]Department of Haematology Oncology and Clinical Immunology, Duesseldorf, Germany

Clonal cytogenetic aberrations in myelodysplastic syndromes (MDS) are an accepted and important prognostic factor of the international prognostic scoring system (IPSS) and have an impact on classification and therapy strategies. Approximately 50% of the MDS patients show a normal karyotype by conventional cytogenetics and are often categorized as ‚low risk'. However, in several cases the clinical course worsens and acute leukaemia develops.

In order to detect submicroscopic imbalances in these cases, 105 high resolution array-based comparative genomic hybridisation (aCGH) analyses were carried out. Using Agilent oligonucleotide microarrays an average spatial genomic resolution of 43 kb, 22 kb and 9 kb was achieved. Up to now, over 40 aberrations were detected and could be confirmed with fluorescence in situ hybridisation (FISH) and/or real-time copy number PCR (qPCR). Besides several nonrecurring gains and losses, 4 recurrent imbalances were found, for example 7q22 deletions in three cases and interstitial 5q and 21q22 deletions in two cases each. Two cases had small interstitial deletions in 4q24 involving the TET2 gene, a recently described hotspot for mutations in myeloproliferative and myelodysplastic diseases. The first one, a heterozygous deletion over 700 kb in size, was verified by FISH and qPCR. In the second case, aCGH and qPCR revealed a smaller homozygous deletion.

To check for mutations in TET2 and loss of heterozygosity (LOH), denaturing high-performance liquid chromatography (DHPLC) analyses in 9 cases (including the one with the heterozygous deletion) were initiated. Three missense mutations and one silent mutation were found. After hybridisation of the DNA sample with the homozygous TET2 deletion to a custom array, achieving a resolution of 1 kb in the proximal and distal rearrangement regions, cloning of the breakpoints became possible. By sequencing, the deletion was determined to be 267.714 kb with end points flanking the TET2 gene at a distance of 46.9 kb and 124.8 kb, respectively. Patient1, 75 years at initial diagnosis, with low risk RCMD (refractory cytopenia with multilineage dysplasia) survived 3 months. Patient2, 57 years at initial diagnosis, suffered from high risk RAEBt/AML (refractory anaemia with excess blasts in transformation/ acute myeloid leukaemia) and died after 10 months.

In conclusion, with the help of high resolution techniques like aCGH hidden aberrations and mutations can be detected. In order to determine the role of these aberrations for disease development, prognosis and therapy response, more patients need to be studied.

P-CancG-022

Search for New Susceptibility Genes for Hereditary Non Polyposis Colorectal Cancer (HNPCC)

Rahner N.[1], Steinke V.[1], Propping P.[1], Nöthen M.M.[1]

[1]Institute of Human Genetics, Bonn, Germany

Hereditary non polyposis colorectal cancer (HNPCC) is the most common entity of hereditary colorectal cancer. By now causative germline mutations have been identified in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. Loss of a mismatch repair protein in tumour tissue by immunohistochemical staining combined with an early age of onset and/or familial occurrence of colorectal cancer point to a pathogenic germline mutation in the respective MMR gene. So far, a pathogenic germline mutation can be identified in only 63 % of these patients. The aim of the study was to find additional genes responsible for HNPCC. Therefore mutation analyses by direct sequencing in the functional candidate genes ESR-alpha, ESR-beta, MAX, KAT2A and PCNA were performed in 64 HNPCC patients with loss of MSH2 expression in their tumour tissues but without germline mutation in MSH2 and in 64 healthy controls. In total 30 genetic variants were identified in the 5 candidate genes. Afterwards these variants were genotyped in 42 additional patients fulfilling the same inclusion criteria and 234 additional healthy controls using Sequenom technology. The results of the association study will be presented.

P-CancG-023

Genome-wide analyses of DNA-methylation patterns in aggressive B-cell lymphomas identify TSPYL5 and G0S2 as genes de novo silenced by 5mC reprogramming

Richter J.[1], Rosolowski M.[2], Kreuz M.[2], Martin-Subero JI.[3], Ammerpohl O.[1], Hasenclever D.[2], Siebert R.[1]

[1]Institute of Human Genetics, University Hospital Schleswig-Holstein Campus Kiel/Christian-Albrechts University Kiel, Germany, [2]Institute for Medical Informatics/Statistics and Epidemiology, University of Leipzig, Germany, [3]Cancer Epigenetics and Biology Program/Bellvitge Institute for Biomedical Research-Catalan Institute of Oncology, Barcelona, Spain

DNA methylation is supposed to be a major epigenetic mechanism involved in initiation and progression of cancer. Nevertheless, the mechanisms underlying aberrant DNA methylation in cancer are widely unknown. By using array-based methylation profiling we have recently identified a set of 969 genes hypermethylated in aggressive B-cell lymphomas as compared to tissues specific controls. These genes were strongly enriched for Polycomb (PcG) targets in stem cells and genes with high CpG content. Somewhat unexpected, the majority of these genes showed low or absent expression in both lymphomas and hematopoietic controls (Martin-Subero et al., 2009). We have now analysed the set of hypermethylated genes with regard to alternative

mechanisms of DNA-methylation recently proposed by Gal-Yam et al. (PNAS, 2008). The majority of genes (64%) hypermethylated in lymphomas were assigned to the class of "epigenetically switched" genes, i.e. such genes silenced by PcG repression already in normal cells. In order to identify genes de novo silenced by "5mC reprogramming" in tumor cells as compared to controls, we analysed Affymetrix gene expression data from a series of aggressive B-cell lymphomas and controls. At an FDR of <0.1, only 53 of the genes aberrantly methylated in lymphomas showed lower expression in lymphomas as compared to controls. Among the top 4 genes were TSPYL5 (TSPY-like 5; FDR: 5e-12, FoldC 5.8) and GoS2 (Go/G1 switch 2; FDR: 0.019, FoldC 2.1). As to the functional role of these both candidates as potential tumor suppressors, we studied DNA methylation and gene expression by bisulfite pyrosequencing and qRT-PCR, respectively, in a set of different B-cell lymphoma cell lines, primary lymphomas and controls. We confirmed frequent hypermethylation of GoS2 and TSPYL5 (83% and 90% of lymphoma cell lines vs. 91% and 76% of primary lymphomas), respectively. Quantitative RT-PCR showed, that this DNA methylation was associated with complete loss of gene expression. Our results suggest that in the vast majority of aberrantly methylated genes in lymphomas, DNA methylation might not alter gene expression. In contrast, TSYPL5 and GoS2 are silenced by 5mC reprogramming in lymphomas as compared to controls and might thus be bona fide tumor suppressor gene candidates undergoing epigenetic inactivation in cancer.

P-CancG-024

Re-Analysis of 25 common risk variants for familial prostate cancer risk in Germany

Rinckleb A.E.[1], Kote-Jarai Z.[2], Luedeke M.[1], Surowy H.M.[1], Easton D.F.[3], Eeles R.A.[2], Vogel W.[1], Maier C.[1]
[1]Institute of Human Genetics, Ulm, Germany, [2]Institute of Cancer Research, Sutton, UK, [3]Department of Public Health and Primary Care, Cambridge, UK

Prostate cancer is the most frequently diagnosed malignant tumour in men in the western world. It is well known that in addition to age, lifestyle and environmental factors a positive family history contributes to an increased prostate cancer risk. However, the genetic fundamentals are still unclear.
Within the international PRACTICAL consortium we had previously analyzed 25 single-nucleotide polymorphisms (SNP) which had been appeared as the most significant on the base of three different genome-wide association studies (sample: a german cohort including 214 age-matched male controls, 189 familial prostate cancer probands (index cases) and 329 sporadic cases). For each SNP the per-allele Odds ratio (OR) had been estimated using logistic regression. Within this primary sample (PS), six SNPs had shown evidence of association with prostate cancer In familial cases [7p15, 8q24 (region 1 and region 3), 11q13, 17q12 and 17q24; per-allele ORs ranged from 1.36 to 2.11].
In the current PRACTICAL phase we contributed a new german sample (VS) composed of 190 new familial PCa index cases and 295 population controls (male/female) to verificate the previous findings. For two SNPs the association was reproducible in the new family set. In detail the SNPs on 8q24 region 3 (rs6983267) [PS: OR 1.42, P = 0,0141; VS: OR 1,40, P = 0,0112] and on 17q12 (rs1859962) [PS: OR 1.40, P = 0,0182; VS: OR 1.47, P = 0,0037] remained significant, while the SNPs on the other regions, especially on 8q24 region 1 [PS: OR 2.11, P = 0,0008; VS: OR 1.4, P = 0,0802] were not significant in the new dataset. On the other hand five new SNPs showed significant association in the verification sample; In detail on 3p12 [OR 1.74, P = 0,0029], 10q11 [OR 1.45, P = 0,0059], 11p15 [OR 1.43, P = 0,0221], 19q13 [OR 1.48, P = 0,0427] and 22q13 [OR 1.68, P = 0,0002]. The overall analysis of our total dataset (the previous and the present samples) yielded 12 strong candidate SNPs (P < 0.1, nine of them P < 0,05) on chromosomes 3p, 4q, 8p, 8q, 10q, 11p 17q, and 19q.
In summary, the validation of the previous loci on chromosomes 8q24 and 17q12 in our relatively small datasets indicates a relevant correlation with prostate cancer risk in our population. The 12 most significant SNPs in our overall sample will be analyzed for cumulative effects in near future.

P-CancG-025

Mutations in the ETS-factor GABP recognition site of the BRCA1 promoter are not a frequent cause of hereditary breast and ovarian cancer

Ripperger T.[1], Gadzicki D.[1], Schlegelberger B.[1], Steinemann D.[1]
[1]Institute of Cell and Molecular Pathology, Hannover, Germany

Monoallelic germline mutations in breast cancer 1, early onset (BRCA1) and breast cancer 2, early onset (BRCA2) cause hereditary breast and ovarian cancer. Besides inactivating mutations in the coding sequence or at splice sites of BRCA1 and BRCA2, deletions in the BRCA1 promoter have been reported in familial breast cancer patients. Moreover, breast cancer can be part of other cancer predisposing syndromes, and rare mutations in intermediate-penetrance breast cancer susceptibility genes and common low-penetrance breast cancer susceptibility single nucleotide polymorphisms have been shown to be associated with an increased breast cancer risk. However, in many families no causative mutations explaining the familial disease burden have been identified.
In this study, we investigated the potential impact of mutations in a cis element of the BRCA1 promoter. The cis element entitled EcoRI band-shift (RIBS) element (g.1377_1422, GenBank: U37574.1) was described as a positive regulatory element of BRCA1. It is located in a CpG island involved in epigenetic silencing of BRCA1 due to promoter hypermethylation, and is bound by a methylation-sensitive trans-activator complex designated as GA-binding protein (GABP). GABPA, the DNA-binding subunit of the heteromeric trans-activator complex, contains a highly conserved ETS domain and binds to a tandem GGAA-binding site within the proximal BRCA1 promoter.
To test our hypothesis of BRCA1 transcriptional downregulation by mutations in the RIBS element, we analysed 160 counselees with a family history of hereditary breast and ovarian cancer. Germline mutations in BRCA1 or BRCA2 and larger deletions in BRCA1 had been excluded by denaturing high-performance liquid chromatography (DHPLC) with subsequent sequencing of aberrant amplicons and multiplex ligation-dependent probe amplification (MLPA), respectively. The RIBS element was amplified from genomic DNA of peripheral blood samples and subsequently sequenced using a GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Krefeld). Obtained sequence electropherograms were analysed using SEQUENCE Pilot 3.3.0 (JSI medical systems, Kippenheim).
None of the 160 investigated counselees had a germline sequence alteration in the RIBS element. Deletions of the promoter element cannot be excluded, since the RIBS element is not covered by MLPA probes used (SALSA MLPA P002, BRCA1 probe mix, MRC Holland). Our result is consistent with sequencing investigations of the BRCA1 promoter in sporadic breast cancer tissue samples. Further investigations of potential deletions of the RIBS element and analyses of other regulatory promoter elements of BRCA1 have to be performed to determine their clinical utility in hereditary breast and ovarian cancer.

P-CancG-026

Analysis of allelic variants in the CHEK2 gene using DHPLC. Study on the Eastern Germany Population.

Scharrer U.[1], Skrzypczak-Zielinska M.[2], Wituszynska W.[3], Froster U.G.[1]
[1]Institute of Human Genetics, University of Leipzig, Germany, [2]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland, [3]Department of Biotechnology, Poznan University of Life Sciences, Poland

CHEK2 gene encodes checkpoint kinase 2, which is a key mediator in DNA damage signalling pathway. Alleles variants of the CHEK2 gene have been found to be associated with several types of cancer such as

breast, prostate, lung and ovarian cancer. In the Polish population three founder mutations of CHEK2 were identified: I157T, IVS2+1G>A and 1100delC. The aim of our study was to establish a simple method to identify founder CHEK2 mutations and determine the prevalence of these changes in the population of Eastern Germany (Saxony, Saxony-Anhalt and Thuringia). In our study, we optimized a DHPLC (Denaturing High Performance Liquid Chromatography) conditions for analysis of intron 2 and exon 3 for two mutations (IVS2+1G>A, I157T) and exon 10 for mutation 1100delC. We tested 251 patients and controls. Mutations show a similar frequency in the general population of Eastern Germany as in the neighbouring Poland (4.95% vs 4.8% concerning the missense mutation I157T and 0.99% vs. 0.5% concerning the truncating mutations IVS2+1G>A and 1100delC). Investigation of these mutations using DHPLC is highly sensitive and less time consuming compared to restriction fragment length polymorphism (RFLP) or allele-specific oligonucleotide (ASO)-PCR. It can be used in diagnostic testing.

P-CancG-027

BRCA associated cancers are phenotypically distinct tumor entities

Schmutzler R.K. (University Clinic Cologne Department of Obstetrics and Gynaecology, Cologne, Germany)

Introduction: Deleterious BRCA mutations confer high risks for the development of breast and ovarian cancer. Extensive evidence suggests that BRCA associated breast and ovarian cancers are different from the sporadic forms.

Material and Methods: The GC-HBOC has so far gathered >9000 families with defined inclusion critieria that account for early age at of onset and multiple affected cases within a family. More than 6000 families have been tested for BRCA mutations and more than 1600 families with deleterious mutations have been identified so far.

In these families we analysed 1. risk of contra-lateral breast cancer, 2. diagnostic accuracy of an intensified surveillance program, 3. outcome of prophylactic surgery and 4. therapeutic response.

Results: To 1: We estimated the risk of contra-lateral breast cancer in a cohort of 2020 affected mutation carriers and demonstrate that contra-lateral breast cancer risk significantly depends on the affected gene and age at first breast cancer. I.e. members of BRCA1 families had a 1.6-fold (95%CI 1.2-2.3) higher risk of contra-lateral breast cancer than members of BRCA2 families. After 25 years, 62.9% (95%CI 50.4 to 75.4) of BRCA1 patients aged <40 years at first breast cancer developed contra-lateral breast cancer, compared to only 19.6% (95%CI 5.3 to 33.9) of those aged >50 years at first breast cancer (M Gräser et al. JCO in press)

To 2: It is already well known that BRCA1 associated breast cancers present with distinct histopathological criteria. We now demonstrate that BRCA1 associated breast cancers also present with distinct imaging criteria that mirror the specific histopathological phenotype and frequently lead to misinterpretation of these tumors. I.e. of 221 mutation carriers that underwent a medium of five screening rounds 27 breast cancers were detected of whom 3 presented as interval cancers. The application of refined diagnostic criteria established by outcome oriented quality control let to an increase of sonographic sensitivity from 33 to 70%.

To 3: Preliminary results from detailed histopathological evaluation of mastectomy and adnexectomy specimens revealed pre-invasive and invasive breast cancer lesions in 10% of the mastectomies and identified fallopian tubes as the main origin of ovarian cancer development.

To 4: Participation in first international studies considering specific therapeutic responses of BRCA associated cancers indicate a high sensitivity to DNA-intercalating agents and a beneficial effect of new molecular targets such as PARP1 inhibitors.

Conclusion: Our results demonstrate that BRCA associated breast cancers represent a distinct tumor entity with a significant spectrum bias that is relevant for the diagnosis and treatment of such tumors. Therefore patients with BRCA associated tumors necessitate tertiary medical care in specialized center that provide specific diagnostic and therapeutic strategies within clinical studies.

P-CancG-028

Heritability of cellular DNA repair capacity

Surowy H.[1], Högel J.[1], Kollak A.[1], Lüdeke M.[1], Rinckleb A.[1], Maier C.[1], Vogel W.[1]
[1]Institute of Human Genetics, Ulm, Germany

Numerous environmental factors and impaired cellular DNA repair capacity have been associated with increased cancer risk and with variants in cancer risk genes. Based on a twin study, we report on the degree of heritability of various measures of DNA repair capacity, providing information on the contribution of genetic and environmental factors to the test results and their possible use as markers for cancer risk.

We used five different tests to evaluate the cellular DNA repair capacity from peripheral blood lymphocytes: The micronucleus assay (MN) visualizes unrepaired DNA double strand breaks, and was performed without and with damage induction by 2 Gray of ionizing radiation. Sister chromatid exchanges (SCE), resulting from mitotic recombinational repair events, were scored with and without induction by the alkylating agent BPDE (0.1 ng/ul). The effects of DNA damage on cell cycle progression were observed by measuring the mitotic delay (MD, G2/S-phase ratios of untreated and irradiated (2 Gy) cell cultures). Whereas tests without damage induction reveal the steady state of cellular DNA repair processes, damage-induced DNA repair measurements lead to an assessment of the system under full stress and are likely to be more robust against exogenic influences. All assays were performed in parallel from the same individual blood sample and in duplicate cultures.

41 monozygous twin pairs (MZ) and 10 pairs of dizygous twins (DZ) have been tested to estimate the heritability (h^2) of the results of each assay (h^2=0: no genetic influence; h^2=1: exclusive genetic influence). To obtain boundaries of heritability estimates, MZ pairs (genotypic correlation=1) were compared to DZ pairs, (genotypic correlation=0.5) and also to age and sex matched random pairs as controls (CP, genotypic correlation=0). Heritability was estimated using different models. The ACE model is based on a decomposition of total variance in genetic and non-genetic components, whereas a further calculation model is based on intrapair variances (IV) comparing MZ vs. DZ or MZ vs. CP. The data were adjusted for age and gender.

The impact of DNA damage on cell cycle progression, measured with the MD assay, shows the highest degree of heritability which is stable for all models (h^2= 0.7-0.8), indicating a strong correlation to the molecular DNA repair system and thus possible cancer risk genes.

Induced and basal MN assays also show high heritability in both models, when MZ are compared to CP (h^2= 0.5-0.6), also indicating a major contribution of genetic factors to these DNA repair measurements. However, when compared to DZ pairs, the heritability is very low (h^2= 0-0.1). Considering the comparison of MZ vs. CP, this estimate is most likely too pessimistic and may be a result of the small DZ pair numbers, although basal MN-frequencies are known to be dependent of various exogenic factors.

Heritability of basal SCE frequencies is moderate (h^2= 0.3), albeit ACE and IV models differ for the MZ vs. DZ comparison. Induced SCE frequencies show the same degree of heritability (h^2= 0.3), however MZ vs. CP comparison yields zero heritability as a result from chance matches in CP.

The strong influence of genetic components on the results of different measurements of cellular DNA repair capacity is consistent with the observation that those assays with the highest degree of heritability, MD and induced MN assays, also have the best discrimination powers in a case and control design.

P-CancG-029

Somatic mosaicism in Families affected by Tuberous Sclerosis: a rare mechanism of inheritance?

Sutter C.[1], Fathali-Zadeh F.[2], Sommer-Ort I.[1], Wellek B.[3], Janssen B.[4], Bartram C.R.[1]

[1]Institute of Human Genetics, University of Heidelberg, Germany, [2]Institute of Human Genetics, Heidelberg, Germany, [3]Institute of Human Genetics, University of Mainz, Germany, [4]Service XS, Leiden, The Netherlands

Tuberous Sclerosis (TSC)is an autosomal dominantly inherited predisposition to benign tumors, caused by alterations of the genes TSC1 and TSC2. Affected individuals may be severely affected by tumors of multiple organs or mildly affected (e.g.skin manifestation exclusively). Transmission of a mutation by a fully affected individual is not often seen in patients carrying a TSC1 mutation and rarely seen in those with a TSC2 mutation, due to the mostly severe phenotype. Therefore, a pattern of somatic mosaicism in the transmitting parent seems to be more common in TSC2 individuals. Unfortunately, many cases of mosaicism will escape detection. Among the 290 TSC families recruited throughout Germany and Europe and investigated at the Institute of Human Genetics, Heidelberg since 2004 in 149 a causative genomic alteration was identified. We found 4 cases of somatic mosaicism, all of them carrying a TSC2 mutation. 3 out of 4 are frameshift mutations (two deletions, one insertion), while one is a pathogenic missense mutation. Although this may be a rare mechanism of inheritance, as germ cell mosaicism has to be considered, the thorough detection of somatic mosaicism by molecular genetic methods has profound impact for genetic counseling.

P-CancG-030

Impact of additional chromosomal aberrations and BCR-ABL kinase domain mutations on the response to nilotinib in Philadelphia chromosome-positive chronic myeloid leukemia

Türkmen S.[1], Kim TD.[2], Schwarz M.[3], Koca G.[3], Nogai H.[3], Bommer C.[1], Dörken B.[3], Mundlos S.[1], Daniel P.[3], Coutre P.[3]

[1]Institut für Medizinische Genetik,Berlin, Germany, [2]Klinik für Hämatologie und Onkologie Charité; Universitätsmedizin Berlin; Campus Virchow-Klinikum, Berlin, Germany, [3]Klinik für Hämatologie und Onkologie Charité - Universitätsmedizin Berlin; Campus Virchow-Klinikum, Berlin, Germany

Background: Additional chromosomal aberrations (ACA) in Philadelphia chromosome-positive (Ph-positive) chronic myeloid leukemia (CML) are nonrandom and strongly associated with disease progression, but their prognostic impact and effect on treatment response is not clear. Point mutations in the BCRABL kinase domain (KD) are probably the most common mechanisms of imatinib resistance.
Design and Methods: We assessed the influence of ACA and BCR-ABL KD mutations on the response to the second-generation tyrosine kinase inhibitor nilotinib after imatinib-failure. Standard cytogenetic analysis of metaphases was performed to detect ACA and sequencing of the BCR-ABL KD was performed to detect point mutations.
Results: Among 53 patients with a median follow-up of 16 months, of whom 38, 5 and 10 were in chronic phase (CP), accelerated phase (AP) and blast crisis (BC), respectively, 19 (36%) had ACA and 20 (38%) had BCR-ABL kinase domain mutations. All patients without ACA had a superior overall survival (89%) than patients with ACA (54%) at 2 years (p=0.0025). In CP, overall survival at 2 years was 100 and 62 % (p=0.0024) for patients without or with ACA, respectively. BCR-ABL KD were associated with lower remission rates to nilotinib with a major cytogenetic remission in 9 of 20 (45%) patients as compared to 26 of 33 (79%) in patients without mutations (p<0.05). However, overall survival was not affected by BCR-ABL KD mutations.
Conclusions: Whereas BCR-ABL KD mutations may confer more specific resistance to nilotinib, which will predominantly affect response rates, the presence of ACA may reflect genetic instability and therefore intrinsic aggressiveness of the disease which will be less amenable to subsequent alternative treatments and thus negatively affect overal survival. Conventional cytogenetic analyses remain mandatory during follow-up of patients with CML under tyrosine kinase inhibito therapy.

P-CancG-031

Comprehensive mRNA analysis of fifteen BRCA1 and nine BRCA2 potential splice mutations in families with hereditary breast and /or ovarian cancer

Weber U.[1], Wappenschmidt B.[1], Krämer V.[2], Köhler J.[2], Meindl A.[3], Schmutzler R.[2]

[1]University Hospital, Cologne, Germany, [2]University Hospital,Cologne, Germany, [3]Klinikum rechts der Isar, Munich, Germany

Summary: Intronic alterations are frequently detected in the BRCA1 and BRCA2 gene and mutations located close to exon/intron boundaries potentially may affect the splice mechanism. Here, we describe the classification of fifteen distinct BRCA1 and nine distinct BRCA2 germline mutations by mRNA analysis.
Methods: Since 1997 1652 families fulfilling the criteria for hereditary breast and/or ovarian cancer were screened for BRCA1 and BRCA2 mutations by denaturing high performance liquid chromatography (DHPLC) followed by direct sequencing analysis. Patients with intronic alterations max. 20 bp up- or downstream or exonic alterations max. 3 bp from exon/intron boundaries were characterized on mRNA level extracted from blood lymphocytes. Quantitative RT-PCR and sequencing was carried out to evaluate expression levels and breakpoints.
Results: We detected four novel BRCA1 (IVS2-1G/C, IVS19+2T/G, IVS19-1G/T, IVS21-1G/T) and four novel BRCA2 (IVS4-6del19, IVS5-8insT, IVS9+2T/G, IVS18-1G/T) intronic germline mutations not previously described. BRCA1 IVS2-1G/C, IVS5+1G/T, IVS5+1G/C, IVS18+1G/C, IVS18-2delA, IVS19+2T/G, IVS19-1G/T, IVS21-1G/T, IVS22+2delT and 5527G/C (G1803A) and BRCA2 IVS4-6del19, IVS9+2T/G, IVS18-2G/T lead to aberrant transcripts. In contrast IVS18-6C/A, IVS19-12G/A and IVS20-14C/G (BRCA1) and IVS5-8insT, 7076C/A (P2283H), IVS14+6G/A, 9344C/T (P3039L), IVS25-12T/G, IVS26-20C/Tdo not disrupt normal splicing. Two BRCA1 alterations (IVS4-18T/G, 710C/T (C197C)) revealed enhanced skipping of Exon 5 and Exon 9, 10.
Conclusions: So fare, ten BRCA1 and three BRCA2 intronic alterations were considered to be causal for the disease. Three BRCA1 and six BRCA2 mutations seem to be neutral. The functional relevance of two BRCA1 mutations is still unknown. These results now allow predictive genetic testing in families carrying a proven deleterious splice site mutation.

P-CancG-032

DNA repair analysis in primary fibroblasts of individuals with childhood malignany and second cancer

Weis E.[1], Schön H.[1], Irmscher B.[1], Ludwig M.[1], Victor A.[2], Spix C.[2], Schneider-Rätzke B.[1], Kaina B.[3], Haaf T.[4], Galetzka D.[1]

[1]Institute for Human Genetics, Mainz, Germany, [2]Institute for Medical Biometry, Epidemiology and Informatics, Mainz, Germany, [3]Institute of Toxicology, Mainz, Germany, [4]Institute for Human Genetics, Würzburg, Germany

Because children with cancer are usually not exposed to environmental hazards or an unhealthy lifestyle, it is plausible to assume that genetic and epigenetic changes play an important role. In particular, modulation or misregulation of DNA repair- and cell cycle-associated genes may predispose to childhood malignancy. With the help of the German Childhood Cancer Registry, we recruited 20 persons who survived childhood malignancy and then developed a second cancer. 20 carefully matched persons with childhood malignancy but without a second cancer served as controls. In order to identify candidate genes, we first compared the DNA repair transcriptomes of primary fibroblasts from one- and two- cancer patients with a pool of healthy controls, using customized cDNA microarrays. We identified 55 genes that were differentially regulated in at least 2 cancer patients versus the control pool.

RT PCR expression analysis was performed for 17 of these genes in all patient and control cell lines without induction of DNA damage. Three genes, VIM, RAD9A, and RFC2, were differentially regulated in the 2-tumor patient group compared to the 1-tumor group. The DNA methylation patterns of selected differentially expressed genes were analyzed by bisulfite pryosequencing. In addition, we irradiated fibroblasts of all cancer patients with a dose of 1 Gray and analyzed gene expression at different time points (1 h, 4 h and 24 h) after DNA damage. Significant expression differences between 2-cancer and 1-cancer patients were detected for CDKN1A and RAD9A. Immunofluorescent staining of gammaH2AX foci did not reveal significant differences in DNA repair capacity and kinetics between the 2-cancer patient group and the healthy control group. However, using the same assay we found a significant difference between two monozygotic twins, one with 2-cancers and one without cancer, after treatment of fibroblasts with UV-C and daunorubicin. UV-C treatment of further patients and molecular karytype analyses are underway.

P-CLINICAL GENETICS

P-ClinG-033

Two different mutations in the ZNF526 gene co-segregate with non-syndromic autosomal recessive mental retardation in independent Iranian families

Abbasi-Moheb L.[1], Jensen L. R.[1], Garshasbi M.[1], Kahrizi K.[2], Ghadami S.[2], Wrogemann K.[3], Hu H.[1], Kariminejad R.[4], Behjati F.[2], Mohseni M.[2], Falah M.[5], Khodai H.[6], Kasiri M.[7], Darvish H.[2], Tzschach A.[1], Ropers H. H.[1], Najmabadi H.[2], Kuss A. W.[1]

[1]Max Planck Institute for Molecular Genetics, Berlin, Germany, [2]Genetics Research Center of the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, [3]Department of Biochemistry & Medical Genetics of the University of Manitoba, Winnipeg, Canada, [4]Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran, [5]Tehran Welfare Institution, Tehran, Iran, [6]Yazd Welfare Institution, Yazd, Iran, [7]Shahre Kord Welfare Institution, Shahre Kord, Iran

Mental retardation (MR) belongs to the most common forms of genetic handicaps, however, still very little is known about the genetic basis of this disorder. Especially the contribution of autosomal recessive hereditary defects remains largely unresolved and so far, only six genes have been found to be directly associated with non-syndromic autosomal recessive MR (NS-ARMR). We have previously identified several new genomic loci for NS-ARMR, including MRT 4-11 (Najmabadi et al., 2007, Hum. Genet. 121(1):43-8) and report now two different single nucleotide changes in the ZNF526 gene, which maps to MRT 11, a 10 Mbp linkage interval on chromosome 19q13.2-13.32. Both mutations cosegregate with moderate to severe NS-ARMR in three large apparently unrelated consanguineous Iranian families and were not found in 572 control chromosomes. ZNF526 encodes a C_2H_2 Zinc finger protein with multiple functional domains. It has DNA binding properties and is assumed to play a role in gene regulation and we confirmed its expression in adult and fetal brain by RT-PCR. Both mutations affect functional domains of the gene product and impair its function, as shown by in silico protein modelling and confirmed by the presence of specific alterations in the gene expression patterns of patient lymphoblastoid cells. A number of zinc finger proteins have previously been found to play a role in the pathogenesis of X-linked MR and may also be involved in autism spectrum disorders. Our results now implicate the first autosomal zinc finger gene in MR. Moreover, ZNF526 is only the second gene where more than one MR causing mutation was found in independent families, which may indicate that ZNF526 mutations are a more common cause of NS-ARMR at least in the investigated population. CHIP-seq experiments are underway to find direct regulatory targets of ZNF526 gene product.

P-ClinG-034

Five patients with Sotos syndrome and missense mutations in NSD1 gene

Albrecht B.[1], Kuechler A.[1], Reiss A.[2], Rossier E.[2], Glaeser D.[3]

[1]Institut für Humangenetik, Essen, Germany, [2]Institut für Humangenetik, Tübingen, Germany, [3]Genetikum, Neu-Ulm, Germany

Compared to deletions, nonsense, frame shift and splice mutations, missense mutations in NSD1 gene are a rare cause of Sotos syndrome. We report on 5 patients with de novo missense mutations in exons 13 to 23 of NSD1 gene.

Not all patients had overgrowth and macrocephaly at birth, but all 5 patients developed macrosomia during the first two years of life. All patients had a high and broad forehead, a long face and a pointed chin. Bone age was accelerated in all five patients and all showed muscular hypotonia and motor and/or speech delay. Two patients had a heart defect (ASD, pulmonary stenosis) and one patient developed a severe scoliosis. Chromosomal analysis was normal in all but one patient, who has Klinefelter syndrome in addition (karyotype 47,XXY).

The five mutations in exons 13-23 (p.Y1615H; p.I1976M; p.D2119G; p.C2159Y; p.I2218N) are all de novo and were not found in SNP databases. The missense mutations in the 3´part of the gene presumably lead to functional changes in the C terminal part of the protein and thus cause Sotos syndrome in these five patients. Functional studies are needed to prove this hypothesis.

P-ClinG-035

Contribution of CDKN1C mutations in Beckwith-Wiedemann syndrome

Bachmann N.[1], Schüller S.[1], Kohlschmidt N.[2], Spaich C.[3], Abou Jamra R.[4], Zabel B.[5], Enklaar T.[6], Prawitt D.[6], Bolz H.[1], Bergmann C.[1]

[1]Bioscientia Center for Human Genetics, Ingelheim, Germany, [2]Institute of Clinical Genetics, Bonn, Germany, [3]Institute of Clinical Genetics, Olgahospital Stuttgart, Germany, [4]Institute of Human Genetics, University of Bonn, Germany, [5]Center for Pediatrics and Adolescent Medicine, University of Freiburg, Germany, [6]Children´s Hospital, Johannes Gutenberg University Mainz, Germany

Beckwith-Wiedemann syndrome (BWS) is a clinically and (epi)genetically heterogeneous overgrowth disorder which mainly occurs sporadically. Cardinal phenotypic features comprise pre- and postnatal gigantism, exomphalos, macroglossia and an increased predisposition for specific embryonal tumors during infancy. The majority of BWS patients show complex alterations in the epigenetically regulated regions ICR1 (H19-DMR) or ICR2 (KvDMR1) in 11p15; most prevalent are methylation specific defects in ICR2. In addition, mutations in CDKN1C (Cyclin-Dependent Kinase Inhibitor 1C) can be found in single sporadic and ~ 40 % of familial cases with BWS. It is of importance that the different (epi)genetic subgroups of BWS are associated with different recurrence and tumor risks.

Here, we present a cohort of 498 patients referred to our department for molecular genetic diagnostics of BWS. After exclusion of methylation defects, paternal uniparental disomy and duplication/deletion in 11p15 by MLPA, we performed mutation analysis of the CDKN1C gene in 42 patients. By this, we detected six CDKN1C mutations of whom four were shown to be familial. In the other two cases, analysis of further family members was not possible.

Our study clearly demonstrates that CDKN1C mutation analysis should be considered in all BWS cases negative in methylation specific MLPA. For genetic counseling and clinical surveillance it is of note that pedigrees with CDKN1C mutation can show autosomal dominant transmission and that the tumor risk is decreased in CDKN1C mutated patients compared to individuals with one of the (epi)genetic alterations described above.

P-ClinG-036

Familial subtle reciprocal translocation t(2;7) with microcephaly- dysmorphism-retardation syndrome due to der(7)t(2;7)(q37.3;q36.3) - a case report.

Bauer I.[1], Caliebe A.[2], Tönnies H.[2], Krüger G.[1]
[1]Universitätsklinikum Rostock Abteilung Medizinische Genetik, Rostock, Germany, [2]Universitätsklinikum Schleswig-Holstein Campus Kiel Institut für Humangenetik, Kiel, Germany

We report on a female patient with short stature, supernumary kidney and hydronephrosis left, microcephaly, craniofacial dysmorphisms including ear abnormalities and upslanting palpebral fissures, and developmental retardation due to a subtle partial trisomy 2q37.3qter in combination with partial monosomy 7q36.3qter identified by FISH with painting and subtelomer-specific probes. The aberration which was not recognized by conventional cytogenetic (up to 700 bph) analysis is of maternal origin: the mother was shown to carry a submicroscopic balanced reciprocal translocation t(2;7)(q37.3;q36.3), the father showed normal conventional cytogenetic and FISH results.
Submegabase resolution array-based comparative genomic hybridization (array CGH) revealed a 9,5 Mb duplication within 2q37 comprising more than 100 genes and a 5,7 Mb deletion within 7q36 comprising about 40 genes.
Cytogenetic and molecular-cytogenetic analyses of two further maternal relatives with up to now unclear malformations and retardations are in progress.

P-ClinG-037

Isolated cardiac manifestation as first presenting symptom in Myotonic dystrophy type 2, Friedreich´s Ataxia and Laminopathy

Beckmann BM.[1], Heck S.[2], Schoser B.[3], Walter MC.[3], Rautenstrauss B.[4], Holinski-Feder E.[5], Klopstock T.[3], Kääb S.[1]
[1]Department of Medicine I, Ludwig Maximilians University of Munich, Germany, [2]Department of Neurology, Ludwig Maximilians University of Munich, Germany, [3]Friedrich Baur Institute of the Department of Neurology, Ludwig Maximilians University of Munich, Germany, [4]Friedrich Baur Institute of the Department of Neurology, Ludwig Maximilians University of Munich and MGZ Medizinisch Genetisches Zentrum Munich, Germany, [5]MGZ, Medizinisch Genetisches Zentrum Munich, Germany

Introduction: The extent and frequency of cardiac involvement in DM2 is not known in detail, but patients are at risk for severe cardiac complications despite mild symptoms of DM2. In Friedreich´s Ataxia (FA) cardiac hypertrophy and repolarisation abnormalities are observed in most patients, but only in rare cases cardiomyopathy appears before neurological symptoms. Laminopathies often are associated with neuromuscular symptoms but isolated cardiolaminopathies and mixed phenotypes within the same family are common.
Case reports: Case 1: A 47y old male patient presented with dilated cardiomyopathy with recurrent ventricular tachycardia and a family history of frequent cardiac death at age 35-42 years. Due to intermittent elevation of creatinkinase (CK) a myopathy had been considered before but due to the absence of specific neurological symptoms abandoned. No one had shown obvious neurological symptoms before. A laminopathy resp. an arrhythmogenic right ventricular cardiomyopathy was suspected but complete sequencing of the LMNA-, the PKP2-, DSP- and DSG2-gene of our patient did not reveal a mutation. A muscle biopsy showed a myopathy with multiple internal nuclei and no sign of mitochondriopathy or inflammation. A CCTG repeat prolongation in the ZNF9-gene, consistent with DM2 was found. Our patient died at the age of 52 years due to electromechanical decoupling of the heart.
Case 2: A 19 years old male patient presented with severe electrocardiographic abnormalities and marked cardiac hypertrophy. Genetic testing for familial hypertrophic cardiomyopathy was performed but no mutation was found in MYH7, TNNT2 or MYBPC3. Two years later family members noted slight speech difficulties and walking instability of our patient, not noted by himself. Neurological examination led to the suspicion of FA, confirmed by detection of 2 prolonged Frataxin alleles.
Case 3: A 33 year old women presented with new onset atrial fibrillation (AF) during pregnancy. Her brother and mother suffered also from AF, her brother had even survived cardiac arrest after treatment with ajmaline and some of her 3rd and 4th degree antecedents had died due to sudden death. A mutation in the LMNA-gene was found which cosegregated with the cardiac phenotype. 2 years later very, mild neuromuscular symptoms presenting as mild limb girdle muscular weakness occurred, and were interpreted by the patient as long-term consequence of delivery. CK levels were nit elevated.
Conclusion: While considering the diagnosis of a familial heart disease a continuous thorough exploration for neurological symptoms within the family should be performed. If in doubt neurological examination, if necessary at intervals, is essential. Neurological symptoms may appear at a later stage, may be mild, absent or mistaken as symptoms of heart failure.

P-ClinG-038

Intragenic Deletions of IL1RAPL1: Report of Two Cases and Review of the Literature

Behnecke A.[1], Hinderhofer K.[1], Bartsch O.[2], Damatova N.[2], Haaf T.[2], Nümann A.[3], Dufke A.[4], Rieß O.[4], Moog U.[1]
[1]Institute of Human Genetics Heidelberg University, Heidelberg, Germany, [2]Institute of Human Genetics Mainz University, Mainz, Germany, [3]Department of Neurology Charité-Humboldt University, Berlin, Germany, [4]Institute of Human Genetics Tübingen University, Tübingen, Germany

Background: The IL1RAPL1 (interleukin-1 receptor accessory protein-like 1) gene is located at Xp22.1, distal to the dystrophin gene, and was repeatedly shown to be deleted in patients with contiguous gene deletion syndromes. IL1RAPL1 is involved in the IL-1 signalling pathway and presumably affects cognitive abilities by modulating the hippocampal memory process. Since 1999, different mutations of IL1RAPL1 or cytogenetic aberrations disrupting the gene, have been reported infrequently in males with non-syndromal X-linked mental retardation (XLMR). Intragenic deletions have been rarely identified. We report on two unrelated males with intragenic deletions of IL1RAPL1 who presented with mild MR and additional features, and give a review of the literature.
Clinical report: From birth on, Patient 1 showed psychomotor retardation and at a young age he developed behavioural problems, a scoliosis, pectus excavatum and strabism but no hypotonia or other neurological symptoms. At the age of 13 years, he was mildly mentally retarded, had a normal growth and in addition mild facial dysmorphic features. Patient 2, a 7-year-old boy, showed mild facial anomalies, muscular hypotonia, and moderate-to-severe developmental retardation. Growth was normal. His carrier mother had a similar squinting of the eyes and mild learning disabilities.
Genetic investigations: Chromosome analysis at a 500 banding level showed a normal karyotype in both patients. In Patient 1, a de novo deletion of exon 2 of IL1RAPL1 was identified by 500k SNP-array and confirmed by multiplex-PCR. In Patient 2, a deletion of exons 3-5 was identified by CGH-array and confirmed by realtime-PCR and multiplex-PCR. It was also present in his mother. X-chromosome inactivation (XCI)-analysis showed a non-skewed pattern in the carrier mother.
Discussion: Recent studies on the biological function of IL1RAPL1 support its crucial influence on neuronal functions and network building-up with loss or confinement of function of IL1RAPL resulting in a cognitive deficit. IL1RAPL1 mutations cause a wide spectrum of neurological impairment, and have lately also been associated with autism. In review of the literature, frameshift and point mutations, as well as inversions disrupting IL1RAPL1 have been reported in males with XLMR and in one female patient. In addition, intragenic deletions have been

described in 3 families (deletion of exons 2-5, exons 3-5, and exons 3-7, respectively), all with several affected males showing MR but no additional dysmorphic features (non-syndromal MR). Two carrier females of point mutations presented with mild learning disabilities, but XCI in carriers in general did not show a specific pattern. We here describe two patients with intragenic IL1RAPL1 deletions (exons 2 and exons 3-5) identified by array techniques and confirmed by multiplex-PCR who presented with syndromic MR showing additional behavioural and dysmorphic features. XCI-analysis in the carrier mother led to normal results and cannot be used for carrier identification.

P-ClinG-039

Congenital Lipoid Adrenal Hyperplasia: Functional characterization of three novel mutations in the STAR gene

Bens S.[1], Bens S.[2], Mohn A.[3], Yüksel B.[4], Kulle AE.[2], Michalek M.[5], Chiarelli F.[3], Özbek MN.[4], Leuschner I.[6], Grötzinger J.[5], Holterhus PM.[2], Riepe FG.[2]
[1]Institute of Human Genetics, Kiel, Germany, [2]Department of Pediatrics, Kiel, Germany, [3]Department of Pediatrics, Chieti, Italy, [4]Division of Pediatric Endocrinology and Metabolism, Balcali/Adana, Turkey, [5]Institute of Biochemistry, Kiel, Germany, [6]Division of Paidopathology, Kiel, Germany

Introduction: The steroidogenic acute regulatory protein (StAR) has been shown to be essential for steroidogenesis by mediating cholesterol transfer into mitochondria. Inactivating StAR mutations cause the typical clinical picture of congenital lipoid adrenal hyperplasia.
Objective: To study the functional and structural consequences of three novel StAR mutations (p.N148K in an Italian patient; p.P129fs and p.Q128R in a Turkish patient).
Methods and Results: Transient in vitro expression of the mutant proteins together with P450scc, adrenodoxin and adrenodoxin reductase yielded severely diminished cholesterol conversion of the p.N148K mutant, the combined p.P129fs and p.Q128R mutant and the p.P129fs mutant by itself. The p.Q128R mutant led to a higher cholesterol conversion than the wild type StAR protein. As derived from three-dimensional protein modelling, the residue N148 is lining the ligand cavity of StAR. A positively charged lysine residue at position 148 disturbs the hydrophobic cluster formed by alpha-Helix4 and the sterol binding pocket. The frameshift mutation p.P129fs truncates the StAR protein. Residue p.Q128 is situated at the surface of the molecule and is not part of any functionally characterized region of the protein.
Conclusion: The mutations p.N148K and p.P129fs cause adrenal insufficiency in both cases, and lead to disorder of sex development with complete sex reversal in the 46, XY case. The mutation p.Q128R, which is not relevant for the patient's phenotype, is the first reported variant showing a gain-of-function. It may be possible that the substitution of neutral glutamine with basic arginine at the surface of the molecule may facilitate the contact to co-factors accelerating cholesterol transfer.

P-ClinG-040

Detection of germline rearrangements in patients with alpha- and beta-thalassaemia using high resolution array CGH

Blattner A.[1], Brunner-Agten S.[1], Hergersberg M.[1], Herklotz R.[1], Huber A.R.[1], Röthlisberger B.[1]
[1]Kantonsspital Aarau, Aarau, Schweiz

Background: Thalassaemia is characterised by a reduced synthesis of genes encoding the globin chains, leading to different phenotypes. Alpha-thalassaemia is primarily caused by deletions on chromosome 16, whereas beta-thalassaemia is caused by point mutations in the beta-globin gene. Larger deletions involving the beta-globin gene cluster on chromosome 11 lead to delta-beta-thalassaemia and hereditary persistence of fetal haemoglobin (HPFH).
Methods: Patients with no mutations found by routine molecular screening were divided into two groups, one with suspicion of a deletion in the alpha-globin gene cluster and one with suspicion of a deletion in the beta-globin gene cluster. Copy number abnormalities were investigated using two different customer defined array CGH formats with 70 bp and 7 bp probe spacing, respectively (NimbleGen®; Roche NimbleGen Inc, Madison, Wisconsin, USA). All results were confirmed by MLPA (Multiplex Ligation-dependent Probe Amplification, MRC Holland, Amsterdam).
Results: Out of 15 samples analysed for copy number abnormalities in the alpha-globin gene cluster, we identified nine deletions. Seven deletions and one duplication were identified out of 21 samples involving the beta-globin gene cluster. Among these deletions were: (1) Deletions of the HS-40 regulatory element which controls alpha-globin gene expression. (2) Deletions of the entire alpha-globin gene cluster reaching to the telomere. (3) Deletions of the beta-globin gene cluster with breakpoint boundaries within repetitive elements. Furthermore, we mapped the exact breakpoint positions in one sample each of the alpha-globin and beta-globin locus using Gap-PCR.
Conclusions: Array CGH is a reliable method to screen for copy number abnormalities in different diseases, such as thalassaemia and haemoglobinopathy. In contrast to MLPA, array CGH offers several advantages, such as a higher resolution with the possibility to characterize breakpoints down to the base pair, and the potential to investigate any customer defined region (and gene) of the human genome.

P-ClinG-041

WNT10A Related Ectodermal Dysplasias: From Oligodontia to Schöpf-Schulz-Passarge Syndrome

Bohring A.[1], Stamm T.[2], Spaich C.[3], Haase C.[4], Spree K.[1], Hehr U.[5], Hoffmann M.[1], Ledig S.[1], Sel S.[6], Wieacker P.[1], Röpke A.[1]
[1]Institut für Humangenetik, Westfälische Wilhelms-Universität Münster, Germany, [2]Poliklinik für Kieferorthopädie, Westfälische Wilhelms-Universität Münster, Germany, [3]Institut für Klinische Genetik, Olgahospital Stuttgart, Germany, [4]Stoffwechselzentrum Thüringen/ Klinische Genetik, Universitätskinderklinik Jena, Germany, [5]Zentrum und Institut für Humangenetik, Universität Regensburg, Germany, [6]Klinik und Poliklinik für Augenheilkunde, Martin-Luther-Universität Halle/S., Germany

The ectodermal dysplasias are a large, heterogeneous and growing group of disorders characterized by defects in morphogenesis of skin, sweat, sebaceous, submucous, and mammary glands, hair, nails and teeth. Numerous more or less distinct entities including also syndromal forms and monosymptomatic oligodontia have been reported. Most of them are very rare and the cause is often unknown. A homozygous WNT10A nonsense mutation was recently recognized as the cause of autosomal recessive inherited odonto-onycho-dermal dysplasia (OODD) in three consanguineous Lebanese families. Here we report on the broad clinical spectrum of WNT10A related ectodermal dysplasias including apparently monosymptomatic severe oligodontia, OODD, and Schöpf-Schulz-Passarge syndrome in 12 patients from 11 unrelated families of German and Turkish origin. We show that WNT10A mutations are a frequent cause of ectodermal dysplasia and were found in homozygous or compound-heterozygous state in about 9% of an unselected cohort of patients. In addition, about half of the heterozygotes show a phenotype manifestation including mainly tooth and nail anomalies which was not reported before in OODD. Thus we conclude, that WNT10A mutation analysis might become an important diagnostic test in many ectodermal dysplasias with severe oligodontia concerning the permanent teeth, with or without sweating problems, palmoplantar hyperkeratosis, or nail and hair anomalies, that are now difficult to classify as well as in isolated oligodontia.

P-ClinG-042

Association of CTLA4 with Systemic Sclerosis: new data and meta-analysis

Burkhardt J.[1], Kirsten H.[2], Melchers I.[3], Ahnert P.[4]
[1]Universität Leipzig, Leipzig, Germany, [2]Fraunhofer IZI and TRM Leipzig, Leipzig, Germany, [3]Universität Freiburg, Freiburg, Germany, [4]IMISE Leipzig, Leipzig, Germany

Objective: Systemic sclerosis (SSc) is an autoimmune disorder that is characterized by massive depositions of collagens in the connective tissue of the skin (scleroderma) and internal organs, as well as vascular alterations and immunological abnormalities. SSc shows great variability in severity and progression, but by now two main subsets are acknowledged: the limited (lSSc) and the diffuse (dSSc) cutaneous type.

Clustering in families indicates SSc as a polygenetic disease that occurs in genetically predisposed individuals. There is also evidence for a role of genetics in specific disease subtypes.
CTLA4 is a prominent candidate gene for involvement in immune diseases like SSc. To date five studies examining association of CTLA4 with SSc were published with partly contradicting results. Therefore, we analyzed within this study association of SSc with 7 CTLA4 polymorphisms (SNPs) including almost all genetic variation in a German European Caucasian SSc case control cohort. Additionally, a meta-analysis of published data on CTLA4 and SSc was conducted.
Methods: A total of 217 German SSc patients were diagnosed at the University Medical Centers of Freiburg and Cologne. The control group consisted of 232 matched German healthy blood donors. Information on disease subtype, antibody status, organ involvement and age of onset was available. Genotyping was done by mass spectrometry.
Allelic distribution was calculated using a chi-square test with one degree of freedom. Standard genotypic distribution was observed using Lathrop tests. Subgroups were also analyzed. To assess significance of differences between subgroups a two-tailed test of interaction was used. Meta analysis was done using a median unbiased estimation.
Results: We revealed an association of rs11571317 (a CTLA4 promoter SNP not previously studied for association with AIDs) with SSc ($p = 0.018$). The association of rs11571317 was also found in various SSc subgroups including ATA+, ACA+, dSSc and lSSc. Association of a haplotype of all 4 promoter polymorphisms (-1661T/-1722T/-318C/rs11571317T) was not stronger than single marker association of rs11571317. Additionally, association of CTLA4 SNPs besides rs11571317 with subgroups was found. The promoter variants -1772CT and -318CT were associated with dSSc ($p = 0.014$ and $p = 0.022$ respectively) and the -1722T/-318C haplotype showed a highly significant association as well (corrected for multiple testing: $p = 0.005$). A test of interaction revealed the association to be highly specific for dSSc compared to lSSc ($p = 0.009$).
Two polymorphisms showed evidence of different genotype distributions between groups with and without organ involvement: -1661CT with heart involvement and CT60 with lung hypertension ($p = 0.020$ and $p = 0.026$, respectively).
Significant influence on age of onset was found for the closely linked +49AG and rs231723 ($p = 0.015$ each).
Meta-analysis did not confirm the general association of -1661CT, -1722CT, -318CT and +49AG with SSc. This was also not the case when only studies on patients of Caucasian origin were included.
Discussion: While meta-analysis did not confirm association of previously investigated CTLA4 variants with SSc in general we found CTLA4 SNPs to be associated with SSc subtypes and a not previously investigated CTLA4 SNP to be associated with SSc in general. Interestingly, SNPs most prominently associated were found in the promoter region of the gene. These SNPs might influence CTLA4 expression levels directly, a mechanism that has been previously proposed for the gene, or they might influence exon splicing and therefore promoting the expression of a soluble CTLA4 isoform missing the third exon. It has been proposed, that low levels of receptor CTLA4 or high levels of soluble CTLA4 might contribute to T cell receptor over-stimulation. In summary, CTLA4 variants might promote autoimmunity and influence patients outcome, reflected by the subtype specific association found in our data.

P-ClinG-043

Clinical and molecular characterization of patients with Waardenburg syndrome

Busch J.[1], Bachmann N.[1], Decker J.[2], Bolz HJ.[3], Bergmann C.[4]
[1]Bioscientia Center for Human Genetics, Ingelheim, Germany, [2]Bioscientia Center for Human Genetics Ingelheim and Department of Internal Medicine, University of Mainz, Germany, [3]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, University of Cologne, Germany, [4]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, RWTH Aachen University, Germany

Hearing impairment is a common condition with a prevalence of approximately 1/600. While most cases of hearing loss are non-syndromic, additional phenotypic features can be found in about 30% of patients. Waardenburg syndrome (WS) is one of those syndromes characterized by varying degrees of hearing loss and associated pigmentary disturbances of the eye, skin and skin appendages, as e. g. a prominent white forelock. Clinically, WS has been categorized into four major subtypes. Type I refers to the first cases described by Waardenburg, while WS type II shows additional lateral displacement of the inner canthi (dystopia canthorum). Musculoskeletal findings with hypoplasia of limb muscles characterize WS type III. Type IV WS, also often called Shah-Waardenburg syndrome or Waardenburg-Hirschsprung disease, combines pigmentation defects, deafness, and Hirschsprung's disease. WS is not only clinically heterogeneous, but also genetically. So far, six genes have been identified, and mutations in some genes can cause more than one specific WS subtype. Here, we report our molecular genetic findings in a large cohort of patients affected with WS types I-III. In total, we were able to identify a disease-causing mutation in 47 unrelated patients (38 PAX3 mutations and 9 MITF mutations). To the best of our knowledge, 25 of the detected PAX3 mutations and 5 of the detected MITF mutations are novel mutations not described in the literature so far. Our study considerably expands the mutational spectrum in PAX3 and MITF and characterizes the close relationship that links the different subtypes of WS.

P-ClinG-044

Clinical and molecular characterization of SHOX mutations in patients with syndromic and non-syndromic short stature

Busch J.[1], Bachmann N.[1], Decker J.[2], Bolz HJ.[3], Rappold GA.[4], Bergmann C.[5]
[1]Bioscientia Center for Human Genetics, Ingelheim, Germany, [2]Bioscientia Center for Human Genetics Ingelheim and Department of Internal Medicine, University of Mainz, Germany, [3]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, University of Cologne, Germany, [4]Institute of Human Genetics, University of Heidelberg, Germany, [5]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, RWTH Aachen University, Germany

Mutations of the SHOX gene constitute a common cause for syndromic and non-syndromic short stature. The phenotypic spectrum is broad and comprises Ullrich-Turner syndrome, Leri-Weill dyschondrosteosis, Langer mesomelic dysplasia and idiopathic short stature without any further syndromic feature. SHOX belongs to the homeobox gene family and is localized in the pseudoautosomal region (PAR1) on the X and Y chromosome. In line with the wide phenotypic spectrum, the encoded SHOX protein plays an important role in chondrocyte organization and skeletal development. The majority of patients with SHOX deficiency harbour partial or complete deletions of the SHOX gene usually detected by MLPA. Recently, we could demonstrate that deletions of a distant 3' enhancer element downregulate SHOX gene

activity and constitute a relatively frequent cause of growth failure in patients of the above clinical spectrum. In contrast, reports of duplications affecting the SHOX gene are very rare and interpretation of their clinical significance if any is difficult. Here, we report our molecular genetic findings by MLPA and direct sequencing in a cohort of 399 unrelated individuals afflicted with one of the above clinical phenotypes. In 49 of these patients, we were able to identify the disease-causing SHOX mutation, among them 17 deletions and point mutations that have been not described in the literature so far.

P-ClinG-045

Phenotypic variability in patients carrying submicroscopic deletions containing TWIST1 but do not present with typical Saethre-Chotzen syndrome

Busche A.[1], Graul-Neumann LM.[2], Zweier C.[3], Hoffmann K.[1], Rauch A.[4], Klopocki E.[1], Horn D.[1]
[1]Charité-Universitätsmedizin Berlin Institute of Medical Genetics, Berlin, Germany, [2]Charité-Universitätsmedizin Berlin Institute of Human Genetics, Berlin, Germany, [3]University Hospital Erlangen Institute of Human Genetics, Erlangen, Germany, [4]University of Zurich Institute of Medical Genetics, Zurich, Switzerland

Saethre-Chotzen syndrome (MIM #101400), a disorder belonging to the group of acrocephalosyndactylies, is usually characterized by coronal synostosis and a characteristic facial dysmorphic pattern. Cutaneous syndactyly is variably present. Loss-of-function mutations in the TWIST1-gene located at chromosome 7p21 are known to cause this syndrome. A few patients described with Saethre-Chotzen syndrome have microdeletions comprising the whole TWIST1. Mental retardation is common for patients carrying larger microdeletions in 7p21.
Here we describe three patients and one clinically unaffected mother who did not present with the classical clinical phenotype of Saethre-Chotzen syndrome. Genome wide screening by array-CGH revealed submicroscopic deletions spanning between 0.5 Mb and 11.6 Mb.
Patient 1 is a 38 months old girl presenting with microcephaly, profound mental retardation, and facial abnormalities including full cheeks, arc-shaped eyebrows, hypertelorism, slight exophthalmos, ptosis, epicanthus, short palpebral fissures, and small, dysplastic ears. The 11.6 Mb deletion of this patient encompasses approx. 50 known genes including TWIST1. Patient 2 is a girl presenting at the age of 21 months with a 526 kb deletion including two genes: FERD3L and TWIST1. Despite the large difference in deletion size, both patients showed striking similarity in their phenotypic appearance aside from psychomotor development in the lower normal range in patient 2. Surprisingly, the phenotypically normal mother of this patient also carried the small 526 kb deletion. Patient 3 carrying a 9.2 Mb deletion encompassing 35 genes including TWIST1 was 16 year old at time of examination. The patient underwent surgery for his craniosynostosis in childhood. He presented with mental retardation and syndactyly but the facial appearance did not meet that of the Saethre-Chotzen syndrome. He showed microcephaly, blepharophimosis, hypertelorism, upslanting palpebral fissures, epicanthus, and prominent nasal bridge.
In our report we illustrate the high variability of the clinical pattern of patients carrying submicroscopic deletions containing TWIST1. Even in patients not showing a typical Saethre-Chotzen syndrome phenotype TWIST1 deletions can be identified. Genome-wide screening by array-CGH is a powerful tool to detect and precisely determine the size of these aberrations.

P-ClinG-046

A novel sporadic COL4A1 mutation causes intracerebral hemorrhage in a healthy young man

Coutts S.B.[1], Matysiak-Scholze U.[2], Kohlhase J.[2], Innes A.M.[3]
[1]Department of Clinical Neurosciences, University of Calgary, Canada, [2]Center for Human Genetics, Freiburg, Germany, [3]Department of Medical Genetics, University of Calgary, Canada

We describe a previously healthy man who presented with an intracerebral hemorrhage, abnormal white matter and cerebral microbleeds. Cerebral angiography revealed that the distal small arteries were tortuous and irregular. A novel, sporadic COL4A1 mutation, c.1537-2delA in intron 24 was subsequently detected in a heterozygous state. This mutation leads to an in-frame deletion of exon 25 and results in deletion of 64 amino acids within the triple-helical domain of the COL4A1 protein. Mutations in COL4A1, the gene encoding the Type IV collagen alpha chain, are an important cause of intracerebral hemorrhage in young people. The possibility of asymptomatic gene carriers and sporadic cases makes this a potentially under-diagnosed condition. This case highlights considering genetic conditions even when the clinical presentation is 'sporadic'.

P-ClinG-047

Expansion of the STAR syndrome phenotype and evidence for genetic heterogeneity

Craig A.[1], Zoll B.[2], Rakenius A.[3], Zappel H.[3], Leube B.[4], Bartz U.[5], Heimdal K.[6], Gener B.[7], Borozdin W.[8], Buiting K.[9], Cobben J.M.[10], Kohlhase J.[8]
[1]Institut für Humangenetik, Universität Freiburg, Germany, [2]Institut für Humangenetik, Universität Göttingen, Germany, [3]Kinderklinik, Universitätsklinikum Göttingen, Germany, [4]Institut für Humangenetik, Universität Düsseldorf, Germany, [5]Praxis für Humangenetik, Universität Düsseldorf, Germany, [6]Dept. for Medical Genetics, Rikshospitalet Oslo, Norway, [7]Hospital de Cruces, Baracaldo, Spain, [8]Praxis für Humangenetik, Freiburg, Germany, [9]Institut für Humangenetik, Universitätsklinikum Essen, Germany, [10]Emma Kinderziekenhuis AMC, Amsterdam, The Netherlands

We have recently delineated the STAR syndrome (Syndactyly, Telecanthus, Anogenital and Renal malformations) phenotype and showed that it results from mutations in the Cyclin family member FAM58A on Xq28. STAR syndrome is extremely rare, and since our original report we collected four further female patients who presented with the characteristic extensive syndactyly of the feet as well as with imperforate anus and further malformations. Of those four patients, two showed a larger multi-exon deletions in FAM58A and one had a novel nonsense mutation, whereas one patient did not show any FAM58A change. The latter also did not show any skewing of the X-inactivation found previously in all patients with FAM58A mutations. Novel features include sacral dysgenesis (2 cases), tumor formation (2 cases), radio-ulnar synostosis (1 case), strabism and Duane anomaly (1 case), retinal hyperpigmentation (1 case), and pulmory valve stenosis (1 case). Two of the three mutation positive patients have compensated renal failure. The patient without a FAM58A defect showed the characteristic toe syndactyly, telecanthus and imperforate anus but no genital or renal malformations and no renal impairment. She had normal CGH array and normal X inactivation studies. To summarize, our results suggest (1) that FAM58A defects are associated with a very high risk for development of renal failure, since all patients known to us older than 10 years developed renal insufficiency, (2) that FAM58A is relevant for the development of the sacral region and (3) that there is likely a similar disease with autosomal (dominant?) inheritance with no renal and genital malformations, identifiable by normal FAM58A studies and normal X-inactivation status.

P-ClinG-048

Myotonia congenita type Becker caused by a putative Roma founder mutation

Czermin B.[1], Horvath R.[1], Lynch B.[2], Shuibne N.[2], Viellieber M.[3], Rating D.[4], van Almsick A.[5], Vorgerd M.[5], Holinski-Feder E.[1], Abicht A.[1]
[1]Medizinisch Genetisches Zentrum, Munich, Germany, [2]Central Remedial Clinic; Clontarf, Dublin, Ireland, [3]Kinderklinik St. Annastift, Ludwigshafen, Germany, [4]Abt. Päditrische Neurologie, Universitätskinderklinik Heidelberg, Germany, [5]Neurologische Universitäts- und Poliklinik, Bochum, Germany

Myotonia congenita is a hereditary muscular disease characterized by slow relaxation (myotonia), hypertrophy, and stiffness of skeletal muscles. Muscle membrane hyperexcitability is caused by reduced chloride conductance due to mutations in CLCN1, the gene coding for the main skeletal muscle chloride channel ClC-1. The disorder is inherited as either autosomal dominant or recessive known as Thomsen and Becker diseases, respectively. In contrast to a number of other neuromuscular disorders known to be frequent among the Roma (Gypsies), Myotonia congenita is so far not known to have an increased incidence in this population.
Here we genetically confirm the clinical diagnosis of autosomal recessive Myotonia congenita in three independent Roma families from different parts of Europe. By PCR and direct sequencing of the CLCN1 gene we identified the same novel splice mutation in the index patients of all three families (c.562+1G>C). This mutation affects the highly conserved splice donor of exon 4 and therefore is predicted to result in aberrant splicing with the consequence of a non-functional channel protein. All affected individuals are homozygous for the disease causing mutation and heterozygous carriers do not report clinical signs of myotonia which is compatible with autosomal recessive inheritance. In concordance with the phenotype of other patients harbouring loss-of-function mutations in the CLCN1 gene the affected patients all exhibit clinical and electrophysiological signs of myotonia since childhood in some with marked muscular hypertrophy. SNP analysis indicates that all affected individuals share a common haplotype which may indicate a founder mutation.
The 8-10 million European Roma/Gypsies are a founder population of common origins that has subsequently split into multiple socially divergent and geographically dispersed groups. Genetically, a string of population bottlenecks and founder effects characterize the Gypsies as a founder population comprising multiple subisolates with differences in mutation frequencies and haplotype divergence. The awareness that Myotonia congenita might be another founder disease of the Roma might lead to the identification of further patients and possibly specific subisolates of high risk due to an increased carrier frequency and facilitate genetic testing and counseling.

P-ClinG-049

Detection and characterization of two sibs with partial trisomy 3q resulting from maternal inherited interchromosomal insertion ins(6;3)(q16.3;q11.2q13.31) by conventional and molecular karyotyping

Dietze-Armana I., Böhrer-Rabel H., Rettenberger G., Schneider M., Mehnert K.
genetikum, Neu-Ulm, Germany

Insertional translocations (IT) are rare chromosomal rearrangements with an 1:80,000 incidence (Van Hemel&Eussen 2000), in which three breaks are required, so an interstitial segment from one chromosome is inserted into one of the arm of another chromosome. Patients with abnormal phenotype are associated with either pure monosomy ore pure trisomy of the inserted segment. Balanced carriers have a high risk of unbalanced offspring, theoretically up to 50%.
Here we report two affected children with the same cytogenetically unbalanced IT inherited from the mother. Probands were studied by cytogenetic analysis, FISH technique and 105k oligo-array-CGH. Chromosome analysis of cultured amniofluid cells caused by advanced maternal age displayed an unbalanced IT der(6)ins(6;3)(q16.3;q11.2q13.31) in the fetus. Parents decided to continue the pregnancy after comprehensive genetic counselling. The same aberration was also found in the 7y old brother with mild dysmorphic signs, borderline mental retardation and speech developmental delay inherited from the unaffected mother with balanced ins(6;3)(q16.3;q11.2q13.31). Array-CGH confirmed the cytogenetic results of partial trisomy 3q11.2->q13.31 and defined the size of 20,4Mb including about 108 genes. Furthermore, array-CGH revealed an additionally small deletion of 475kb including part of the gene GRIK2 (MIM138244) in the region of the insertion-breakpoint 6q16.3 in both affected offspring which has been enlarged compared to the mother, who showed only a 190kb deletion on 6q16.3. Interpretation of data and genetic counselling in this family is difficult, nevertheless, correct identification is important because of the high risk associated with insertions and possible recombination during meiosis. Array-CGH helps to detect relevant genes which may be important for interpretation and identification their function in this way. These new findings have implications in genetic counselling and patient management.

P-ClinG-050

Genotype-phenotype associations in autosomal recessive cutis laxa syndromes and their contribution to differential diagnosis

Dimopoulou A.[1], Rajab A.[2], Kayserili H.[3], Al-Gazali L.[4], Brancati F.[5], van Maldergem L.[6], Robertson S.[7], Horn D.[1], Mundlos S.[1], Fischer J.[8], Morava E.[9], Kornak U.[1]
[1]Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, Germany, [2]Genetic Unit-DGHA-Ministry of Health, Muscat, Sultanate of Oman, [3]Medical Genetics Department, University of Istanbul, Turkey, [4]Department of Paediatrics-Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates, [5]Ospedale Casa Sollievo della Sofferenza-San Giovanni Rotondo and Istituto CSS-Mendel, Rome, Italy, [6]Centre de Génétique Humaine-Centre Hospitalier Universitaire du Sart-Tilman, Université de Liège, Belgium, [7]Department of Paediatrics and Child Health, University of Otago-Dunedin, New Zealand, [8]CEA-Institut de Génomique-Centre National de Génotypage-Dermatologic Diseases, Evry, France, [9]Department of Pediatrics-Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

The term cutis laxa denotes a skin anomaly with loose, redundant skin folds and abnormal elasticity of the skin. Among the different forms of congenital cutis laxa, the autosomal recessive cutis laxa (ARCL) appears to be the most heterogeneous regarding either the clinical picture as well as the underlying molecular defects. Here we propose a diagnostic pathway, which could contribute to a more effective differential diagnosis of ARCL subtypes. Common features of all ARCL-phenotypes are inelastic and wrinkly skin, joint laxity, delayed motor development and fragmented elastic fibers in skin biopsies. Involvement of inner organs can differentiate the main types of ARCL (I, II and III). At the most severe end of the spectrum lies type I, showing lung disease and anomalies of the vascular, gastrointestinal and genitourinary system. These patients were found to bear mutations in fibulin-5 (FBLN5) or in fibulin-4 (EFEMP2). The milder phenotypes of ARCL Type II (Debré Type) and Wrinkly Skin Syndrome (WSS), were found to be caused by mutations in the gene encoding the a2 subunit of the V-type H+ ATPase (ATP6V0A2). These patients show a combined N- and O-glycosylation defect (Congenital Disorder of Glycosylation, CDG type II). This feature is unique and therefore facilitates differential diagnosis, which is especially useful for the differentiation from the highly similar phenotype of ARCL with progeroid appearance, also referred to as ARCL Type IIb. These patients show the most prominent mental retardation among the cutis laxa phenotypes and were recently found by our group to bear mutations in the pyrroline-5-carboxylate reductase gene (PYCR1). Individuals affected by PYCR1 mutations have been previously categorized variably as WSS, de Barsy syndrome and gerodermia osteodysplastica. However, patients with bona fide gerodermia osteodysplastica caused by

mutations in GORAB (SCYL1BP1) do usually not show cognitive impairment but a more severe bone phenotype with osteoporosis and increased fracture risk. The molecular basis of several cases categorized as de Barsy syndrome (DBG; also referred to as ARCL type III) not showing PYCR1 mutations remains unknown. DBG overlaps with ARCLII, but shows no signs of CDG. Besides a prominent mental retardation an important hallmark is corneal clouding. Although ARCL comprises a heterogeneous group of phenotypes, we think that a reliable differential diagnosis based upon these clinical and biochemical features is possible.

P-ClinG-051

Molecular characterisation and phenotypic presentation of a large family with isolated thoracic aortic aneurysms and dissections (TAAD) caused by a novel missense mutation in the TGFBR2 gene

Doelken S.C.[1], Horn D.[1], Fahsold R.[2], Mundlos S.[1], Robinson PN.[1]
[1]Institute of Medical Genetics Charité, Berlin, Germany, [2]Laboratory Prager & Junge for Medical Genetics, Dresden, Germany

Approximately 20% of thoracic aortic aneurysms and dissections result from a genetic predisposition. Thoracic aortic aneurysms are the cardinal feature of connective tissue diseases such as Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome vascular type (EDS type IV), and congenital contractural arachnodactyly. Nevertheless, familial thoracic aortic aneurysms and dissections (TAAD) also occur as an isolated trait in families without other anomalies of a connective tissue disease, further complicating the process of differential diagnosis for affected families. Inherited as an autosomal dominant trait with decreased penetrance, onset and rate of progression of aortic dilation is highly variable, even within families. Diverse mutations in genes such as TGFBR1, TGFBR2, FBN1, MYH11 and ACTA2 have been described in families with isolated familial aortic aneurysms. Mutations in the TGFBR2 gene are responsible for approximately 5% of these cases, which according to current nomenclature are then specified as TAAD2. Mutations previously found in familial TAAD2 typically affected the amino acid arginine at position 460 (NM_003242).
We report on a large four generation family including four individuals with aortic aneurysms and a woman considered to be an obligate carrier. Performing mutation analysis by direct sequencing we detected a novel missense mutation in the protein kinase domain of the TGFBR2 gene at position 525 (p.Pro525Thr) not previously described in patients with Loeys- Dietz syndrome or isolated TAAD. In the past, biochemical analysis performed by Carcamo et al (1995) demonstrated loss of kinase activity by a proline to leucine missense mutation at the same position. The Pro525Thr mutation fully segregates with the phenotype in our family and supports the diagnosis of TAAD2. Cerebral strokes and unexplained syncopal episodes occurred in some affected family members and a female considered to be an obligate carrier apparently died at the age of 53 years after three cerebral strokes. Further surveillance of cerebral arteries in affected family members was recommended but presence of cerebral aneurysms could not be confirmed in any family member so far.
The family reported here illustrates the importance of determining the correct diagnosis of TAAD or other forms of isolated aneurysmal arterial diseases in families with absence of other distinctive phenotypic features. Risks for either aortic aneurysms, cerebral aneurysms or multiple arterial aneurysms may vary depending on the underlying disease causing mutation. With appropriate surveillance, aortic dissection or rupture can be prevented by prophylactic repair of aneurysms once the dilation progresses to a critical point, usually when the aortic diameter reaches about 5.0 cm. However there are indications that individuals with mutations in the TGFBR2 gene may be at increased risk of rupture or dissection at aortic diameters less than 5 cm. Therefore making the correct molecular genetic diagnosis may be critical for surveillance as well as treatment of manifestations especially concerning the decision on whether and when to perform surgery for aneurysms occurring in affected members of these families.
We will report in detail on clinical and molecular genetic aspects of our family and review other forms of isolated thoracic aortic aneurysms.

P-ClinG-052

Detection of a 1,4 Mb duplication on 17p13 with the distal breakpoint disrupting the LIS1 gene in a female patient with lissencepahly

Drechsler M., Schaefer N., Classen S., Goecke T., Gebauer H.-J., Beier M., Royer-Pokora B.
Institute of Human Genetics and Anthropology, Duesseldorf, Germany

We describe a female patient with mental retardation, dysmorphic signs e.g. short nose, hypertelorism, long philtrum, uvula bifida and muscular hypotonia and lissencephaly.
Classical cytogenetic analysis showed an interstitial deletion on chromosome 20p which was described as a mosaic of 80 %. Due to the clinical finding of lissencephaly a FISH analysis with the Miller-Dieker probe (17p13) was performed, but a microdeletion was excluded.
To confirm the cytogenetic results and to look for smaller aberrations an array-based comparative genomic hybridisation (aCGH) was determined. The 244K Agilent array we used presents 60mer oligonucleotides covering the whole genome with an average distance of 6,5 kb.
Although a mosaic of 80 % would be detected with an array analysis, the cytogenetic result could not be confirmed. Instead the patient showed a 1,4 Mb duplication with the proximal breakpoint disrupting the LIS1 gene. This implicates a disturbance of the LIS1 gene causing the clinical phenotype of the patient.

P-ClinG-053

Screening of breast cancer families for Fanconi anemia mutations

Eirich K.[1], Gehrig A.[1], Schuster B.[1], Hoehn H.[1], Schindler D.[1]
[1]Department of Human genetics, Wuerzburg, Germany

Breast cancer is the most common cancer in women. Inherited monoallelic mutations in BRCA1 and BRCA2 are frequently underlying familial cases. In addition, germline mutations in other DNA caretaker genes have been reported to increase the risk for breast and ovarian cancer. Three of those susceptibility genes were found to be identical to Fanconi anemia (FA) genes: BRCA2 is FANCD1, BRIP1 (BACH1) is FANCJ and PALB2 is FANCN. FA is a rare autosomal or X-chromosomal recessive disease with great genetic and phenotypic heterogeneity. FA is characterized by bone marrow failure, high cancer risk and various, non-obligatory, yet typical developmental anomalies. Hypersensitivity of FA cells to DNA crosslinking agents such as mitomycin C results in chromosomal breakage and G2-phase arrest in the cell cycle. To date, 13 FA genes have been identified: FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M and -N. Their products and additional proteins interact in the FA/BRCA DNA damage response network. While biallelic mutations lead to FA, monoallelic mutations in three of these genes increase the risk for breast cancer up to 85%. Notwithstanding the high general risk for cancer, FA patients do not show particularly high rates of breast cancer. In this study, we investigated twenty families that gained our attention because of increased incidence of breast and ovarian cancer. From each family we selected one affected woman for analysis. Initially we screened for mutations in the most common breast cancer genes, BRCA1 and BRCA2. Because all of the twenty women lacked mutations in these two genes, we extended our attempt to identify other disease-causing genes in these families. Because of the relationship of breast cancer susceptibility to FA genes we started sequencing of the known FA genes and other genes associated with the FA/BRCA pathway for genomic stability. These genes included PALB2 and the RAD51 family of genes involved in homologous recombination. Apart from a frequently occurring annotated single nucleotid polymorphism in RAD51C we did not yet disclose any change. This ongoing study is intended to include screens of additional genes of the FA/BRCA DNA damage response network.

P-ClinG-054

An unusual case of terminal monosomy 11q and terminal duplication 16q in a boy with ocular coloboma

Evers C.[1], Janssen J.W.G.[1], Jauch A.[1], Holzmann-Mau U.[2], Bonin M.[2], Moog U.[1]

[1]Institute of Human Genetics Heidelberg University, Heidelberg, Germany, [2]Department of Medical Genetics Tübingen University, Tübingen, Germany

Background: The 11q terminal deletion disorder (Jacobsen syndrome) is a contiguous gene syndrome caused by a terminal deletion of 11q. The deletion size varies between ~5 to 20 Mb and the proximal breakpoint is localised within or telomeric to 11q23.3. The spectrum of clinical symptoms is variable and depends on the extent of the deletion. Typical clinical signs include developmental delay, pre- and postnatal growth retardation, thrombocytopenia or pancytopenia, cardiac defects, trigonocephaly and dysmorphic facies. Further common findings are gastrointestinal, genitourinary, ocular, hearing and immunological problems. In contrast to the 11q terminal deletion disorder, the phenotype of a terminal duplication of 16 is less well defined. It shows an unspecific picture of varying malformations and mental retardation.
Case report: We describe a one year and two month old boy with unilateral ocular coloboma and mild muscular hypotonia at birth without any other anomalies or dysmorphic features. He was found to have a partial deletion of chromosome 11q25-qter and a partial duplication of chromosome 16q22.3-qter due to a reciprocal translocation t(11;16)(q25;q22.3) in his father. Using high resolution SNP array analysis the proximal deletion breakpoint in 11q was mapped between 132.251.238 and 134.449.982 bp. The terminal 11q deletion has a size of ~2.2 Mb and contains 21 genes. The proximal deletion breakpoint in 16q was localised between 73.092.442 and 88.815.024 bp, resulting in a ~15.7 Mb terminal duplication of 16q.
Discussion: To our knowledge this is the smallest terminal 11q deletion reported to date. Interestingly no typical features of Jacobson syndrome or other congenital anomalies were found in this patient, except for the ocular coloboma. This suggests that the critical region responsible for the clinical abnormalities of Jacobson syndrome is localised proximal to 11q25. Additionally, our case report shows that a duplication of 16q22.3-qter can be associated with only minor anomalies.

P-ClinG-055

Deletion Xp22.11: PTCHD1 is a candidate gene for X-linked complex neurodevelopmental disability without recognizable physical phenotype

Filges I.[1], Weber P.[2], Röthlisberger B.[3], Wenzel F.[1], Huber AR.[3], Heinimann K.[1], Miny P.[1]

[1]Division of Medical Genetics University Children's Hospital and Department of Biomedicine, Basel, Switzerland, [2]Division of Neuropediatrics and Developmental Medicine University Children's Hospital, Basel, Switzerland, [3]Center of Laboratory Medicine Cantonal Hospital, Aarau, Switzerland

Copy number variation has been shown to play a significant role in the etiology of developmental disabilities including non-syndromic intellectual impairment and/or autism disorders.
We describe a family of Italian origin compatible with a history of X-linked complex developmental disability. The index patient, fourth child of healthy non-consanguineous parents, is a non-dysmorphic 6.5 year-old boy. He presents with severe global developmental delay. He was born at 38 weeks of gestation after an uneventful pregnancy. Birth weight was 3750 g (P90), length was 51 cm (P90). At 6 months celiac disease was diagnosed, all developmental milestones were significantly delayed with free walking at 3.5 years and absent expressive speech until today, limited receptive speech, generalized persistent muscular hypotonia and ataxic movement patterns since the age of 2.5 years. Autistic traits noticed since the age of 3 years improved, and the boy shows limited emotional non-verbal contact today. Toilet training has not been successful to date. Growth parameters are in the upper normal range (P75-90). Mild cerebral atrophy was suspected at MRI at 3.5 years, EEG was normal.
The eldest child is a 20-year-old man, non-dysmorphic, presenting with severe intellectual disability (IQ 30-40) compatible with an intellectual development at 5 years. Generalized psychomotor retardation has been recognized since early childhood with delayed walking at 2.5 years, first words at 4.5 years. At 16 years he was able to write his name and to read words with 4-5 letters. He continues to present with hypotonia of upper and lower extremities as well as behavioural problems. The 19-year-old daughter has passed basic school and professional education with considerable difficulties. A 14-year-old son is healthy and attending normal school with success, his development was within normal range.
Standard karyotyping revealed normal chromosome count 46,XY in the index patient. Array-CGH analysis (NimbleGen HG18 WG Tiling 385k CGH v2.0) showed a small 200kB interstitial deletion on the short arm of chromosome X. High-resolution X-chromosomal specific array analysis (NimbleGen HG18 WG, median probe spacing 340 bp) refined the breakpoints to a telomeric breakpoint between 23,185,818 und 23,190,300 bp and the centromeric breakpoint between 23,381,367 und 23,387,114 bp.
The deleted region can be cytogenetically described as 46,XY,del(X)(p22.11). The mother is a heterozygous carrier of the identical deletion whereas the deletion is absent in her healthy son. Analysis of her affected eldest son, whom we suspect to harbour the familial deletion, is ongoing. A possible heterozygous manifestation for the daughter has been discussed.
Interestingly, the deleted region comprises only one gene, PTCHD1, a highly conserved gene coding for the patched-domain-coding-protein 1 playing a role in hedgehog receptor activity. No clear disease-phenotype has been related to the gene today. As the transmembrane protein patched transduces hedgehog signals and as its expression ratio is highest in the cerebellum a functional consequence for the neurodevelopmental phenotype can be suggested.
Nullisomy of PTCHD1 is therefore likely to cause the male phenotype with severe intellectual disability and speech impairment. Thus PTCHD1 can be considered as a candidate gene for X-linked complex neurodevelopmental disability without recognizable physical anomalies. Our findings support recent suggestions of an inherited deletion (Marshall et al. 2008) or rare sequence variants in PTCHD1 (Noor et al., Abstract 225, ASHG2008) to be causative of autism disorders.

P-ClinG-056

Novel mutations of the AR gene result in complete androgen insensitivity syndrome (CAIS) in two unrelated patients

Fleischer S.[1], Groß U.S.[1], Möller-Krull M.[1], Wittmann S.[1], Richter-Unruh A.[2], Schulte H.M.[1]

[1]ENDOKRINOLOGIKUM Hamburg; Center for Hormonal and Metabolic Diseases; Reproductive Endocrinology and Prenatal Medicine, Hamburg, Germany, [2]ENDOKRINOLOGIKUM Ruhr; Center for Hormonal and Metabolic Diseases, Wattenscheid, Germany

Introduction: Complete androgen insensitivity is characterized by an endogenous unresponsiveness to androgenic steroids in 46,XY individuals due to mutations of the androgen receptor. In CAIS patients completely female habitus is achieved by aromatization of high testosterone levels to estrogens. Less severe disruptions of androgen receptor function (partial androgen insensitivity syndrome, PAIS) results in more mildly affected patients, characterized by incomplete male sex differentiation to varying degrees. We here describe three German females suspected to have CAIS.
Patients: Three unrelated girls were referred to the department of pediatric endocrinology due to primary amenorrhea at ages of 14, 17 and 19 years. All three presented with female external genitalia, breast stages Tanner B 4-5, pubic hair stages P1. Gonads were bilaterally seen in the inguinal regions by ultrasound. Karyotype was 46,XY. Hormonal

evaluation revealed markedly elevated gonadotropin levels with a high testosterone.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding regions (exons 1-8) and corresponding exon-intron boundaries of the AR gene. PCR products were sequenced directly. CAG repeats have been determined by fragment length analysis.
Results: Sequence analysis of the AR gene of the first patient showed a novel hemizygous germline deletion of one nucleotide in Codon 780 that results in a frame-shift leading to premature terminal signal in exon 6. The sister (46,XX) of this patient was identified as carrier of this single nucleotide deletion mutation.
Furthermore the sequence analysis of the AR gene of the second patient showed a novel hemizygous germline mutation in exon 4, codon 682 (GAG>GAT), leading to the substitution of glutamic acid (Glu) to aspartic acid (Asp) and therefore to an alteration of the amino acid sequence within the enzyme.
Sequence analysis of the AR gene of the third patient showed the novel hemizygous germline splice site mutation c.2173+1G>C in intron 4.
Conclusion: These findings suggest that the previously undescribed mutations in the AR gene are the cause of CAIS in these patients. For this purpose genetic counseling and recognition of germline mutations within the AR gene is important to confirm the diagnosis, to provide the basis for therapeutic decisions and to define asymptomatic gene carriers.

P-ClinG-057
MECP2 duplication syndrome - a frequent finding in patients with severe mental retardation, seizures, spasticity and absence of speech
Franke K.[1], Demuth S.[2], Weidensee S.[2], Fahsold R.[1]
[1]Mitteldeutscher Praxisverbund Humangenetik, Dresden, Germany, [2]Mitteldeutscher Praxisverbund Humangenetik, Erfurt, Germany

The Xq28 chromosomal region is frequently affected by rearrangements. The duplication of MECP2 gene has been recently discovered, and thus only about 50 affected males have been reported. The clinical phenotype of MECP2 duplication syndrome is consistent in all reports and encompasses severe mental retardation, absence of or severely retarded speech, hypotonia, progressive spasticity, recurrent respiratory infections and seizures. Until now, all affected males have inherited the MECP2 duplication from their mother. Most females heterozygous for MECP2 duplication are asymptomatic because of an extreme up to complete skewing of X-chromosome inactivation.
The majority of Xq28 duplications varies in size from 0,2 to 1,3 Mb and normally includes at least the MECP2 and the L1CAM gene. Thereby, the severity of the phenotype appears to be independent from the size of the duplication. Several previous studies suggest that the similar phenotype of all patients is mainly due to overexpression of MECP2.
Here, we report the identification of submicroscopic duplications in Xq28 in three non-related male patients out of a cohort of fifteen. Each of the duplication, identified by MLPA (Multiplex Ligation-dependent Probe Amplification), shows different expansions distal and proximal of the MECP2 gene. Due to the frequent finding (3/15) of this aberration in our male patients with severe mental retardation and neurological symptoms, the testing of the DNA copy number in the MECP2 region should be included in the current diagnostic testing in this group of patients and in their female relatives.

P-ClinG-058
Mutation analysis of the genes AHI1, CEP290 and TMEM67/MKS3 in patients with Joubert syndrome and related disorders.
Frost S.[1], Korenke GC.[2], Marquardt I.[2], Kläs R.[3], Janecke A.[4], Kohlhase J.[5], Goecke T.[6], Uyanik G.[7], Güzünler M.[8], Rudnik-Schöneborn S.[9], Gillessen-Kaesbach G.[1], Hellenbroich Y.[1]
[1]Institut für Humangenetik, Lübeck, Germany, [2]Klinik für Neuropädiatrie und Stoffwechselerkrankungen,Oldenburg, Germany, [3]Zentrum für Humangenetik, Mannheim,Germany, [4]Division of Clinical Genetics and Department of Pediatrics II, Innsbruck, Austria, [5]Praxis für Humangenetik, Freiburg, Germany, [6]Institut für Humangenetik und Anthropologie, Düsseldorf, Germany, [7]Institut für Humangenetik, Hamburg, Germany, [8]Department of Pediatrics, Aydin, Turkey, [9]Institut für Humangenetik, Aachen, Germany

Joubert syndrome (JS) and related disorders (JSRD) are autosomal recessive conditions characterised by developmental delay, ataxia, hypotonia, irrgeular breathing pattern, eye movement abnormalities and cerebellar vermis hypoplasia with accompanying brainstem malformations resulting in the "molar tooth sign" on axial images. Other variable frequent features include retinal dystrophy, coloboma, cystic renal disease, hepatic fibrosis and polydactyly. JS and JSRD are genetically heterogeneous disorders, so far 9 different causitive genes have been identified. We analysed 23 patients with JS or JSRD for mutations within the genes AHI1 (JBTS3), CEP290 (JBTS5) and TMEM67 (JBTS6). We found mutations within the AHI1 gene in 2 patients, within the CEP290 gene in 4 patients and within the TMEM67 gene in 4 patients. In 5 of the 10 cases consanguinity of the parents was reported, and initial genome-wide linkage analyses were helpful to highlight one disease gene in 2 of the 5 consanguineous families. 3 of the 4 patients with mutations within the TMEM67 gene had the clinical diagnosis of COACH syndrome (Cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma and hepatic fibrosis). In conclusion, mutations in these 3 genes seem to be the most frequent known causes of JS and JSRD. The TMEM67 gene should be analysed first in patients with COACH syndrome. In consanguineous families a homozygosity mapping before starting a mutation analysis should be considered.

P-ClinG-059
Clinical Genetics of Möbius Syndrome
Gaspar H.[1], Chang S.[2], Scott S.[3], Edelmann L.[3], Jabs E.W.[3]
[1]Institute of Human Genetics Heidelberg University, Heidelberg, Germany, [2]Johns Hopkins University, Baltimore, USA, [3]Mount Sinai School of Medicine Department of Genetics and Genomic Sciences, New York, USA

Background: Möbius Syndrome is a rare, congenital disorder with partial or complete agenesis of the 6th and 7th cranial nerves (CN), which control eye movements and facial expression. There is a broad spectrum of associated features such as limb malformation and mental retardation. This heterogeneous condition is usually sporadic, but can be inherited in a dominant or recessive manner. Different abnormal karyotypes have been reported in patients with Möbius Syndrome which may give clues to the location of genes that can cause Möbius syndrome.
Hereditary Congenital Facial Paresis (HCFP) involves only the 7th cranial nerve and loci have been identified at 3q21-q22 and 10q21.3-q22.1. There are overlapping features for Möbius and HCPF and to date, a clear clinical definition of both has been in dispute.
Methods: Our goal is to better define both the spectrum of features associated with Möbius syndrome and HCFP and the clinical diagnostic criteria by reviewing the so far largest cohort of 75 patients referred by the Möbius Syndrome Foundation. Clinical information was obtained from a questionnaire, medical records, and physical examinations on patients. In order to find candidate regions, we also screened 18 patients with Möbius Syndrome and different associated features and their parents by high resolution array CGH (244K array, Agilent).

Results: 60 patients (80%) had the classic Möbius features of 6th and 7th CNs, and 15 patients (20%) had atypical features with 7th CN involvement only. We observed 3 families with autosomal dominant and 4 families with autosomal recessive inheritance. Classic Möbius Syndrome patients are not clearly distinguishable from HCFP patients, but there are three exceptions: vision problems (univariate logistic regression, p value 0.015) and clubfeet (p value 0.003) seem to be more often associated with the Möbius phenotype and familial occurrence is more common in patients with 7th CN only (p value 0.0025).
By array CGH, we did not find consistent aberrations in 18 complex cases, but we identified specific aberrations in multiple patients involving regions of chromosomes 4, 5, 7, 9, 10 and 14.

P-ClinG-060

Co-occurrence of two microaberration-syndromes in a patient with mental-retardation and multiple congenital anomalies

Goehring I.[1], Wiesener A.[1], Ekici A. B.[1], Rauch A.[2], Reis A.[1]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute of Medical Genetics, Schwerzenbach-Zurich, Switzerland

We report on a 26 month old boy presenting with delay of speech development, discrete right sided hemiparesis, generalized muscular hypotonia, anal stenosis, celiac disease, bilateral inguinal hernia, and unilateral undescended testicle. He also showed dysmorphic facial features including a high forehead, epicanthal folds, low set ears, a broad nasal bridge, a bulbous nasal tip, retrognathia, a wide mouth and discrete facial asymmetry. High resolution GTG-banding showed a normal karyotype and fragile X-syndrome testing was unremarkable. Molecular karyotyping using an Affymetrix GeneChip SNP array 6.0 revealed a 2.7 Mb microduplication in 22q11.21 and a 1.5 Mb microdeletion in 17q12. The 22q11.2 duplication was confirmed by MLPA using the P250-Kit from MRC Holland and the 17q12 deletion was confirmed by fluorescence in situ hybridization (FISH) with the BAC probe RP11-27K22. MLPA and FISH were also performed on the parents' samples demonstrating that both well described microaberrations had occurred de novo.
The duplication of the typical DiGeorge region in 22q11.2 is associated with mental retardation of variable degree (97%), delayed psychomotor development (67%), growth retardation (63%), muscular hypotonia (43%), seizures, behavioural problems and malformations such as heart defects and cleft palate. The recurrent 17q12-deletion- or RCAD syndrome (renal cysts and diabetes) encompasses a wide clinical spectrum comprising developmental kidney abnormalities such as renal cysts and renal dysplasia usually present in early life, and diabetes (MODY 5) typically diagnosed in the early 20s, as well as uterine or genital anomalies like undescended testicles. The current phenotype of the patient is compatible with the duplication 22q11.2 syndrome, but renal cysts and diabetes have to be expected in the further course due to the 17q12 microdeletion. This observation demonstrates that co-occurrence of common microaberration syndromes may modify the phenotype. We assume that such double de novo events may be underdiagnosed in cases specifically tested for one microaberration syndrome.

P-ClinG-061

Primary ovarian failure due to two novel mutations in the FSHR gene

Groß U.S.[1], Dornauf I.[2], Mann W.A.[2], Fleischer S.[1], Wittmann S.[1], Möller-Krull M.[1], Schulte H.M.[1]
[1]Endokrinologikum Hamburg; Center for Hormonal and Metabolic Diseases; Reproductive Endocrinology and Prenatal Medicine, Hamburg, Germany, [2]Endokrinologikum Frankfurt; Center for Hormonal and Metabolic Diseases and Reproductive Endocrinology, Frankfurt, Germany

Introduction: The follicle-stimulating hormone (FSH) and the FSH receptor (FSHR) are essential for normal development and reproduction in females and males. In females FSH regulates follicle maturation and estrogens production by granulosa cells. Furthermore inactivating mutations within the FSHR gene are known to be the cause of primary and secondary amenorrhea, hypergonadotropic hypogonadism and ovarian hyperstimulation syndrome in rare cases.
Patient: The index case is a 18-year-old female patient. She was referred to our center with primary amenorrhea. An endocrine analysis confirmed hypergonadotropic hypogonadism. Her medical record showed normal start of pubarche and thelarche at the age of 13 years but primary amenorrhea. She also had a medical history of anorexia nervosa during the last years. So nobody wondered about the primary amenorrhoea.
The patient's parents and her brother have shown no symptoms of hypergonadotropic hypogonadism until now and are apparently healthy (clinically and biochemically).
Genetic testing for the novel mutation was done on the parents subsequent to the results from the patient.
Methods: Genomic DNA was extracted from peripheral blood leukocytes. The entire coding region (10 exons) of the FSHR gene including corresponding exon-intron boundaries was amplified by PCR amplification, followed by direct sequencing.
Genetic testing for the novel mutations was done on the parents and the brother subsequent to the results from the patient.
Results: Sequencing analysis of the FSHR gene revealed two heterozygous mutations in exon 10 (p. Ala501Thr and p.Tyr626Stop) of the patient. Suspected compound heterozygosity was confirmed by genetic testing of the healthy patents for these mutations.
Conclusion: These data confirm the importance of mutations within the FSHR gene in primary ovarian failure and FSH resistance in females. We therefore recommend that existence of FSHR mutations should be considered in the clinical workup and genetic counselling of these patients.

P-ClinG-062

High frequency of mutations in THAP1 (DYT6) in patients with sporadic dystonias

Grundmann K.[1], Glöckle N.[2], Soehn A.[2], Duarte Doetzer A.[2], Deuschl G.[3], Felber U.[4], Topka H.[5], Schoels L.[6], Riess O.[1], Müller U.[7], Bauer P.[1]
[1]Dept.of Medical Genetics, Tuebingen, Germany, [2]Dept. of Medical Genetics, Tuebingen, Germany, [3]Dept. of Neurology, University of Kiel, Germany, [4]Institute of Human Genetics, University of Greifswald, Germany, [5]Department of Neurology and Clinical Neurophysiology, Academic Hospital Bogenhausen, Germany, [6]Hertie-Institute for Clinical Brain Research, University of Tuebingen, Germany, [7]Institute of Human Genetics, Giessen, Germany

Dystonias are a heterogeneous group of movement disorders which are clinically characterised by involuntary muscle contractions causing twisting movements and abnormal postures.
Whereas most cases (~ 70%) of early-onset dystonia are caused by a mutation in the DYT1/TOR1A gene, the majority of cases of adult-onset forms appear to be sporadic and only a small proportion seem to have a genetic basis. Unlike DYT1 dystonia, DYT6 dystonia has a later onset and primarily involves the craniocervical muscles. Most patients have some degree of progression to other body regions, but final distribution varies widely and includs focal dystonia, segmental dystonia, and generalized or multifocal dystonia.
Mutations in THAP1 have recently been shown as the cause of DYT6 dystonia. To further investigate the prevalence of mutations in the THAP1 gene and the phenotypic variability, we established a rapid, sensitive and inexpensive high resolution melting assay to analyze a cohort of 610 patients with various forms of dystonia for mutations in the THAP1 gene. We identified seven mutations in this large series of patients and one sequence variation in a control. The mutations were not detected in 537 healthy controls. Surprisingly, four out of seven patients presented with sporadic cervical dystonia as a sole condition, however with young onset. Conclusively, mutations in THAP1 have to be considered in the differential diagnosis of cervical dystonia.

P-ClinG-063

The CFTR alteration M348K is a polymorphism rather than a CF-causing mutation

Hentschel J.[1], Riesener G.[1], Nelle H.[2], Stuhrmann M.[3], Schöner A.[3], Sommerburg O.[4], Fritzsching E.[4], Mall M. A.[4], von Eggeling F.[1], Mainz J.[5]
[1]Institute of Human Genetics, Universital Hospital Jena, Germany, [2]ZAM Jena, Jena, Germany, [3]Medizinische Hochschule Hannover, Hannover, Germany, [4]CF-Centre Paediatrics, University of Heidelberg, Germany, [5]CF-Centre, University-Children´s Hospital, Germany

Cystic fibrosis is the most common recessive disorder in Western countries and is caused by mutations in the CFTR gene. Depending on the kind of CFTR mutation, this disease shows very high clinical variability. Here, we report on a 6 month old boy who was born prematurely at week 26+5 of gestation with necrotizing enterocolitis with pneumatosis intestinalis, hyperbilirubinemia, respiratory distress and failure to thrive. CF diagnostics was performed using the INNO-LiPA-System to test for the 36 most common CFTR mutations in Caucasian populations. Although no mutation was detected, the wild-type signal of the mutation R347P was lacking. Sequencing of this region revealed the homozygous substitution 1175T>A in exon 7 of the CFTR gene resulting in the homozygous amino acid change p.M348K. This alteration has never been reported in homozygosity before. Case reports in the literature of individuals who are compound heterozygous for M348K and a second CFTR mutation provide some evidence that M348K may not be a CF-causing defect, but rather a rare polymorphism. However, online biometric analysis tools produced controversial results/predictions. 'MutationTaster' (http://neurocore.charite.de/ MutationTaster/ index.html) and 'SIFT Sequence' (http://sift.jcvi.org/www/SIFT_seq_ submit2.html) classified the M348K substitution as 'presumably disease causing' and as 'not tolerated', respectively, whereas 'SIFT Blink' (http:// sift.jcvi.org/ www/SIFT_BLink_submit.html) predicted this alteration to be 'tolerated'. The healthy consanguineous parents and siblings of the patient are heterozygous for this alteration. Sequencing of the complete CFTR gene of the patient detected no further CFTR mutation. A subsequent MLPA analysis uncovered no greater deletion or duplication in the CFTR gene. Sweat testing was normal in repeated measurements (Cl- concentrations of 27 mmol/l, 12 mmol/l and 13 mmol/l). Further, electrophysiological assessment of CFTR function in rectal biopsies, as determined by Ussing chamber measurements, demonstrated that CFTR-mediated Cl- secretion was normal in native epithelium. Taken together, the clinical and functional assessment demonstrate that CFTR function was normal in our patient indicating that the alteration M348K is a polymorphism rather than a CF-causing mutation.

P-ClinG-064

Pathogenic mechanism of three novel PAX8 mutations in patients with thyroid dysgenesis.

Hermanns P.[1], Grasberger H.[2], Refetoff S.[2], Pohlenz J.[1]
[1]Department of Pediatrics of the Johannes Gutenberg-University, Mainz, Germany, [2]Department of Medicine of the University of Chicago, IL, USA

Thyroid dysgenesis is the cause of approximately 80% of patients diagnosed with congenital hypothyroidism. So far mutations in five transcription factors (PAX8, TSHR, TTF1, TTF2 and NKX2.5, respectively) have been identified to cause thyroid dysgenesis. We have identified three mutations in the PAX8 gene in three independent families with the diagnosis of thyroid dysgenesis that have not been described previously. We also functionally characterized these three new PAX8 mutations in in vitro assays. Two of the three mutations are located within the DNA binding domain of the PAX8 gene. The third mutation lies outside of this domain. The two mutations inside the DNA binding domain show a significantly decreased activation activity of the TG and the TPO promoters in in vitro analysis, respectively. The first mutation does not show any activation activity at all, but when TTF1 is cotransfected a very low activation of the TG and TPO promoter activity can be observed. Whereas, the third mutation that is outside the DNA binding domain exhibits the same activity when compared to the WT control. The first mutation is expected to cause a loss of DNA binding activity as the previously described with a similar mutation that has been published before, whereas the other two mutations are thought to have no influence on the DNA binding ability. The pathogenic mechanism of the first two mutations might be a dominant negative effect as indicated in an in vitro assay. In silico analysis showed that the third mutation, which is located outside the DNA binding domain, results in an addition of a new exonic splice enhancer. Therefore the latter mutation might lead to an altered splicing of the PAX8 gene. The resulting protein might have an altered function, no function at all or might be degraded by e.g. nonsense mediated decay.

P-ClinG-065

Genotype-phenotype correlations in Pompe disease

Herzog A.[1], Hartung R.[1], Mengel E.[1], Hermanns P.[2], Runz H.[1], Gökce S.[1], Pohlenz J.[2], Beck M.[1]
[1]Villa Metabolica; Zentrum für Kinder- und Jugendmedizin der Universitätsmedizin, Mainz, Germany, [2]Zentrum für Kinder- und Jugendmedizin der Universitätsmedizin, Mainz, Germany

Background: Pompe disease is an autosomal recessive lysosomal storage disorder caused by a deficiency of the acid a-glucosidase. There is a broad spectrum of phenotypes that is divided in infantile and late onset forms. Disease severity primarily depends on residual enzyme activity that relates to the different mutations in the GAA gene. All patients show progressive skeletal muscle weakness which results in a decline in locomotive and respiratory functions. In addition, the infantile form is characterised by hypertrophic cardiomyopathy. c.-32-13T>G is the most common mutation in late onset patients which leads to alternative splicing of exon 2 with about 10% of normally spliced products. Objective: To characterise the genotypes of 3 patients with infantile and 37 patients with late onset Pompe disease and correlate them with the respective phenotype.
Methods: We clinically evaluated the patients, measured their enzyme activities in lymphocytes and determined the molecular defects in the GAA gene by direct sequencing.
Results: On a total of 71 alleles 31 different mutations were found, 13 of which are new. In all patients two mutant alleles were identified except one pair of siblings.
The infantile patients had genotypes that allow at most a very low enzyme activity. Their measured activities were 0.25, 0.96 and 1.2 mU/mg (normal range 8.95-42.14 mU/mg). The enzyme activities of the late onset patients were in the range of 0.22-4.93 mU/mg. Thirty of those were compound heterozygous and one patient was homozygous for c.-32-13T>G. Four patients showed two other mutations each.
The first complaints of the c.-32-13T>G positive patients started between 0 and 56 years with a median of 21.5 (3.0 / 33.8) years, whereas the late onset patients with two other mutations all had first symptoms in childhood at the median age of 5.3 (1.5 / 12.0) years.
A milder affection of the patients with the common splicesite mutation also was seen by means of their mobility graduated by the Walton & Gardner-Medwin scale. About one third of them (31%) were able to walk and run freely (grade 0) and 51 % were able to climb stairs without banisters (grade 0-2). This wasn't achieved by any of the four patients with other mutations. Two of them could climb stairs only with banisters (grade 3) and two of them weren't able to walk (grade 7 and 8).
Furthermore, the c.-32-13T>G compound heterozygous patients had higher forced vital capacities with a median of 72% (50 / 98). One of the other patients needed an invasive ventilation and therefore wasn't able to perform spirometry. The others had forced vital capacities of 32%, 50% and 56%.
Conclusions: This study confirms that residual enzyme activity as well as the phenotypic expression of Pompe disease primarily depends on the genotype. The common c.-32-13T>G splicesite mutation thereby is

associated with a milder phenotype than other mutations leading to the late onset form of the disease. Yet there is a broad variability in the enzyme activities as well as in disease severity between the patients bearing this mutation. Since this variability couldn't be explained by the mutations on the second allele, it is likely that there are existing secondary disease modifying factors.
Annotation: This work contains parts of the doctoral thesis of A. Herzog.

P-ClinG-066
Diagnosis and differential diagnosis in mentally retarded children referred for Fragile X testing
Hesse M.[1], Tönnies H.[1], Kautza M.[2], Sternkopf A.[1], Husemeyer N.[1], Gesk S.[1], Caliebe A.[3], Stephani U.[4], Siebert R.[1], Caliebe A.[1]
[1]Institut für Humangenetik, Kiel, Germany, [2]Praxis für Humangenetik, Kiel, Germany, [3]Institut für Medizinische Informatik und Statistik, Kiel, Germany, [4]Klinik für Neuropädiatrie, Kiel, Germany

In mentally retarded males Fragile X syndrome is one of the most common diagnoses. However, to our experience in most patients testing for Fragile X syndrome is negative.
Based on published screening programms [1-4] we tried to adapt a clinical checklist for routine testing of mentally retarded children for Fragile X syndrome. Additionally we screened several mentally retarded patients with negative Fragile X results for submicroscopic deletions and duplications using Array-CGH and FISH.
The adapted six-feature-score (large/dysplastic ears, positive family history of mental retardation, hyperactivity, attention deficit, autistic like behaviour und delayed development of speech) turned out to be significant with regard to a positive Fragile X test and should be further tested in a prospective setting. By applying Array-CGH and FISH 5 out of 54 patients tested showed microdeletions with assumed correlation to the phenotype. Among those were two well described deletions, del(16p11.2) [MIM 611913] and del(22q13.3) [MIM 606232]. Moreover we detected microdeletions in 10p12.2 and 18p11.2 that have not been described yet in combination with a Fragile X phenotype. Additionally a familial aberration (microduplication 1q21.1) was found.
We propose a new simplified six-feature-score for clinical screening for Fragile X syndrome and demonstrate that submicroscopic chromosome aberrations can overlap with symptoms of Fragile X syndrome.

1. Butler, M.G., et al., A 15-item checklist for screening mentally retarded males for the fragile X syndrome. Clin Genet, 1991. 39(5): p. 347-54.
2. Arvio, M., M. Peippo, and K.O. Simola, Applicability of a checklist for clinical screening of the fragile X syndrome. Clin Genet, 1997. 52(4): p. 211-5.
3. Hagerman, R.J., K. Amiri, and A. Cronister, Fragile X checklist. Am J Med Genet, 1991. 38(2-3): p. 283-7.
4. Giangreco, C.A., et al., A simplified six-item checklist for screening for fragile X syndrome in the pediatric population. J Pediatr, 1996. 129(4): p. 611-4.

P-ClinG-067
Hyperphosphatasia with mental retardation, brachytelephalangy, and a distinct facial gestalt: delineation of a recognizable syndrome
Horn D.[1], Schottmann G.[2], Meinecke P.[3]
[1]Institut für Medizinische Genetik Charité-Universitätsmedizin, Berlin, Germany, [2]Kinderklinik Charité-Universitätsmedizin, Berlin, Germany, [3]Medizinische Genetik Altonaer Kinderkrankenhaus, Hamburg, Germany

A combination of hyperphosphatasia, mental retardation, and various neurological problems, mainly seizures, was reported by different authors, and this rare condition was summarized till now by the term "hyperphosphatasia with mental retardation" (OMIM 239300). Autosomal recessive inheritance of this condition seems to be likely as affected sibs as well as parental consanguinity have been documented. From the data published so far, it is not clear whether "hyperphosphatasia with mental retardation" is a single disease entity.
We report three sibs with an identical phenotype consisting of severe mental retardation, considerably elevated serum levels of alkaline phosphatase, hypoplastic terminal phalanges, and distinct facial features including hypertelorism, long palpebral fissures, a broad nasal bridge and tip, and down turned corners of the mouth. The degree of persisting hyperphosphatasia varied among these three sibs. Elevation of alkaline phosphatase was found to be 1.5 to 2.8 times of the age-adjusted upper limit of the normal range. Radiologically, shortness of distal phalanges could be demonstrated in all of them. Their particular facial appearance led to recognition of phenotypic similarities of two earlier reported familial cases. Judging from the clinical and radiological appearance, we suspect that there is a specific clinical entity within this group of patients with mental retardation and elevated serum levels of alkaline phosphatase which can be delineated by a recognizable facial gestalt and hypoplasia of distal phalanges.

P-ClinG-068
Infantile reversible COX deficiency myopathy caused by the m.14674T>C mutation in mt-tRNAGlu in a German family
Horvath R.[1], Abicht A.[1], Czermin B.[1], Walter M.C.[2], Günther-Scholz A.[3], Holinski-Feder E.[1]
[1]Medizinisch Genetisches Zentrum, Munich, Germany, [2]Friedrich-Baur-Institut, Munich, Germany, [3]Department of Pediatrics, Itzehoe Hostpital, Germany

Childhood-onset mitochondrial encephalomyopathies are usually severe, relentlessly progressive conditions, and have a fatal outcome. However, a puzzling infantile disorder, long known as "benign cytochrome c oxidase deficiency myopathy" is an exception because it shows spontaneous recovery if infants survive the first months of life. We have recently defined the principal molecular basis of the disorder by identifying a maternally inherited, homoplasmic m.14674T>C mt-tRNAGlu mutation in 17 patients from 12 families. Here we describe the clinical presentation of this disease in 4 members of a German family homoplasmic for the m.14674T>C mutation in mt-tRNAGlu. Although 10 maternal family members carry the homoplasmic mtDNA mutation, the clinical symptoms developed in only 4 of them in early childhood. The severity of symptoms was variable, but all affected patients showed a remarkable spontaneous recovery. We present the histological and biochemical findings in follow-up muscle biopsies of 2 affected brothers, confirming reversibility. Cell culture experiments for mt-tRNAGlu steady state levels, mitochondrial translation and immunoblotting for mitochondrial proteins provide further evidence for a spontaneous recovery.

Early differential diagnosis between fatal and benign mitochondrial myopathies is of critical importance for prognosis and management of these infants, because the benign form is initially life threatening but ultimately reversible. Supportive care should not be withdrawn from these children early in life.

P-ClinG-069
Atypical duplication 12p in a patient with mental retardation, frontotemporal hairlessness and onycholysis confines the trisomy 12p syndrome critical region
Hoye J.[1], Trautmann U.[1], Zweier M.[1], Rauch A.[2]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute of Medical Genetics, Zurich, Switzerland

We report on a 3 years and 10 months old girl with developmental delay, frontotemporal hairlessness and onycholysis affecting fingernails and toenails. She also showed a high arched palate but no major dysmorphic features. High resolution GTG-banding revealed a duplication in 12p12-p13.3. Her mother is reported to have had similar hair growth problems until age 4 years. However, chromosomal analyses in

the parents showed no aberration indicating that the duplication in the patient has occurred de novo. Molecular karyotyping using an Affymetrix GeneChip SNP array 6.0 was performed in the patient showing a 21.8 Mb duplication in 12p13.31-p11.21.
In the literature overlapping duplications are already described. These patients often present a high birth weight, macrocephaly and muscular hypotonia, a short neck and anomalies of ears, fingers and feet. Nevertheless, in all reported patients region 12p13.1-p13.33 was affected which is considered to be the critical region for trisomy 12p syndrome. As 12p13.32-p13.33 shows no copy number changes in our patient and she is not presenting the typical phenotype the critical region may be narrowed down to these chromosomal bands.
Although a gene which is responsible for hair growth was assumed in 12p, up to now sparse scalp hair was reported only in triplication 12p.

P-ClinG-070

Novel autosomal recessive mental retardation/multiple congenital anomalies syndromes in three Iranian families: linkage data and clinical reports

Kahrizi K.[1], Najmabadi H.[1], Kariminejad R.[1], Jamali P.[1], Malekpour M.[1], Garshasbi M.[2], Ropers H.-H.[2], Kuss A. W.[2], Tzschach A.[2]
[1]Genetics Research Center, Tehran, Iran, [2]Max Planck Institute for Molecular Genetics, Berlin, Germany

We report on three large consanguineous Iranian families with hitherto undescribed autosomal recessive mental retardation/multiple congenital anomalies (MR/MCA) syndromes.
In family I, two brothers and a sister who were born to first-cousin parents suffered from mental retardation, pectus excavatum, short stature and brachydactly; the sister also had primary amenorrhoea and ovarian insufficiency. Homozygosity mapping revealed a large linkage interval of 24 Mb in 5q23.2q32.
The affected members of family II, a 16-year old female patient and two younger brothers, had moderate mental retardation, severe kyphoscoliosis and congenital heart defects (ventricular septal defect). The parents were first cousins, and linkage analysis revealed a single homozygous locus of 28 Mb in 11q14.2q23.2.
The distantly related parents of family III had three affected children who suffered from severe mental retardation, short stature, cataracts with onset in late adolescence, kyphosis, contractures of large joints, facial dysmorphisms (bulbous nose and thick lips) and uni- or bilateral iris coloboma. Homozygosity mapping revealed a locus in the pericentromeric region of chromosome 4 (4p12q12).
The complex phenotypes and the linkage data strongly suggest that these three families constitute novel and clinically recognizable entities. We will present the clinical features in detail and discuss differential diagnoses as well as candidate genes within the linkage intervals and the results of the ongoing mutational screening.

P-ClinG-071

Biometric Analyses of Mutations in patients with Marfan-Syndrom

Keyser B.[1], Hellwig T.[1], Schulze S.[1], Arslan-Kirchner A.[1], Schmidtke J.[1], Stuhrmann M.[1]
[1]Hannover Medical School, Institute of Human Genetics, Germany

Marfan syndrome (MFS, OMIM 154700) is an autosomal dominant hereditary disorder of connective tissue. The clinical phenotype is heterogeneous with manifestations in the skeletal, cardiovascular and ocular systems. The diagnosis is based on a set of well-defined clinical criteria, the Ghent nosology. Marfan syndrome is mainly caused by mutations in the fibrillin 1 gene (FBN1), which is located on chromosome 15q21.1 and spans 65 exons. Overlapping syndromes such as the Loeys-Dietz syndrome (LDS1A, OMIM 609192; LDS1B, OMIM 610168; LDS2A, OMIM 608967; LDS2B, OMIM 610380) or Marfan syndrome type II (MFSII) are caused by mutations in TGFBR1 or TGFBR2 genes, coding for the transforming growth receptor type I and II, respectively.
To date, 954 FBN1 mutations are described in the HGMD database (www.biobase-international.com). These mutations are located all over the gene. Especially in the case of missense or potential splice mutations, which had not been described before, it is difficult to conclude if the mutation is disease causing or not. Biometric analyses tools can assist here to come to a decision. In the present study we investigated how trustworthy the predictions of these programs are.
In the last 10 years, we identified 223 different FBN1 mutations, 118 of which had been described previously and 105 were novel. These mutations were found in a cohort of clinically "proven" (according to the Ghent nosology) or highly suspected MFS patients. We analysed 191 missense/nonsense/small deletions/small insertions with the online-software "Mutation Taster" (www.mutationtaster.org). For 187 of these mutations, the program predicted a disease causing effect, the other 4 mutations were judged as polymorphisms. None of these 4 mutations (including two missense mutations) was previously described. We also analysed 22 possible splice mutations with Mutation Taster, and for 20 of these mutations, a relevant splice site change was predicted, while no disease causing effect was predicted in the remaining two cases. Further biometric analyses of the two missense and the two possible splice mutations with "SIFT" (www.sift.jcvi.org) and "ASSP" (www.es.embnet.org), respectively, revealed that both missense mutations were tolerated, but one of the possible splice mutations was classified as disease causing. Genetic analysis of further family members and functional investigations are required before final conclusions can be drawn on the relevance of these mutations.
We conclude that biometric analyses can provide evidence for or against the assumption that a mutation is disease causing. However, further genetic or functional analysis is warranted in doubtful cases.

P-ClinG-072

Array-CGH detects mosaic tetrasomy 12p (Pallister-Killian syndrome) in peripheral blood without invasive skin biopsy

Koehler U.[1], Balg S.[1], Reiter K.[2], Holinski-Feder E.[1]
[1]Medizinisch Genetisches Zentrum München, Munich, Germany, [2]Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderspital, Munich, Germany

We report on a 3 month old girl presenting with multiple dysmorphic features including inner epicanthic folds, low set ears with auricular fistulae, retrognathy, and four-finger folds; contractural arachnodactyly, impaired hearing and seizures were reported. Karyotyping of peripheral blood and molecular genetic testing for Beals Hecht Syndrome both revealed normal results. Array-CGH with genomic DNA from peripheral blood using 105K oligo arrays, however revealed an amplification of the complete short arm of chromosome 12, which lead to the assumption of an additional marker chromosome 12 and most probably of a mosaic isochromosome 12p which is common in patients presenting with Pallister-Killian syndrome. Karyotyping of 100 metaphases and interphase FISH analysis on nuclei from cultured T-lymphocytes using a chromosome 12 centromeric probe failed to detect the additional isochromosome 12, most probably due to the preferential stimulation of T-lymphocytes. Interphase FISH on peripheral blood smear was not performed due to depletion of the blood sample. The subsequent karyotyping and FISH analysis on fibroblasts of a cultured skin biopsy confirmed the presence of an isochromosome 12p in 56% of cells. It is known that detection of an isochromosome 12p in stimulated T-lymphocytes is hard to achieve as only 1-2% of lymphocyte metaphases contain the additional isochromosome 12p. Up to now, analysis of fibroblasts from invasive skin biopsies was the only opportunity to detect this chromosome anomaly. Array-CGH with genomic DNA from peripheral blood, however is an adequate and powerful tool to investigate children suspected of having Pallister-Killian syndrome.

P-ClinG-073

Osteopathia striata with cranial sclerosis. Follow up of two male patients

Koenig R.[1], Mehler K.[1], Schäfer D.[1]
[1]Institut für Humangenetik, Frankfurt, Germany

Osteopathia striata with cranial sclerosis (OSCS) is a bone dysplasia characterized by macrocephaly, hypertelorism, cleft palate, hearing deficits, psychomotor retardation and various other malformations. Typical radiographic findings include the name-giving linear striations of tubular bones and iliac wings and sclerosis of craniofacial bones. OSCS is X-linked and recently the gene encoding WTX, a repressor for wnt signaling, was identified. Here we describe two male patients from birth to adulthood.

The first patient was born with macrocephaly, facial anomalies, Robin sequence, tracheomalacia and flexion contractures of fingers and toes. Cranial CT showed ventricular dilatation without signs of increased intracranial pressure. Mixed conductive-sensorineural deafness required hearing aids. Radiographs of the skull showed increased density of the cranial base and maxilla. At examination at 18 years he presented with macrocephaly (8 cm >97-perc.), mild dysmorphic facial features, hypodontia, conical teeth, pectus excavatum, cubitus valgus and clubbing of fingers. Despite retarded development in the first years of life, the patient attended regular school and achieved his graduation. Roentgenograms of the femur and tibia do not show the typical linear striations. Mutation analysis revealed an 811C>T mutation in the WTX gene.

At birth, the second patient showed macrocephaly, bilateral cleft-lip-palate, dysplastic ears, short neck, umbilical hernia, flexion contractures of the fingers II-V, broad halluces. Cranial CT revealed hypoplasia of the corpus callosum and hydrocephalus internus. Roentgenograms showed severe cranial sclerosis, short broad clavicles, hypoplasia and deformations of the ribs and bilateral fibular hypoplasia. An atrium septum defect was corrected at 1 year. Because of bilateral sensorineural deafness he got hearing aids. Psychomotor development was severely retarded. Recurrent apnoes and seizures, beginning at the age of 4 years, were explained by an Arnold–Chiari I malformation and improved after decompression. Progressive scoliosis was treated by an exercise program and bracing. Examination at 17 years: length 158 cm (5cm <3-perc.), weight 54 kg (10-25 -perc.), CFC 67 cm (9 cm > 97-perc.), macro-dolichocephalus, hypertelorism, downslanting palpebral fissures, hypodontia, dysplastic teeth, scoliosis, normal pubertal development and hallux valgus. The patient required a special education program and now, at 20 years, got a sheltered work. We discuss the features, course and management of this rare syndrome.

P-ClinG-074

Dravet Syndrome/GEFS+: Mutational Analysis of SCN1A – Diagnostic Clue with Pitfalls

Kohlschmidt N.[1], Gross C.[1], Kuczaty S.[2], Sassen R.[2], Kunz W.[2]
[1]Institut fuer Klinische Genetik, Bonn, Germany, [2]Universitaetsklinik und -poliklinik fuer Epileptologie, Bonn, Germany

Background: Dravet syndrome or SMEI comprises severe myoclonic epilepsies starting in the late first year of life which are difficult to treat and show a poor developmental prognosis. Since 2001 mutations in the SCN1A gene are known to cause Dravet syndrome and the closely related GEFS+. Molecular analysis may confirm the clinical diagnosis, though some cases bear marked difficulties in DNA data interpretation.

Material and Methods: 72 cases aged 10 months to 24 yrs with Dravet syndrome/GEFS+ were analysed by DNA sequencing of the entire coding region.

Results: In 32 cases a pathogenic mutation was unveiled; 15 of which were missense, 5 were nonsense, and 6 were frame shift mutations. Additional findings were a complex deletion-insertion and five splice site changes. The mutations were spread over the entire gene; no clustering around hot spots was noted. 24/32 mutation had not been described elsewhere. 12/32 mutations were de novo with little risk for future siblings.

One, healthy, mother of two affected siblings bore a mosaicism, another likewise affected sibship also had an identical mutation. The mother in a third family with a splice site mutation had very mild symptoms whilst their children were more severely affected.

Message: Mutational analysis of the SCN1A gene clarifies the cause of severe myoclonic epilepsy in about half of the cases and enables specific genetic counselling.

The class of mutation (missense/nonsense) seems not to have a prognostic value.

Though spontaneous de novo events cause the majority of mutations a considerable number of cases are due to parental germ cell mosaicism with significant recurrence risk for future siblings.

P-ClinG-075

LRRC7 encoding densin-180 is disrupted in a patient with clinical features of Marfan syndrome

Kortüm F.[1], Tönnies H.[2], Albrecht B.[3], Kutsche K.[1]
[1]Institut für Humangenetik, Hamburg, Germany, [2]Institut für Humangenetik, Kiel, Germany, [3]Institut für Humangenetik, Essen, Germany

Marfan syndrome, Loeys-Dietz syndrome and the vascular type of Ehlers-Danlos syndrome constitute a group of multisystem connective tissue disorders with a pattern of overlapping congenital anomalies. These disorders are caused by mutations in the FBN1, TGFBR1 and TGFBR2, and COL3A1 genes, respectively. TGFBR1 and TGFBR2 code for the transforming growth factor (TGF)-beta type 1 and 2 receptors. Individuals with these genetic syndromes are predisposed to thoracic aortic aneurysms leading to type A dissections (TAAD). Mutations in MYH11 and ACTA2, encoding smooth muscle-specific beta-myosin and alpha-actin, respectively, could be linked to isolated TAAD. Both beta-myosin and alpha-actin are part of the smooth muscle cell (SMC) contractile unit, while the ligand of the TGF-beta receptors, TGF-beta, is important for SMC differentiation. Thus, disturbances in the SMC mechanotransduction complex or SMC differentiation would be predicted to lead to decreased SMC contractility and may represent a general pathomechanism underlying connective tissue disorders. We report a female patient with mitral valve prolapse, scoliosis, striae distensae, preaxial hexadactyly of feet, and long and thin fingers, a phenotype resembling Marfan syndrome. Sequencing of FBN1, TGFBR1 and TGFBR2 was negative, but the patient was found to carry a balanced de novo translocation with breakpoints in 1p31.1 and 2q31.3. Array CGH analysis did not reveal any genetic imbalance. Breakpoint mapping revealed that the 1p31.1 breakpoint directly disrupts the LRRC7 gene. LRRC7 encodes the synaptic protein densin-180 which is important for the formation, maintenance, and plasticity of neuronal synapses. Recently, a ubiquitously expressed splice variant of densin-180 has been described. Interaction of densin-180 with the Ca2+/calmodulin-dependent protein kinase II, involved in SMC proliferation and migration, and alpha-actinin, a component of SMC dense bodies, may suggest that LRRC7/densin-180 could be a candidate for the Marfan-like phenotype in the translocation patient.

P-ClinG-076

A de novo t(1;8)(p36.11;p12) Translocation Associated with Severe Obesity and Hypogonadotropic Hypogonadism

Kraft M.[1], Bauhuber S.[1], Trautmann U.[1], Thiel C.T.[1], Ekici A.B.[1], Rauch A.[2]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute of Medical Genetics, Schwerzenbach-Zurich, Switzerland

We report on an 18 years old female patient with severe obesity (BMI: 46 kg/m2) combined with hypogonadotropic hypogonadism resulting in amenorrhea. Cytogenetic analysis using high resolution GTG-band-

ing revealed an apparently balanced de novo translocation initially described as t(1;8)(p35.2;p11.2). Delineation by fluorescence in situ hybridization (FISH) mapping and subsequent sequencing of a breakpoint-spanning long-template PCR product from derivative chromosome 8 revealed the exact location of the breakpoints and corrected the description of our translocation to t(1;8)(p36.11;p12). It was found that the gene FGFR1 (fibroblast growth factor receptor 1) on chromosome 8 was disrupted by one chromosomal break. Consistent with the patients phenotype, mutations in FGFR1 were previously described as causative for both normosmic idiopathic hypogonadotropic hypogonadism and autosomal dominant Kallmann syndrome of which hypogonadotropic hypogonadism is a major sign. In proximity to the breakpoint on chromosome 1 the gene NR0B2 (nuclear receptor subfamily 0, groub B, member 2) was found which encodes for an integrated factor of the cholesterol and glucose metabolism and was previously found to be mutated in patients with obesity. In subsequent quantitative PCR analysis both genes showed significantly reduced expression levels in our patient. Thus we suggest that our patient suffers from two distinct genetic disorders which account for the combined phenotype observed. Interestingly we also detected significantly reduced expression for LETM2 (leucine zipper-EF-hand containing transmembrane protein 2) which is located directly upstream of FGFR1 but cannot be linked to the observed phenotype of our patient. Expression analysis of ADRB3 (beta-3-adrenergic receptor) which is also located upstream of FGFR1 is pending. ADRB3 is coding for a receptor found mainly in adipose tissue involved in the regulation of lipolysis and thermogenesis and is therefore discussed as a candidate for susceptibility to obesity. Copy number variation studies using the Affymetrix 6.0 SNP platform revealed a known recurrent microduplication 15q13.2-13.3 (phenotype: autism, learning difficulties, dysmorphic features, seizures), which cannot be linked to the major phenotype of our patient.

P-ClinG-077

Genetic counseling in breast and ovarian cancer – problematic pedigrees

Kroll P.[1], Bechtold A.[1], Grimm T.[1]
[1]Department of Human and Medical Genetics, Würzburg, Germany

Confirmatory and predictive testing for mutations in the high-risk breast and ovarian cancer genes BRCA1 and BRCA2 has become routine in many countries. Each pedigree represents a unique constellation of healthy and affected indviduals scattered throughout multiple generations, leading to widely varying risk estimates and test results which need to be interpreted by the genetic counselor. Although mostly trivial, we wish to illustrate that there are a number of problems and pitfalls in such at risk pedigrees which seriously limit scope and impact of the counseling process.
Confirmatory and predictive testing for mutations in the high-risk breast and ovarian cancer genes BRCA1 and BRCA2 has become routine in many countries. Each pedigree represents a unique constellation of healthy and affected indviduals scattered throughout multiple generations, leading to widely varying risk estimates and test results which need to be interpreted by the genetic counselor. Although mostly trivial, we wish to illustrate that there are a number of problems and pitfalls in such at risk pedigrees which seriously limit scope and impact of the counseling process.
Pedigree 1 illustrates that if all affected family members are deceased or otherwise unavailable, there is no possibility of confirmatory testing. Moreover, predictive testing of individuals at risk might yield negative results in such families, rendering reliable risk prediction difficult. Pedigree 2 shows that affected members within a single family may be discordant for a given type of mutation. This possibility has to be taken into account and complicates predictive testing. Pedigree 3 deals with the problem of an extensive pedigree with only a single affected individual who tested positive for a BRCA1 mutation. Such a situation may be indicative of a parental de novo germline mutation, germ line mosaicism, silent transmission throughout the paternal lineage, and/or strongly reduced penetrance. Pedigree 4 empasizes that both parents can be affected, each conveying a formal genetic risk to their offspring. Given the unavailability of the parents for testing, a negative test result in the offspring does not permit a definitive risk estimate. Pedigree 5 demonstrates a family structure in which the possibility of unknown genes or the possibility of phenocopies have to be taken into account. Even within families with known mutations in BRCA1 or BRCA2, the coincidental concurrence with sporadic types of breast cancer should be considered. Pedigree 6 illustrates that pedigrees with predominantly male gender family members are less informative because of the problem of silent transmission through the male lineage. Pedigree 7 emphasizes the trivial but nevertheless rather frequent fact that conclusive genetic counseling depends on the availability and accuracy of pedigree information. Pedigree 8 illustrates the unsolved problem of so called "unknown variants" (UV) and demonstrates that genetic testing can sometimes cause concern and confusion rather than establish clarity. Pedigree 9 shows that computer programs and calculation tables are only as good as the basic calculation model. They can´t replace the experience of a genetic counselor. Because of the high cost of mutation screening, we have to set certain limits for who should undergo molecular testing.

P-ClinG-078

Clinical and genetic challenges in patients with prenatally diagnosed Pallister-Killian syndrome

Kron A.[1], Seipel B.[1], Bolz HJ.[2], Decker J.[3], Schmitt-Heidsieck S.[4], Bahlmann F.[5], Bergmann C.[6]
[1]Bioscientia Center for Human Genetics, Ingelheim, Germany, [2]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, University of Cologne, Germany, [3]Bioscientia Center for Human Genetics Ingelheim and Department of Internal Medicine, University of Mainz, Germany, [4]Practice for Prenatal Diagnosis, Trier, Germany, [5]Department of Obstetrics & Gynaecology, Bürgerhospital Frankfurt, Germany, [6]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, RWTH Aachen University, Germany

Pallister-Killian syndrome is a rare sporadic chromosomal aberration caused by a tissue-limited mosaicism for a supernumerary isochromosome 12p (i12p), leading to a tetrasomy of 12p. In line with mosaicism, a postzygotic defect with nondisjunction during meiosis might be the most likely pathomechanism. The chromosome aberration is usually absent in cultured lymphocytes, but present in fibroblasts and prenatally in amniotic fluid and chorionic villi. The proportion of abnormal cells at prenatal diagnosis can vary considerably.

Clinically, frequent ultrasonographic features in favour of the diagnosis of Pallister-Killian syndrome are polyhydramnios, increased nuchal translucency, diaphragmatic hernia, short (rhizomelic) limbs, and a recognizable fetal facial profile. Other malformations like cardiac defects and anal atresia are also not uncommon. Children born with Pallister-Killian syndrome usually display profound motor and mental retardation, seizures, typical craniofacial dysmorphisms, and streaks of hypo- or hyperpigmentation. Visual and hearing impairment may also occur.
We present seven prenatally diagnosed patients with Pallister-Killian syndrome and will discuss clinical and genetic findings. An early and correct diagnosis of Pallister-Killian syndrome is important for appropriate genetic counseling; all cases of Pallister-Killian syndrome reported so far occurred sporadically, an information that should be shared with affected families.

P-ClinG-079

Rett-like Syndrome Patient: CDKL5 deletion detected with arrayCGH

Kuhn M.[1], Lott A.[1], Schell-Apacik C.[2], Gabrie[1] H.-D.[1], Gencik M.[1]
[1]Zentrum für Medizinische Genetik, Osnabrück, Germany, [2]Praxis für Humangenetik am DRK-Klinikum Westend, Berlin, Germany

Array-CGH is now a worldwide well implemented diagnostic tool for the detection of submicroscopic chromosomal imbalances. Our lab has a lot of experience with oligo-based array-CGH analysis in the postnatal diagnostics of mental retardation. Array-CGH is routinely performed using whole genome 44k, 105k or 244k Oligo-arrays (Agilent).
Here we report on a 3 8/12 year old female patient, who was born to unrelated German parents and has an unaffected brother. At the age of three months first signs of psychomotor delay were observed. She was able to walk with assistance at the age of 2 ½ years. Furthermore, swallowing difficulties and apnoea attacks were observed. MR imaging of the brain and EEG diagnostics showed normal findings. At present, the patient presents muscle hypotonia and growth retardation (< P10), and delayed speech development. Strabismus, astigmatism, as well as facial dysmorphism were present.
Chromosome analysis was normal. Array-CGH (105k Oligo-array) revealed a 100kb deletion on the small arm of chromosome X (arr Xp22.13(18.264.816-18.362.063)x1)(hg17;ISCN2009) including exon 3-10 of the CDKL5 gene. The deletion was confirmed by MLPA. Mutations in the CDKL5 gene are known to cause a Rett-like syndrome with severe seizures in the first 6 month of life. Genomic deletions involving CDKL5 were described in up to 8% of patients with Rett(-like) Syndrome in the literature.
Currently, investigations concerning the mutational status in the parents and exact breakpoint characterization are being performed in our laboratory.

P-ClinG-080

A large family with Rubinstein-Taybi syndrome (RTS): vertical transmission of RTS due to somatic mosaicism in the father

Lechno S.[1], Zechner U.[1], Kempf O.[1], Haaf T.[2], Kress W.[2], Bartsch O.[1]
[1]Institute of Human Genetics, Mainz, Germany, [2]Institute of Human Genetics, Würzburg, Germany

Rubinstein-Taybi syndrome (RTS) is a rare (1 in 100,000 to 125,000 newborns) but well characterised disorder of broad thumbs and halluces, facial anomalies, mental and growth delay. Dominant mutations or deletions of the CREBBP and EP300 genes can be identified, respectively, in 50-70% and 3% of clinically diagnosed patients, causing haploinsufficiency of these transcriptional regulator genes. Even though more than 1,000 cases have been reported, vertical transmission (parent-to-child) is extremely rare with only five cases currently known. We present a two-generation family from Germany with three girls aged 6, 7, and 12 years with typical RTS. The 42-year-old father presented with learning difficulties and broad thumbs and was suspected of having mild or incomplete RTS. CREBBP mutation analysis in the four patients showed a mutation of the first nucleotide of coding exon 25, c.4134G>T, predicting a splice mutation (no amino acid change). Bioinformatic analysis with ESEfinder 3.0 (http://rulai.cshl.edu/tools/ESE) (Smith et al., 2006) predicted most probably exon 25 skipping or false splicing because the mutation relocates the tttcagG sequence motif for SRp40 (arginine/serine-rich splicing factor, 40-KD) to an alternative position (14 nt later). Quantitative analysis of sequence chromatograms (Mutation Surveyor 3.1; Softgenetics, State College, PA, USA) showed that the mutant T allele peak of the father, but not that of the three affected daughters, was consistently much smaller when compared with the normal G allele peak, indicating somatic mosaicism and thus a somatic de novo mutation in the father. The finding was validated using a quantitative pyrosequencing analysis of blood DNAs of all affected family members and, in addition, of buccal smear DNA of the father. The father´s blood DNA and buccal DNA showed mutant allele frequencies of 38% and 31% (mosaicism), respectively, whereas his daughters showed mutant allele frequencies around 50% (constitutional heterozygosity for the mutated allele).
This is the sixth and largest family with RTS reported so far. The case demonstrates that in familial cases of dominant mental retardation syndromes, the affected parent may be much milder affected due to somatic mosaicism. Here, the diagnosis of the mosaic state of the mutation was only possible through the affected children. Our observation has implications on genetic counseling. Despite our findings, we assume that the recurrence rate of RTS is low in general, but nevertheless we would advise prenatal diagnosis in all families with RTS. This and previous observations indicate for RTS a recurrence risk around 0,5%, which is low but significantly higher than the so far estimated empiric risk for sibs of approximately 0,1% (Hennekam et al., 1990; Wiley et al., 2003).

Hennekam et al. Am J Med Genet 1990; S6:56–64.
Smith et al. Hum Mol Genet 2006; 15:2490–2508.
Wiley et al. Am J Med Genet 2003; 119A:101–110.

P-ClinG-081

An unusual case of fragile X syndrome due to partial deletion of FMR1

Lott A.[1], Gabriel H.[1], Hikkel I.[2], Gencik M.[1]
[1]Zentrum für Medizinische Genetik, Osnabrück, Germany, [2]medgene, Bratislava, Slovakia

We report on a 9 year old boy, whose blood was referred to our laboratory by a pediatrician for testing of Prader-Willi syndrome and submicroscopic chromosomal imbalances by array-CGH.
The boy presented with moderate mental retardation without family history, delayed attainment of motor milestones and speech, hyperactivity, normal stature with slight obesity, hyperphagia, full lips and large dysmorpic ears.
After Prader-Willi syndrome was excluded by MS-MLPA testing, we performed array-CGH using a whole genome 44k Oligo-array (Agilent). The array-CGH analysis revealed a deletion of 70-170kb on Xq27.3, including the gene FMR1NB and possibly a part of FMR1:
arr Xq27(146.844.547-146.915.743)x1 (ISCN 2009, hg18)
To validate and further characterize this result and to determine if the deletion was maternally transmitted, we performed MS-MLPA for fragile X syndrome (Kit ME0289-B1, MRC Holland).This analysis confirmed a normal methylation pattern in the patient and his mother and a deletion of exons 15 to 17 of the FMR1 gene in the patient but not in his mother. Furthermore, we tried to amplify all exons of FMR1 (1-17) of the patients DNA by PCR and got no PCR product with primers for exons 15, 16 and 17, whereas the other exons were present. Summarized, these results prove in our patient a de novo deletion of the 3‘ part of the FMR1 gene with the proximal deletion breakpoint between exons 14 and 15, protruding 70-160 kb distal including the FMR1NB gene.

The most common cause of fragile X syndrome is the expansion of a CGG trinucleotide repeat in the 5‘ UTR region of FMR1, which leads to functional silencing of this gene by hypermethylation. However, in rare cases (< 1%) the loss of FMR1 gene function is due to partial or whole gene deletions, ranging in size from single nucleotides to several Mb. The smaller deletions (< 10 kb) described so far are restricted to the 5‘ end of FMR1 and due to instability of the CGG repeat, whereas larger deletions seem not to be associated with CGG repeat instability. A lot of patients with large deletions spanning FMR1 have a typical fragile X phenotype. But in most of these cases other genes proximally and/or distally are also lost resulting in additional symptoms in the patient. To our knowledge, this is the first case of a deletion of only the 3‘ part of FMR1 and the distally neighboring gene FMR1NB, resulting in a phenotype compatible to fragile X syndrome. We are now performing a PCR analysis to determine the exact positions of the proximal and distal breakpoints of this deletion.

P-ClinG-082

Albright hereditary osteodystrophy (AHO)-like syndrome and associated schizophrenic psychosis

Mehraein Y.[1], Wichnalek M.[1], Bubendorff V.[2], Mannhard A.[2], Steinlein O.[1], Müller S.[1]

[1]Insitute of Human Genetics, Munich, Germany, [2]Heckscher Clinic for Child and Adolescence Psychiatry, Munich, Germany

Albright hereditary osteodystrophy (AHO)-syndrome is a dominantly inherited genetic syndrome due to GNAS1-gene germ line mutations. It is characterized by short stature, obesity, round face, brachydaktyly with shortening of phalanges and metacarpals, developmental retardation, intellectual deficit, and eczema with variable biochemical changes (pseudohypoparathyroidism).

In a small number of cases with a phenotype termed AHO-like syndrome a small terminal deletion of chromosome 2q37 has been identified. Different from classical AHO these patients lack renal parathyroid hormone resistance and soft tissue ossification. Here we report a novel case of AHO-like syndrome with associated psychiatric features.

The 18 year old woman presented with acute symptoms of paranoid schizophrenia. A mild intellectual impairment with learning disability had been preexisting. Physical examination showed brachydaktyly type E and nail anomalies in hands and – less severe – feet, as well as mild facial dysmorphic features suggestive of AHO-syndrome. Further features were pectus excavatus and uterus bicornis. Conventional chromosome analysis revealed a normal female karyotyp 46,XX. A microdeletion 22q11.2 was excluded by FISH-analysis, but 2q subtelomere FISH using a 2q37.3 probe (D2S447; TelVysion, Abbott Molecular) showed a terminal deletion consistent with the diagnosis of AHO-like syndrome. So far schizophrenia has not been described as a regular feature of AHO-like syndrome. Previously reported linkage studies in small genetic isolates, however, support the existence of a schizophrenia susceptibility locus on 2q37. We therefore discuss the possibility that the schizophrenic disorder in our patient may be due to haploinsufficiency of a gene located in 2q37.

P-ClinG-083

Further evidence on genetic heterogeneity of medullary cystic kidney disease: report on a large Portuguese family.

Miltenberger-Miltenyi G.[1], Almeida E.[2], Teixeira C.[2], Jorge S.[2], Carvalho F.[3], Gomes da Costa A.[2]

[1]Institute of Molecular Medicine University of Lisbon, Lisbon, Portugal, [2]Department of Nephrology and Dialysis Hospital Santa Maria University of Lisbon, Lisbon, Portugal, [3]Department of Anatomy and Pathology Hospital Santa Maria University of Lisbon, Lisbon, Portugal

BACKGROUND: Medullary cystic kidney disease type 2 (MCKD2) is a rare autosomal dominant syndrome characterized by gout, tubulointerstitial nephropathy and end-stage renal failure (ESRF). The disease is caused by mutations in the uromodulin gene (UMOD, 16p12.3). Besides, MCKD1, with overlapping phenotype to MCKD2, but milder symptoms and with later age-at-onset is associated with a candidate disease locus on chromosome 1q21.

PATIENTS: We investigated a Portuguese family of 4 generations suffering of MCKD2 syndrome. The proband of the family developed ESRF at the age of 28 years. Further nine family members were affected. The disease showed a typical autosomic dominant trait in the family.

RESULTS: Affected family members showed elevated serum uric acid levels. Renal biopsy, carried out in one affected family member revealed signs of a tubulointerstitial disease. Immunohystochemistry of the kidney showed staining of uromodulin in tubule profiles. Genetic analysis of the UMOD gene showed three non-pathogenic variants but did not reveal any pathogenic mutations.

CONCLUSION: The clinical and pathological characteristics of this family suggested a clear MCKD2 syndrome. The missing of pathogenic mutations in the UMOD gene points out the genetic heterogeneity of this disease and broadens the phenotypic spectrum of non-UMOD related MCKD. Further investigations of this family might be helpful in identifying candidate genes for MCKD1 and describe genotype-phenotype correlations.

P-ClinG-084

Genotype-Phenotype Correlation in Eight New Patients with a Deletion Encompassing 2q31.1

Mitter D.[1], Delle Chiaie B.[2], Lüdecke H.-J.[1], Gillessen-Kaesbach G.[3], Bohring A.[4], Kohlhase J.[5], Caliebe A.[6], Siebert R.[6], Roepke A.[4], Ramos-Arroyo M.A.[7], Nieva B.[8], Menten B.[2], Loeys B.[2], Mortier G.[2], Wieczorek D.[1]

[1]Institut für Humangenetik, Universitätsklinikum Essen, Germany, [2]Center for Medical Genetics, Ghent University Hospital, Belgium, [3]Institut für Humangenetik Lübeck, Universität zu Lübeck, Germany, [4]Institut für Humangenetik, Westfälische Wilhelms-Universität Münster, Germany, [5]Center for Human Genetics, Freiburg, Germany, [6]Institut für Humangenetik, Christian-Albrechts-Universität zu Kiel, Germany, [7]Department of Medical Genetics, Hospital Virgen del Camino Pamplona, Spain, [8]Unidad de Genética, Policlinica San Sebastian, Spain

Microdeletions of the 2q31.1 region are rare. We present the clinical and molecular findings of eight previously unreported patients with overlapping deletions in 2q31.1. The patients have a variable clinical phenotype and present with developmental delay (7/8), growth retardation (5/8), seizures (2/8) and a craniofacial dysmorphism consisting of microcephaly (4/8), short palpebral fissures (7/8), broad eyebrows with lateral flare (7/8), low-set ears with thickened helices and lobules (5/8), and micrognathia (6/8). Additional congenital anomalies were noted, including limb abnormalities (8/8), heart defects (3/8), genital anomalies (3/8) and craniosynostosis (1/8). Six of these microdeletions, ranging in size from 1.24 to 8.35 Mb, were identified by array CGH, one larger deletion (19.7 Mb) was detected by conventional karyotyping and further characterized by array CGH analysis. The smallest region of overlap in all eight patients spans at most 88 kb and includes only the WIPF1 gene. This gene codes for the WAS/WASL interacting protein family member 1. The patients described here do not present with clinical signs of the Wiskott-Aldrich syndrome and the deletion of this single gene does not allow explaining the phenotype in our patients. It is likely that the deletion of different but overlapping sets of genes from 2q31 is responsible for the clinical variability in these patients. To further dissect the complex phenotype associated with deletions in 2q31, additional patients with overlapping phenotypes should be examined with array CGH. This should help to link particular phenotypes to specific genes, and add to our understanding of the underlying developmental processes.

P-ClinG-085

Functional analysis of a novel I71N mutation in the GJB2 gene among Southern Egyptians with autosomal recessive hearing loss

Mohamed M.R.[1], Bress A.[2], Alesutan I.[3], Föllre M.[3], Sopjani M.[3], Baur M.[2], Salama R.H.M.[4], Bakr M. S.[1], Mohamed M. A.[1], Blin N.[5], Lang F.[3], Pfister M.[2]

[1]Department of Otolaryngology, Assiut University, Egypt, [2]Department of Otolaryngology, University of Tübingen, Germany, [3]Institute of Physiology, University of Tübingen, Germany, [4]Department of Biochemistry, Assiut University, Egypt, [5]Institute of Human Genetics, University of Tübingen, Germany

Mutations in GJB2, a gene encoding the gap junction protein connexin 26 (Cx26), are a major causes for inherited and sporadic non-syndromic hearing loss, albeit with highly variable clinical effects. Screening of GJB2 in a Southern Egyptian population aimed at determining new mutations and their frequencies. GJB2 variations were analyzed using restriction fragment length polymorphism (RFLP), gene sequencing, and single strand conformational polymorphism (SSCP). Only 2 mutations were found in the study group: c.35delG and pI71N.

The allelic frequency of the c.35delG mutation was 8.7% as it was found in 27 out of 310 investigated alleles. Eleven patients were homozygous for c.35delG and 5 patients were heterozygous. This result indicates a carrier frequency of 1.6% in Upper Egypt, a number in the lower range of the list among other Mediterranean and central-European populations in comparison. The new mutation, a substitution of isoleucin (I) (a non-polar amino acid) by the polar amino acid asparagin (N), was localized within the conserved Cx26 structure. The functional significance of p.I71N was tested by injection of wild type and mutated cRNA into Xenopus laevis oocytes. Cx26 hemi-channel activity was measured by depolarization activated conductance in non-coupled oocytes. As a result, the p.I71N mutated channel was non-functional. The study discloses a novel, functionally relevant GJB2 mutation and defines the contribution of Cx26 alterations to the hearing loss in the Egyptian population providing evidence-based risk assessment in genetic counseling

P-ClinG-086

Influence of variations of the SHOX gene and PAR1 region on the phenotype in families with short stature

Möller-Krull M.[1], Groß U.S.[1], Fleischer S.[1], Wittmann S.[1], Richter-Unruh A.[2], Schulte H.M.[1]
[1]ENDOKRINOLOGIKUM Hamburg; Center for Hormonal and Metabolic Diseases; Reproductive Endocrinology and Prenatal Medicine, Hamburg, Germany, [2]ENDOKRINOLOGIKUM Ruhr; Center for Hormonal and Metabolic Diseases, Wattenscheid, Germany

Introduction: The short stature homeobox gene (SHOX gene) is an essential transcription factor for the development of the skeleton. Mutations and deletions of the SHOX gene encompassing the pseudo-autosomal region 1 (PAR1) are known to lead to idiopathic short stature as well as Léri-Weill dyschondrosteosis (LWD).
The frequency of SHOX deficiency in the caucasian population is about 1:1000. It is assumed that about 5% of the persons with short stature feature changes in the SHOX gene and PAR1 region with deletions covering about 80% of these changes.
Patients: Regarding the possibility of growth hormone therapy we analyzed the SHOX gene of 40 children within the last two years. All patients exhibit the typical phenotype indicating SHOX gene deficiency.
Methods: Genomic DNA was extracted from peripheral blood leukocytes. Multiplex Ligation-Dependent Probe Amplification (MLPA) was performed using the SALSA P018 probemix by MRC-Holland for detection of deletions and duplications in exons 1 to 6a and near exon 6b of the SHOX gene as well as of the encompassing PAR1 region.
Samples negativ for deletions according to the MLPA were further analysed by direct sequencing of PCR products of all coding exons of the SHOX gene including corresponding exon-intron boundaries.
Results: In the cohort of our 40 patients, MLPA analysis and sequencing revealed point mutations, deletions of the SHOX gene with the encompassing PAR1 region as well as sole deletions of the PAR1 region without effecting the coding region of the SHOX gene. Interestingly, members of a family with the identified point mutation p.Tyr199Stop show the same phenotype as members of a family featuring only a loss of the PAR1 region without effecting the coding region of the SHOX gene.
Conclusion: Our findings suggest that screening for large deletions of the PAR1 region encompassing the SHOX gene (e.g. by MLPA) has to be essentially included in the diagnostic of a SHOX gene deficiency. Due to this fact, genetic counselling and the detailed analysis of the SHOX gene and the encompassing PAR1 region is important to confirm the diagnosis and to provide the basis for the therapeutic decisions.

P-ClinG-087

Genotype-phenotype correlation in MYH9-related platelet disorders

Najm J.[1], Althaus K.[2], Felbor U.[1], Greinacher A.[2]
[1]Institut für Humangenetik des Universitätsklinikums Greifswald, Greifswald, Germany, [2]Institut für Immunologie und Transfusionsmedizin des Universitätsklinikums Greifswald, Greifswald, Germany

Myosin heavy chain 9 (MYH9)-related platelet disorders belong to the group of autosomal dominantly inherited thrombocytopenias caused by heterozygous mutations in MYH9. This gene encodes for non-muscle myosin heavy chain IIA (NMMHC-IIA), a cytoskeletal contractile protein. Four overlapping syndromes, known as May-Hegglin anomaly, Epstein syndrome, Fechtner syndrome, and Sebastian platelet syndrome, describe different clinical manifestations of MYH9 gene mutations. Macrothrombocytopenia and inclusion bodies in neutrophils are present in all patients, whereas only some of them develop sensorineural deafness, presenile cataracts, and/or progressive nephritis leading to end-stage renal failure. MYH9 mutations in the C-terminal coiled-coil region have been associated with a hematological-only phenotype while mutations of the head ATPase domain have frequently been related to nephritis and/or hearing loss. Mutations of other regions show an intermediate expression of non-hematological characteristics. To date, more than 200 families with MYH9 disorders have been studied. Most of the about 30 different MYH9 mutations cluster in a limited region of the gene (exons 1, 16, 25-26, 30 and 40). More than 20% of cases are sporadic indicating recurrent mutational events at these sites rather than a founder effect. We here report on cases misdiagnosed as having chronic autoimmune thrombocytopenia (ITP). In these cases, inappropriate treatment with intravenous immunoglobulin G and corticosteroids as well as splenectomy increases susceptibility to infections and further decreases platelet count.

P-ClinG-088

Stickler syndrome type 1–with predominantly ocular features with autosomal dominant inheritance- in a six generation family and a novel mutation in the COL2A1 gene

Neumann LM.[1], Porstmann AU.[2], Kaminski E.[3], Velhagen KH.[2]
[1]Institute of Human Genetics Charité Universitätsmedizin, Berlin, Germany, [2]Department of Ophthalmology Charité Universitätsmedizin, Berlin, Germany, [3]Practice of Human Genetics, Hamburg, Germany

Introduction: Predominantly ocular Stickler syndrome (OMIM 108300) is a rare syndrome which is known to result from mutations in the COL2A1 gene. Autosomal dominant inheritance is described, additional features such as premature osteorthrosis, cleft palate, hearing impairment and craniofacial abnormalities may be present.
Case Report: We present a six generation German family with ocular Stickler syndrome. The index patient, a 23 year old male patient had bilateral retinal ablation and a marfanoid habitus, hypermobile elbow and finger joints. He had a bifid uvula. Echocardiographic examination was normal.
Retinal ablation was present in most adult patients of our family. One patient had ablatio retinae at an early age, 7 years and 12 years respectively resulting in blindness. The mother as well as the maternal grandmother of the index patient had camptodactyly. One patient had cleft palate. Hearing as well as body measurements and psychomotor development was normal in all patients.
In exon 50 of the COL2A1 gene an up to now undescribed mutation Arg992Stopp(R992X;CGA-TGA) which is most probable pathogenic could be verified in 5 patients of this family–the index patient, the mother, the maternal grandmother and her brother and his son.
Comment: Our family shows autosomal dominant inheritance of Stickler syndrome also called nonsyndromic ocular membraneous vitreous type in six generations. All patients had ocular symptoms, some presented with additional mild skeletal features. Until now ocular Stickler syndrome was predominantly associated with mutations in

exon 2 of the COL2A1 gene. This is the first report of a causative mutation in another region of the COL2A1 gene.

P-ClinG-089

Network 'Imprinting defects'

Rademacher K.[1], Buiting K.[1], Albrecht B.[1], Küchler A.[1], Gillessen-Kaesbach G.[2], Eggermann T.[3], Prawitt D.[4], Siebert R.[5], Horsthemke B.[1]
[1]Institut für Humangenetik, Essen, Germany, [2]Institut für Humangenetik, Lübeck, Germany, [3]Institut für Humangenetik, Aachen, Germany, [4]Kinderklinik, Mainz, Germany, [5]Institut für Humangenetik, Kiel, Germany

The network investigates imprinting defects, which are a rare cause of genetic diseases. Genomic imprints are gamete-of-origin specific chromatin marks (DNA methylation and histone modifications), which make the two parental alleles functionally different. Errors in imprint erasure, establishment and maintenance lead to aberrant gene expression and disease. Imprinting defects contribute to several recognizable syndromes (Prader-Willi syndrome, Angelman syndrome, Beckwith-Wiedemann syndrome, Silver-Russell syndrome, Transient Neonatal Diabetes Mellitus, upd(14)mat syndrome) and probably to other disorders as well (macrosomia and early-onset morbid obesity, small for gestational age). Whereas most imprinting defects occur without any DNA sequence change (primary imprinting defects), some are the result of a mutation in an imprinting control element (secondary imprinting defects). Using locus-specific and genome wide DNA methylation and gene dosis studies, we will identify patients who have single or multiple imprinting defects and search for genetic variants in cis-regulatory elements and trans-acting factors that are involved in genomic imprinting. At the phenotypic level, we will define the clinical spectrum of imprinting defects and compare patients with an imprinting defect with those who have a chromosomal aberration or DNA sequence mutation affecting the imprinted domain. Clinical and molecular data are being collected in a special designed database (supported by the BMBF).

P-ClinG-090

A novel NEMO mutation causes X-linked osteopetrosis and lymphoedema combined with anhidrotic ectodermal dysplasia and immunodeficiency (OL-EDA-ID) and additional metaphyseal irregularities

Reicherter K.[1], Lausch E.[1], Unger S.[2], Superti-Furga A.[1], Zabel B.[1]
[1]University of Freiburg: Centre for Pediatrics and Adolescent Medicine, Freiburg, Germany, [2]University of Freiburg: Centre for Pediatrics and Adolescent Medicine; Institute for Human Genetics, Freiburg, Germany

The transcription factor NF-kB regulates the expression of genes involved in control of immune and stress responses, inflammatory reactions and protection against apoptosis. NF-kB is inactivated when bound to the inhibitor IkB and controlled by NEMO (NF-kB essential modulator, IKGKB). Cell lines defective of NEMO do not activate NF-kB in response to most stimuli. Mutations in the X-linked NEMO gene account for the human diseases Incontinentia pigmenti (IP), X-linked anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) and the more severe phenotype with EDA-ID plus osteopetrosis and/or lymphoedema (OL-EDA-ID).
A male patient was initially referred to us with the possible diagnosis Cartilage-hair hypoplasia (CHH) to perform RMRP mutation analysis. He suffered from recurrent infections with low immunoglobulin levels and persistent eosinophilia. Additional findings were an enteropathy with failure to thrive, and epidermal hyperplasia. The hair was fine and sparse. The X-rays showed a metaphyseal dysplasia as well as increased bone density. The baby boy died at the age of 6 months of a septic shock.
The diagnostic key features were 'short stature' and 'severe immune deficiency' but the RMRP gene test excluded CHH. As Omenn syndrome is also characterized by a severe immune phenotype, including eosinophilia, and association with short-limbed dwarfism, we performed mutation analysis for Omenn syndrome, excluding RAG1, RAG2 and ARTEMIS gene mutations. By extending the diagnostic features including the symptoms 'ectodermal dysplasia', 'epidermal hyperplasia' and 'abnormal bone density' we focused on OL-EDA-ID as possible clinical diagnosis and sequenced the NEMO gene. Thereby we identified a novel hemizygous insertion (c.1237insT) in exon 10 of the NEMO gene that presumably affects the zinc finger domain of the NEMO protein. The clinically unremarkable mother carried the same mutation, the father, an unaffected brother and a maternal aunt showed a normal NEMO sequence. Then we got to know that the mother was again pregnant with a male fetus. The parents refused prenatal NEMO analysis but the newborn boy was tested directly after birth, although he appeared to be clinically normal. In contrast the X-rays of this boy showed metaphyseal dysplasia and increased bone density as observed in his deceased brother. DNA and cDNA sequencing revealed the presence of the c.1237insT NEMO mutation. In consequence, the boy was already treated before having evolved recurrent infections due to immunodeficiency, or inflammatory enteropathy. An antibiotics prophylaxis and substitution of immunoglobulins was started directly after diagnosis and bone marrow transplantation is scheduled.
In conclusion, we identified a novel c.1237insT NEMO mutation in the index patient, his mother and the newborn brother. This mutation causes OL-EDA-ID associated with metaphyseal dysplasia in males. The combination of features (ectodermal dysplasia with epidermal hyperplasia and sparse hair; severe immunodeficiency; abnormal bone density) distinguishes this condition from Cartilage-hair hypoplasia and Omenn syndrome. OL-EDA-ID should be diagnosed as soon as possible as defective NF-kB signaling leads to death within the first two years of life, if male patients are not transplanted.

P-ClinG-091

SYCP3 mutations are uncommon in aneuploid conceptions

Roos A.[1], Eggermann T.[1], Schwanitz G.[2], Zerres K.[1], Caliebe A.[3], Kautza M.[3], Löffler C.[4], Heidemann S.[3]
[1]Institute of Human Genetics, Aachen, Germany, [2]Institute of Human Genetics, Bonn, Germany, [3]Institute of Human Genetics, Kiel, Germany, [4]Department of Human Genetics, Chemnitz, Germany

The SYCP3 gene (12q23.3) encodes a structural protein component of the synaptonemal complex (synaptonemal complex protein 3) which mediates the synapsis between homologous chromosomes during meiosis I. Clinical studies of knock-out mice revealed azoospermia in male animals (Yuan et al., 2000) and increased aneuploid fetuses in female animals (Yuan et al., 2002). Miyamoto et al. (2003) identified a recurrent SYCP3 frameshift mutation in 2 out of 19 males with azoospermia. Recently, Bolor et al. (2009) identified SYCP3 mutations in 2 out of 26 females with recurrent pregnancy losses. To clarify, whether mutations of the SYCP3 gene may predispose for aneuploid conceptions in humans, we investigated the coding and immediately flanking regions of the SYCP3 gene by direct sequencing in 46 fetal materials (chorionic villi and/or amniotic cells) with different aneuploidies. For that purpose we preselected fetal materials with an advanced probability of a genetic predisposition according to three clinical subgroups: I. aneuploid fetal materials of women with consecutive aneuploid conceptions (n=8), II. fetal materials with double or multiple aneuploidies (n=13) and III. aneuploid/polyploid fetal materials of women younger 35 years (n=25). No mutations were detected within the coding and flanking sequences of the SYCP3 gene in all three clinical subgroups. Even considering the limitation, that only 50% of parental mutations are detectable in fetal materials our results suggest that SYCP3 mutations are no common reason for aneuploid conceptions. Therefore we assume that other meiotic genes account for the genesis of aneuploidies in humans.

P-ClinG-092

Brugada syndrome may be part of the cardiac disease in myotonic dystrophy type 2: report of two unrelated patients

Rudnik-Schöneborn S.[1], Schaupp M.[2], Lindner A.[2], Kress W.[3], Schulze-Bahr E.[4], Elbracht M.[1], Zerres K.[1]
[1]Institute of Human Genetics, RWTH Aachen, Germany, [2]Department of Neurology, Marienhospital Stuttgart, Germany, [3]Institute of Human Genetics, University of Würzburg, Germany, [4]Department of Cardiology, UKM Münster, Germany

Myotonic dystrophy type 2 (DM2) is an autosomal dominant multisystem disorder caused by CCTG repeat expansions within intron 1 of the ZNF9 gene on chromosome 3q. Cardiac conduction disturbances, supraventricular arrhythmias and cardiomyopathy are described in DM2 but Brugada like features have not yet been reported.
Brugada syndrome (BS) is a genetically heterogeneous cardiac conduction disorder which is characterized by a significant ST segment elevation upon ECG evaluations and bears an increased risk for sudden cardiac death.
We observed two unrelated patients with genetically confirmed DM2 who developed clinical relevant cardiac arrhythmias on the basis of ECG findings typical for BS. Patient 1 had elevated liver enzymes from 24 years, hand myotonia from 34 years and was diagnosed DM2 at 38 years. After ajmalin provocation at age 35 years he showed increasing right bundle branch blocking and developed ventricular tachycardias and atrial fibrillation which were self-terminating. He received beta blockers for treatment. Patient 2 had muscle stiffness since 20 years of age but no weakness when last examined at 49 years. At 35 years of age, bilateral cataracts were removed. He was treated with beta blockers for cardiac rhythm disturbances since age 44, nonetheless he had two episodes of near syncopes at 47 years. Following ajmalin provocation at 48 years, ventricular tachycardia with right bundle branch block was induced. He received a dual-chamber cardioverter-defibrillator-pacemaker. Family history was negative for BS, but the mothers of both index patients were also affected by DM2 and had different ventricular rhythm disturbances.
Recently, a high incidence of BS was found among French patients with myotonic dystrophy type 1 (DM1). We hypothesize that Brugada like features may be part of the cardiac disease in myotonic dystrophy and may be explained by alternatively spliced isoforms of ion channel genes.

P-ClinG-093

Paternally inherited deletion 1q21.1 involving TAR region without TAR features: a case report

Samuelsson L.[1], Andreasson AC.[2], Samuelsson B-O.[2], Henricsson M.[1], Ahlfors F.[1], Stefanova M.[1]
[1]Department of Clinical Genetics, Gothenburg, Sweden, [2]Department of Paediatrics, Alingsas, Sweden

Here we report 5 years-6-months-old boy with a deletion of 1q21.1 sized 1.9 Mb and encompassing Thrombocytopenia-Atresia Radii (TAR) critical region. He presented with moderate mental retardation, speech delay, brachycephaly, minimal skeletal changes, conductive hearing impairment, flat haemangioma, recurrent infections and following dysmorphic features: broad face, slightly slant up almond shaped palpebral fissures, hypertelorism, epicanthic folds, slight strabismus, narrow nose, prominent base of nose, hypoplasia alae nasi, short philtrum, high arched palate, low-set posteriorly rotated dysplastic ears, thick lobules with transversal creases, tragus hypoplasia, low posterior hairline, short broad hands, fingers and toes, clinodactyly, and syndactyly. The chromosome aberration was detected by high-resolution array analysis, 250k SNPs array (Affymetrix) and extends between the following base pairs: 144404740-146281318. A deletion 1q21.1 with the exact same location and size was found with the healthy father.
To our knowledge there are very few previous reports of inherited deletion 1q21.1 syndrome from a healthy parent. Additionally, this patient is one of the rare examples of 1q21.1 deletion involving TAR critical region and without any features of TAR syndrome.

P-ClinG-094

NOTCH2 mutations in patients with Alagille syndrome

Schmidt C.[1], Wandt D.[1], Schulz S.[2], Gläser C.[1]
[1]Institute of Human Genetics and Medical Biology Martin-Luther University Halle-Wittenberg, Halle, Germany, [2]University School of Dental Medicine Department of Operative Dentistry and Periodontology Martin-Luther University Halle-Wittenberg, Halle, Germany

Aim: Alagille syndrome (ALGS) is an autosomal dominant multisystemic disorder. Main symptoms of this complex disease with reduced penetrance and variable expressivity are chronic cholestasis, congenital heart anomaly, skeletal defects, eye and kidney abnormalities and a characteristic face. Disease associated genetic background was found within the NOTCH-signalling pathway: about 80% of the patients are associated with mutations of JAG1 (ALGS1; MIM #118450) and some few cases with NOTCH2 (ALGS2; MIM #610205). We report on five novel NOTCH2 mutations in ALGS2 patients with varying severity of clinical symptoms.
Methods: We performed a step-wise molecular analysis starting with JAG1 investigation. Unless the detection of a JAG1 mutation cycle sequencing of NOTCH2 was performed.
Result: The first case is a three years old girl who already has undergone a liver transplantation. The heterozygous amino acid exchange p. 1759V>A is situated within exon 29 in the cytoplasmic domain of NOTCH2 receptor. Her clinically unaffected mother is heterozygous for the mutation as well. The 2nd case is a two years old female with liver transplantation and a heterozygous amino acid exchange in exon 24 (p.1327D>G, EGF-like domain 34, involved in the receptor-ligand interaction), which was also identified in her clinically unaffected father. 3rd case: a liver transplantation was considered for a five years old boy with a heterozygous base pair deletion nearby the acceptor splice site of intron 3 (IVS3-6delC). The 4th patient, a four month old male with clinical symptoms compatible with ALGS carries a heterozygous exon 34 mutation (p.2032H>N) affecting the 6th Ancyrin-domain which is involved in the protein-protein interplay. His clinically unaffected mother is heterozygous as well. 5th case: a 51 years old woman suffering from hepaticolithiasis and considered for liver transplantation was identified to have an intron 9 mutation (IVS9-17C>T). This heterozygous base pair exchange is situated close to the acceptor splice site and was not found in 115 healthy controls.

P-ClinG-095

Array-based genome-wide genotyping: an approach to identify candidate genes contributing to the development of anorectal malformations

Schramm C.[1], Draaken M.[2], Bartels E.[1], Boemers T.[3], Ludwig M.[4], Nöthen MM.[2], Reutter H.[5]
[1]Institute of Human Genetics, Bonn, Germany, [2]Institute of Human Genetics and Department of Genomics - Life & Brain Center, Bonn, Germany, [3]Department of Pediatric Surgery and Pediatric Urology, Cologne, Germany, [4]Department of Clinical Chemistry and Pharmacology, Bonn, Germany, [5]Institute of Human Genetics and Department of Neonatology, Bonn, Germany

Anorectal malformations (ARM), syndromic and non-syndromic, comprise a broad clinical spectrum from mild anal to severe anorectal anomalies including complex cloacal malformations with an overall incidence of about 1 in 2500 live birth. Observation of familial cases and genetically determined syndromes associated with structural or numeric chromosomal anomalies which include ARM as part of their

phenotypic spectrum, e. g. trisomy 18 or 21, indicate the importance of genetic factors in the assumed multifactorial etiology of ARM. However, main genetic factors causing ARM could not be elucidated so far. Since ARM are often associated with reduced reproduction it is reasonable to assume that a significant proportion of patients carry de novo mutations. We used Illumina's Human660W-Quad-v1 BeadChip containing 657,367 markers for genotyping 10 patients with different forms of ARM and their non-affected parents. The parallel investigation of the patients' parents allows efficient identification of de novo mutations.
Investigating the initial data set we were able to identify a 15 Mb de novo duplication of 18p11.21 – 18q12.1 in a female patient presenting with rectovesical fistula and patent ductus arteriosus (PDA). Anal anomalies, especially anal fistulas, have not been reported in association with partial trisomy 18 before. Therefore our results narrow the candidate region for the anorectal malformations observed in trisomy 18. The identification of candidate genes in this region might lead to better understanding of the biology of anorectal development.

P-ClinG-096
MRNET TP7 – Summary of high resolution molecular karyotyping of patients with mental retardation – Identification of regions containing candidate genes
Schrock E.[1], Tyshchenko N.[1], Neuhann T.[1], Tinschert S.[1], Hackmann K.[1]
[1]Institute of Clinical Genetics, Dresden, Germany

The German Mental Retardation Network (MRNET) is a nationwide consortium that aims for elucidation of genetic causes of intellectual disability (ID). Currently, ten centers (TP="Teilprojekt") are involved. Participating patients are carefully examined in the clinical genetics departments of each center. Eligible causes like Fragile X syndrome or RETT disease as well as other phenotypically noticeable syndromes are excluded by standard diagnostics before chromosome analysis is performed. If this examination does not reveal any major genomic rearrangements array CGH is carried out. We are using Agilent's array CGH platform which is at present being updated to the 1M chip and has been used with the standard 244k chip so far. Every suspicious copy number variation (CNV) is validated by FISH analysis, customized array CGH, quantitative PCR, or quantitative genotyping in patients as well as in parents to see if a certain variation is of familial origin or arose de novo. All obtained data sets are fed into a database that is accessible for every participating center to carry out comparisons to distinguish between unpublished common CNVs and CNVs that contain candidate genes. Disease causing mutations that have been confirmed as plausible reason of a patient's feature will be further investigated for functional analysis for example in animal models. Out of 78 patients we found eight de novo mutations and 17 patients without suspicious CNVs while all others carry familial rearrangements of unclear meaning. Candidate loci we are currently investigating include one locus with four genes for frontotemporoparietal polymycrogyria and one gene for perisylvian polymicrogyria, one candidate gene for Shprintzen-Goldberg syndrome/Loeys Dietz syndrome type 1, one gene for microcephaly as well as several other genes for non-syndromal intellectual disability.

P-ClinG-097
Microdeletion syndrome 16q11.2q12.2: a Case Report
Shoukier M.[1], Wickert J.[1], Burfeind P.[1], Pantazi A.[1], Schneegans S.[1], Weise D.[2], Zoll B.[1]
[1]Institute of Human Genetics, Göttingen, Germany, [2]Department of Paediatrics and Paediatric Neurology, Göttingen, Germany

he 16q11.2q12.2 deletion syndrome is a newly recognized microdeletion syndrome (Ballif et al. 2008). To the best of our knowledge, till now 5 patients have been described. Our patient here shows a high similarity to the published cases.

We report on a 21-month-old boy with severe mental retardation, amblyacousia and microcephaly, muscular hypotonia, epilepsy, widespread and inverted nipples, facial dysmorphism like microretrognathy, telecanthus, hypotelorism, deep-set ears with auricle dysplasia like dysplastic helices, median cleft of the soft palate, broad nasal root (wide nasal bridge), cranial asymmetry and a prominent protuberance of the occipital bone. Also, a heart defect was detected (DD: residual patent foramen ovale (PFO) and an ostium secundum atrial septal defect (ASD)). In medical examination he shows no eye-contact, but shows a light reaction. Multiple periventricular cysts were found in cranial sonography. The brain cavities were enlarged and the corpus callosum was absent. The parents and the three siblings of the patient are healthy. Genetic analyses with normal results included chromosome analysis (350–400 band level), analysis of the subtelomeric regions by qPCR, and FISH analysis for DiGeorge-locus. Array CGH revealed a 16q interstitial deletion of approximately 4.7 Mb with breakpoints in bands 16q12.1q12.2, spanning from position 48017848 to 52725934. This deletion contains 17 RefSeq genes including the SALL1-, CHD9-, TMEM188-, FTO-, CYLD-, NOD2-, ZNF423 and SLI1-genes. Mutations in SALL1-gene are the cause of Townes-Brocks-syndrome (TBS), which is a rare autosomal-dominant condition characterised by malformations of anus, hands and ears. The absence of anus and hand malformations excludes clinically TBS in our patient. This is consistent with previously published data, which supports that a dominant-negative action is the predominant mechanism underlying TBS.

P-ClinG-098
Interstitial deletion 11p detected by array CGH in a boy presenting with BNS seizures and mental retardation
Siebers-Renelt U.[1], Müller-Hofstede C.[1], Bohring A.[1], Wieacker P.[1], Röpke A.[1]
[1]Institut für Humangenetik, Münster, Germany

We report on a 2 years old boy with profound mental retardation, muscular hypotonia and BNS epilepsy since 3 months of age. Cerebral MRT showed a hypoplastic corpus callosum and cysts of the plexus chorioideus. No major malformations of inner organs were present. Dysmorphic features include plagiocephaly, frontal bossing, epicanthic folds, telecanthus, short palpebral fissures, depressed nasal bridge and dyplastic ears. Conventional cytogenetic analysis (GTG-banding, 600 bands) and screening for subtelomeric rearrangements by FISH were inconspicuous (46,XY.ish subtel(41x2)). However, array CGH analysis using a 244 K chip revealed a 7.906 Mb deletion spanning the chromosomal region 11p11.2 to 11p12. This deletion encompasses 27 genes including the EXT2 and ALX4 gene. The deletion was confirmed by realtime PCR and not found in both parents. Deletions of proximal 11p have been described in patients with multiple exostoses and enlarged parietal foramina. The phenotype is also known as Potocki-Shaffer syndrome. Some, but not all patients show mental retardation and about half of them suffer from hypotonia and seizures. Interestingly, in our patient the predominant clinical feature are therapy resistent BNS seizures and he does not show obvious exostoses at age 2 years. Possibly, he will develop this characteristic feature later in life, so that a thorough follow-up of the skeletal phenotype is recommended.

P-ClinG-099
Buccal smear MS-MLPA as a tool for the detection of 11p15 hypomethylation in Silver-Russell syndrome
Spengler S.[1], Begemann M.[1], Binder G.[2], Bachmann N.[3], Eggermann T.[1]
[1]Institute of Human Genetics, Aachen, Germany, [2]Children's Hospital, Tübingen, Germany, [3]Bioscientia Centre for Human Genetics, Ingelheim, Germany

Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous disorder which is primarily characterized by intrauterine and postnatal growth restriction. Further typical features include a trian-

gular shaped face with a prominent forehead, a relative macrocephaly, asymmetries of the body and the limbs, and clinodactyly of the fifth digits.
In > 38 % of SRS patients hypomethylation of the telomeric imprinting control region 1 (ICR1) on chromosome 11p15 can be detected. The ICR1 controls the expression of two imprinted genes: insulin-like growth factor 2 (IGF2) is expressed from the methylated paternal allele while H19 is expressed from the unmethylated maternal allele. Hypomethylation of the ICR1 results in repression of IGF2 and bi-allelic expression of H19.
Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is a well established method for the detection of (epi)mutations in 11p15 in SRS patients. Usually, DNA samples derived from peripheral blood lymphocytes are used for the standard diagnostic procedure. We analysed buccal smear DNA taken from both cheek sides of 22 SRS patients to check whether i) ICR1 hypomethylation can also be detected in other tissues and ii) the detection rate can be increased due to the discovery of a tissue-specific mosaicism of methylation patterns.
In the six SRS patients with ICR1 hypomethylation in blood the epimutation was also visible in buccal smear. Interestingly, a slight difference in the degree of hypomethylation was seen in samples taken from the right and the left cheek and from blood indicating the presence of a tissue-specific as well as body-side-specific hypomethylation mosaicism. The detection rate could not be increased in the examined cohort since no ICR1 hypomethylation was found in buccal smear of the 16 patients without epimutation in blood.

P-ClinG-100

Spondyloperipheral dysplasia - Clinical and radiographic delineation of a novel COL2A1 missense mutation in the C-Propeptide

Thiel C.T.[1], Doerr H.G.[2], Sticht H.[3], Rauch A.[4]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Childrens and Adolescents Hospital, Erlangen, Germany, [3]Institute of Biochemistry, Erlangen, Germany, [4]Institute of Medical Genetics,Schwerzenbach-Zurich, Switzerland

Mutations in the type II collagen gene (COL2A1) result in a broad spectrum of skeletal dysplasias. Most of these are located in the coding region for the helical part of the alpha1(II)-chain and lead to alterations of the triple helix formation or to nonsense-mediated decay (NMD) as observed in Stickler syndrome. In contrast, truncating mutations coding for the C-propeptide have been recently identified as the underlying cause of spondyloperipheral dysplasia [SPD, MIM 271700].
We present an 11 year-old female with characteristic features of SPD-like short-trunk dwarfism and brachydactyly E-like changes. Growth retardation was first noted at 25th week of gestation and she was born at 36th weeks of gestation with a birth weight of 1990 g (-1.7 SDS) and length of 41 cm (-2.9 SDS). Psychomotor development was normal. Her HC followed the 50th to 97th c. whereas her growth remained below the 3rd c. with a proposed adult height of 145 cm. Growth hormone treatment was futile. There have been no signs of hearing impairment or eye problems as reported in other cases of SPD. X-ray evaluation at 11 years of age confirmed a spondyloepiphyseal dysplasia with an accelerated bone age, delayed ossification of the pubic and ischial bones and degenerative changes of the femoral head, horizontal acetabulae, epiphyseal changes and shortening of the metarcapals and the distal phalanges, mild metaphyseal widening at the knee joint and a platyspondyly with fish-mouth vertebrae.
Mutational analysis of the COL2A1 gene revealed the de-novo missense mutation p.F1486C (NM_033150: c.4250T>G, p.F1417C) within the C-propeptide coding region. All known mutations in patients with SPD result in a premature stop codon within the C-propetide disrupting disulfide bonds formed by cysteine residues or trimer association specific recognition sequences. Therefore, it has been proposed that the C-propeptide plays a crucial role in the association of the procollagen chains in helix formation, which might be disturbed by truncating mutations. As our patient presented with mild clinical and characteristic radiographic features of SPD caused by the first reported missense mutation forming a new cysteine residue, our results support the hypothesis of a conformational change of the C-propeptide by alteration of the disulfide bonds composition. This is of special relevance as accumulation of free procollagen II chains with a calcification-promoting effect of the C-propeptide has been proposed as the underlying cause of SPD.

P-ClinG-101

Definition of the critical region in chromosome band 1q25 for patients with mental retardation and cleft lip / palate.

Tinschert S.[1], Tyshchenko N.[1], Hackmann K.[1], Klinik B.[1], Rump A.[1], Schrock E.[1]
[1]Institut für Klinische Genetik, Dresden, Germany

Microdeletions and microduplications are a known cause of mental retardation with and without further clinical findings. Interstitial deletions of the long arm of chromosome 1 have so far been classified as either proximal deletion ranging from 1q21 to 1q25 or intermediate deletion from 1q24 to 1q32. The phenotypes of the patients in each group overlap partially (Descartes et al., 2008).
Here, we report on one patient with an interstitial deletion in 1q25.2-q25.3 combined with duplication in 1q25.2. The four-year-old girl was referred to the clinical genetics center because of mild/moderate developmental delay, cleft palate, microcephaly, epilepsy, and mild facial dysmorphic features. The unspecific combination of the features did not allow for a diagnosis. Therefore, whole genome array CGH using an Agilent 244 A chip was performed, with the result of a de novo duplication accompanied by an allelic de novo deletion in chromosome band 1q25.
All features present in our patient have been reported in other patients with larger interstitial deletions including band 1q25 and demonstrate the minimal clinical presentation of this syndrome. It was therefore possible, based on our data, to reduce the smallest region of overlap (SRO) from 14 MB to 6.5 Mb. Moreover, 1q25.2-q25.3 may be considered as a new cleft palate associated locus, since over 70% of the patients with overlapping interstitial deletions in 1q25 have lip and or palate anomalies. The definition of the candidate gene is still difficult, since the overlapping region has a size of 6.5 Mb and contains 48 genes with many of them of unknown function.
We expect that the identification of further patients with 1q25 deletions will either provide evidence to classify the 1q25 deletion as a contiguous gene deletion syndrome or to define single genes causing lip/palate abnormalities and developmental delay.

P-ClinG-102

Case report of a patient with SMC1A Mutation and review of probands with X-Linked Cornelia de Lange Syndrome

Uhrig S.[1], Pfleger A.[2], Weber-Mzell D.[2], Gruber-Sedlmayr U.[2], Schwarzbraun T.[1], Gabriel H.[3], Speicher M.R.[1]
[1]Institut für Humangenetik, Graz, AT, [2]Universitätsklinik für Kinder und Jugendheilkunde, Graz, AT, [3]ZMG Osnabrück, Osnabrück, Germany

Cornelia de Lange syndrome (CdLS) is a dominant genetic disorder with variable expressivity and multiple organ system abnormalities which is classically characterized by typical facial features, growth and mental retardation, upper limb defects, hirsutism, gastrointestinal and other visceral system involvement. Mutations in three genes, NIPBL, SMC1A and SMC3, etiologically account for about 65% of individuals with CdLS. So far a total of 29 probands with 26 different mutations of the SMC1A gene have been reported. Here we describe a male patient with a new missense mutation c.2078G>A resulting in the previously described aminoacid change p.Arg693Gln (Mannini et. al. 2009, Hum

Mutat, Sep 23). Our patient is a 15-month-old boy with CdLS who was delivered in the 35th week of gestation by caesarean section due to fetal distress and pathologic Doppler ultrasound. Birth weight (2 280 g), length (49 cm) and head circumference (32,5 cm) were all in the lower normal range. Post partum a right side diaphragmatic hernia with diaphragma aplasia, lung hypoplasia and pulmonal hypertension was noted and required surgery and long term assisted ventilation. Malnutrition and gastroesophageal reflux needed surgical correction with fundoplication and gastrostomy tube placement when the boy was 18 month old. At the age of 15 month the boys weight (9043 g; P10) , length (76 cm; P10) and head circumference (46 cm; P18) were all in the lower normal range. The boy showed a coarse face with brachycephaly, arched eyebrows, long, thick eyelashes, broad nasal bridge, upturned nasal tip with anteverted nares, low-set posteriorly rotated ears, long smooth philtrum and short neck. Upper and lower limbs showed no malformation but hands and feet are small with proximally placed thumbs. The boy had cryptorchidism and orchidopexy was done. G-banding analysis of peripheral lymphocytes revealed a normal male karyotype (46,XY). Array CGH on the 44k-Chip (Agilent) was done to rule out chromosomal imbalances. Due to phenotype features mild type CdLS was suspected. Sequencing of the NIPBL gene revealed no mutation. Sequencing of the SMC1A gene identified a new missense mutation c.2078G>A resulting in an aminoacid change. This aminoacid change was previously described however due to a different mutation (c.2077C>G). Here we report the first patient with a new missense mutation in the SMC1A gene resulting in a CdLS phenotype with severe intestinal malformations (CHD and cryptorchidism) requiring long term assisted ventilation.

P-ClinG-103

Horizontal Gaze Palsy with Progessive Scoliosis (HGPPS): Clinical and neuroradiological findings in 4 patients from three consanguineous families and identification of three novel mutations in the ROBO3 gene

Volk A.[1], Carter O.[1], Fricke J.[2], Herkenrath P.[3], Poggenborg J.[4], Eysel P.[5], Kubisch C.[1], Neugebauer A.[2]
[1]Institute of Human Genetics, University of Cologne, Germany, [2]Department of Ophthalmology, University of Cologne, Germany, [3]Department of Pediatrics, University of Cologne, Germany, [4]Department of Radiology, University of Cologne, Germany, [5]Department of Orthopedics, University of Cologne, Germany

Horizontal gaze palsy with progressive scoliosis (HGPPS, OMIM 607313) is a rare autosomal recessive neurological disorder characterized by congenital absence of conjugate horizontal eye movement, progressive scoliosis, and failure of the corticospinal and somatosensory axon tracts to decussate in the medulla. Major clinical complications are elicited by progressive scoliosis. HGPPS is caused by homozygous or compound heterozygous mutations in the ROBO3 gene located on chromosome 11q23-q25.
We present 4 patients of 3 consanguineous families with typical clinical and neuroradiological findings of HGPPS (aged 8 months – 12 years). Molecular analysis revealed three novel mutations of the ROBO3 gene: a homozygous frameshift mutation (c.1014delAinsTGC; p.Ile305CysfsX21), a larger homozygous deletion (c.2770_2279del10, 2279+1_+21del21) and a splice site mutation at the end of exon 22 (c.3509A>C/p.S1107R) confirmed by cDNA analysis.
Interestingly, in one patient the typical ophthalmologic phenotype and neuroradiological findings led to the diagnosis before scoliosis developed. Accordingly, early diagnosis of HGPPS by ophthalmologic and molecular investigations allows early orthopedic treatment which may help to attenuate scoliosis, i.e. the symptom responsible for the major complications of HGPPS.

P-ClinG-104

Analysis of a complex chromosome rearrangement t(13;14;17) by conventional cytogenetics, Fluorescence In Situ Hybridization (FISH), Spectral Karyotyping (SKY), and Molecular Karyotyping

Werner W.[1], Frensel A.[1], Schröck E.[1], Klink B.[1], Brocke K.[2], Hackmann K.[1]
[1]Institut für Klinische Genetik, Dresden, Germany, [2]Klinik und Poliklinik für Kinder- und Jugenmedizin, Dresden, Germany

Individuals carrying an apparently balanced chromosomal rearrangement most often appear unaffected. However, in carriers with a severe phenotype, it is assumed that the disruption of a gene (or a small imbalance) at one of the breakpoints could be causative for the observed phenotype. Alternatively, the chromosomal rearrangement could be coincidental to another defect causing the abnormal phenotype. In this sense, complex chromosome rearrangements (CCRs) involving more than two chromosome breapoints are of special interest.
We report on a 2 1/2-year-old girl with global developmental delay, especially of speech development, and microcephaly. The cytogenetic analysis revealed a complex karyotype 46,XX,inv(11)(q22.2q24),t(13;14;17)(q22;q24.2;p13.3). Employing FISH and SKY analysis, we could delineate the complex rearrangement t(13;14;17) in two separate rearrangements, an insertional translocation ins(17;13)(p13.2-p13.3;q22q33) and a reciprocal translocation t(13;14)(q33-q34;q24.2). Possibly, the insertional translocation ins(17;13) and the reciprocal translocation t(13;14) share an (nearly) identical breakpoint at 13q33-q34. Further, Array-CGH analysis has been applied to answer, if a submicroscopic imbalance at one breakpoint or at another site in the genome would be causative for the patients phenotype.
In conclusion, the combined approach of conventional cytogenetic, molecular cytogenetic (FISH, SKY), and Array-CGH analysis should underline their usefulness to resolve the genotype-phenotype correlations in patients with apparently balanced rearrangements. MRNET

P-ClinG-105

Report of five novel CHD7 mutations in patients with CHARGE syndrome supporting the variability of the clinical phenotype

Wessels K.[1], Bohnhorst B.[2], Luhmer I.[3], Morlot S.[4], Bohring A.[5], Jonasson J.[6], Epplen J.T.[7], Gadzicki D.[8], Glaser S.[8], Göhring G.[8], Arslan-Kirchner M.[1], Stuhrmann M.[1], Schmidtke J.[1], Pabst B.[1]
[1]Institute of Human Genetics, Hannover Medical School, Germany, [2]Clinic for Paediatric Pneumology and Neonatology, Hannover Medical School, Germany, [3]Clinic for Paediatric Cardiology and Internal Medicine, Hannover Medical School, Germany, [4]MVZ wagnerstibbe, Hannover, Germany, [5]Institute of Human Genetics, Westphalian Wilhelms-University, Germany, [6]Clinical genetic, University Linköping, Sweden, [7]Human Genetic Department, Ruhr University Bochum, Germany, [8]Institute of cell and molecular pathology, Hannover Medical School, Germany

CHARGE syndrome (OMIM 214800) is a multiple malformation disorder. The acronym summarizes the main clinical features of the syndrome, which are coloboma, heart defect, choanal atresia, retardation in development and growth, genital hypoplasia and ear anomalies. Further common clinical findings include hypoplasia of the semicircular canals, rhombencephalic dysfunction, facial nerve palsy, cleft lip/palate, tracheo-esophageal fistula, arhinencephaly and distinctive facial features. Occasional findings are renal anomalies, thymic/parathyroid hypoplasia and hand and spine anomalies. Diagnostic criteria were set up and redefined by Blake et al. (1998) and later by Verloes (2005). Mutations in the chromodomain helicase DNA-binding protein 7 (CHD7) gene cause most cases of CHARGE syndrome. The majority of CHD7 gene mutations is unique and occurs de novo. Occasional familial transmission and germline mosaicism have been described.
Here we report on the mutation analysis in 19 patients either with typical features of CHARGE syndrome or suspected to have CHARGE syndrome. Altogether eight pathogenic mutations were identified, five of which were new and contribute to the mutation spectrum found in

CHARGE patients. The mutations were scattered throughout the gene with no mutational hotspot. The main mutation type recognized is truncation mutation of the CHD7 gene. All patients who fulfilled the diagnostic criteria according to Blake's et al. and/or Verloes' scoring schemes were heterozygous carriers of a disease-causing CHD7 mutation. In five children under the age of one year mutations in the CHD7 gene were identified. Applying the scoring scheme of Verloes in two of the five infants no major criterion was reported. Thus it is advisable to consider a CHARGE syndrome in infants even if they do not fulfill the diagnostic score. The variability of clinical features found in recurrent mutations confirms and emphasizes the wide variability of the clinical phenotype of patients with CHARGE syndrome.

P-ClinG-106

Williams Beuren-like phenotype in a boy with a de novo microdeletion 15q22.1-q22.2

Wohlleber E.[1], Zink A.[1], Betz R.C.[1], Hoffer M.J.V.[2], Breuning M.H.[2], Ruivenkamp C.[2], Engels H.[1]
[1]Institute of Human Genetics - Rheinische Friedrich-Wilhelms-University, Bonn, Germany, [2]Department of Clinical Genetics - Leiden University Medical Center, Leiden, The Netherlands

Patients with mental retardation (MR) of unknown etiology are increasingly analyzed by molecular karyotyping using SNP arrays or array-CGH in order to identify disease-causing microaberrations and candidate genes for MR. Here, we report on a 14 6/12 year old boy who was referred with suspected Williams Beuren syndrome (WBS). He presented moderate MR, growth retardation, truncal obesity, strabismus and mild dysmorphisms, such as edema of the upper eyelids, a long philtrum, a thin upper lip and a prominent, everted lower lip. His length was 7 cm below the 3rd centile. His bone age at the age of 14 years was delayed by three years. In relation to his trunk, his legs seemed disproportionally short. A SON-R test (age 6 8/12 years) gave an IQ of 55-60. However, his language skills at age of 14 6/12 were significantly better in comparison. He was highly verbal with a sociable and outgoing personality.
Molecular karyotyping using an Illumina 610k SNP array detected a 5.33 Mb deletion in 15q22.1-q22.2 (NCBI 36). qPCR confirmed the deletion and demonstrated a de novo occurrence. The deletion contained 29 genes including several candidate genes with central nervous expression such as the forkhead transcription factor FOXB1 and the retinoic acid receptor RORA.
A phenotype-genotype correlation was hampered by the rareness of comparable deletions. A clinical comparison to patients with cytogenetically visible deletions of 15q22 (without molecular-cytogenetic work-up, i.e. without proven overlap) resulted in only few common clinical symptoms such as growth retardation, strabismus and thin upper lip. A comparison with two published patients with microdeletions which proximally overlap the microdeletion described here yielded only MR as a feature common to all microdeletion patients. A search of the DECIPHER database (http://decipher.sanger.ac.uk/) resulted in a microdeletion which overlaps the proximal 1.87 Mb of the microdeletion described here. Interestingly, the carrier of the said microdeletion was analyzed because of renal failure and displayed neither MR nor obesity nor dysmorphisms described here.
Several features of our patient such as his MR with comparatively good language skills and outgoing character, the pronounced growth retardation and the appearance of his mouth (long philtrum, thin upper and prominent lower lip) are reminiscent of WBS. Taken together, the said comparisons with other deletion carriers may be interpreted as an indication that our patient's WBS-like symptoms may be attributed to the haploinsufficiency of one or more genes such as FOXB1 and RORA which are localized in the distal part of the deletion described here.

P-ClinG-107

Mutation analysis of the SLC26A4 gene in 133 patients from Germany with nonsyndromic hearing loss

Zechner U.[1], Herrmanns P.[2], Keilmann A.[3], Napiontek U.[3], Bartsch O.[1], Haaf T.[4], Pohlenz J.[2]
[1]Institute of Human Genetics, Mainz, Germany, [2]Children´s Hospital, Mainz, Germany, [3]Department of Communication Disorders, Mainz, Germany, [4]Institute of Human Genetics, Würzburg, Germany

It is generally believed that mutations in the human SLC26A4 gene represent the second most frequent cause of autosomal recessive non-syndromic hearing loss (NSHL) after GJB2 mutations and affect 4% of the Caucasian population with NSHL. The determination of the SLC26A4 mutation spectrum and frequency in different populations with NSHL is crucial for the establishment of appropriate genetic testing and counseling services for congenital deafness. We performed a mutation analysis of the SLC26A4 gene in 133 NSHL patients from Germany without GJB2 mutations by direct sequencing of the SLC26A4 coding exons. In 8 patients (6,0%, 8/133) SLC26A4 mutations were identified. Only one patient (0,8%) was found to have a potential biallelic mutation being homozygous for the novel missense variant p.P525L. The other 7 patients were heterozygous for one SLC26A4 variant including the known mutations p.L597S, p.M283I, p.F354S and three novel variants p.M147I, p.N324I, p.M775I (two cases with p.L597S, and each one case with the other mutations and variants, respectively). The affected patients will now be subjected to a high-resolution temporal bone computerized tomography scan to verify the presence of an enlarged vestibular aqueduct. Our data argue that mutations in the SLC26A4 gene are a less frequent cause of NSHL in the German population than in other Caucasian populations and question the need for SLC26A4 mutation analysis in German NSHL patients not carrying biallelic GJB2 mutations.

P-ClinG-108

DNA diagnosis in Potter sequence – a rational approach

Zerres K.[1], von Bothmer J.[1], Rudnik-Schöneborn S.[1], Ortiz Brüchle N.[1], Bergmann C.[2]
[1]Institute of Human Genetics, Aachen, Germany, [2]Bioscientia Center for Human Genetics & Institute of Human Genetics, Aachen, Germany

Potter sequence (PS) refers to the typical appearance of a fetus due to oligohydramnios irrespectively of the underlying cause with a typical facial appearance and arthogryposis. Most often PS is caused by a kidney malformation but can also be a result of amniotic rupture or uteroplacental insufficiency. PS can be a manifestation of many syndromes (e.g. Kallmann- and BOR-, Meckel-, Jeune-, Joubert- syndromes, VATER association, and chromosomal disorders). Typical kidney lesions in PS are: renal agenesis often in combination with renal dysplasia (RD), autosomal recessive (ARPKD), early onset autosomal dominant (ADPKD) polycystic kidney disease, and as a rare cause renal tubular dysgenesis (RTD). To identify the underlying cause, a thorough clinical and pathoanatomical examination should be required. A detailed family history including a renal ultrasound in close family members should be performed before starting with a possible DNA diagnosis. Fetuses with enlarged kidneys with increased hyperechogenicity can be found in ARPKD as well as in early onset ADPKD. The most useful diagnostic information is a positive parental ultrasound in ADPKD, whereas normal parental ultrasound is essential for assuming the diagnosis of ARPKD. In early onset ADPKD mutations can be usually detected in the PKD1 gene and only exceptionally in the PKD2 gene. Mutations in the PKHD1 gene are found in ARPKD. Fetuses with renal agenesis and/or RD may be part of familial renal adysplasia or renal cyst and diabetes (RCD) syndrome with mutations in the HNF1ß gene and in rare cases even in the RET gene. Autosomal recessive RTD is a severe disorder of renal tubular development characterized by persistent fetal anuria. Absence or paucity of differentiated proximal tubules

is the histopathologic hallmark of the disorder. Mutations can be found in different genes: REN, AGT, ACE, or AGTR1 encoding components of the Renin-Angiotensin System. We will describe a diagnostic algorithm and report typical families with proven mutations.

P-ClinG-109

Coincidence of two constitutional de novo mutations, KRAS mutation and trisomy 8 mosaicism, in a patient with Noonan syndrome and JMML

Zoll B.[1], Pauli S.[1], Kühnle I.[2], Schubert C.[3], Bartels I.[1]
[1]Institut für Humangenetik, Universitätsmedizin Göttingen, Germany, [2]Pädiatrie II, Universitätsmedizin Göttingen, Germany, [3]Zentrum Kinder- und Jugendmedizin, Medizinische Hochschule Hannover, Germany

Noonan Syndrome is a highly variable dominant disorder affecting 1 in 1000 to 1 in 2500 newborns that has significant phenotypic overlap with Costello syndrome and cardio-facio-cutaneous syndrome. Mutations in genes of the RAS/mitogen-activated protein kinase signaling pathway (PTPN11, SOS1, KRAS; RAF1) are responsible for Noonan syndrome.
We report on a girl who developed juvenile myelomonocytic leukemia (JMML) at the age of 5 months. She was born at 35 weeks of gestation (weight 2400g, length 45 cm, head circumference 33 cm). Postpartal cardiomyopathy and multiple facial dysmorphism led to the suspected diagnosis Costello or Noonan syndrome.
The girl`s development (all milestones) is delayed. At the age of 15 months she presents with cardiomyopathy, short stature, macrocephalus and facial dysmorphism. She sits without attendance but does not stand or walk nor speaks any word. She behaves attentive and friendly.
A heterozygous KRAS 173C->T transition predicting a 58T->I amino acid substitution was detected in bone marrow cells and in buccal cells as well. Chromosome analysis revealed mosaicism for trisomy 8 in 50% of bone marrow cells. The trisomic cell line was also present in peripheral blood (30%) and cultivated fibroblasts (10%). No trisomic cells were detected in buccal mucosa smear. In the patient, both mutations occurred de novo.
A mutation in KRAS is detected in 2% of the patients. Constitutional trisomy 8 is a very rare chromosomal constitution in lifeborn, and only observed as mosaicism. There is a wide range of phenotypes associated with mosaic trisomy 8. We suggest that the developmental capacities of the child are mainly determined by the Noonan mutation rather than trisomy 8. Both mutations are predisposing to malignancies.

P-COMPLEX GENETICS, COMPLEX DISEASES

P-Compl-110

Mapping of functional DNA variants for human hippocampal gene expression (eQTL analyses)

Alexander M.[1], Hillmer A.[1], Kluck N.[1], Pernhorst K.[2], Eibl E.[2], Schoch S.[2], Müller-Myhsok B.[3], Nöthen M. M.[1], Becker A.[2], Cichon S.[1]
[11] Department of Genomics - Life & Brain Center - University of Bonn; [2] Institute of Human Genetics - University of Bonn, Bonn, Germany, [23] Department of Neuropathology, University of Bonn Medical Center, Germany, [34] Max Planck Institute of Psychiatry, Munich, Germany

Recent studies have stressed the extent to which gene expression varies within and between populations, and have shown that allele-specific expression is relatively common among non-imprinted autosomal genes. Much of the genetic component of human phenotypic diversity has been proposed to be the result of cis-acting influences on gene expression. In the present study, we aim to identify genetic factors that influence gene expression in the human hippocampus. For systematic mapping of determinants for hippocampal gene expression, we isolated genomic DNA and RNA of hippocampus samples taken from the Bonn tissue bank and performed a genome-wide association study (GWAS) and gene expression (GEX) analysis. Each individual DNA sample is genotyped with >600,000 SNPs. Individual gene expression levels for more than 99,9% of all known human genes (approx. 25,000 annotated RefSeq and UniGene genes) are interrogated with microarrays containing more than 48,000 probes. Gene expression levels are then systematically correlated with individual genotype information to identify expressed quantitative trait loci (eQTLs). Data analysis is currently underway and results will be presented. In addition we are establishing lymphocyte cell assays to characterize regulatory elements via Chromatin immune-precipitation (ChIP). One important application of our findings will be the interpretation of SNP association findings for brain phenotypes, in particular neuropsychiatric disorders.

P-Compl-111

Genome wide copy number determination with oligonucleotide arrays in psoriatic arthritis patients

Apel M.[1], Uebe S.[1], Ekici A.B.[1], Lascorz J.[1], Behrens F.[2], Lohmann J.[3], Wichmann H.E.[4], Burkhardt H.[2], Reis A.[1], Hüffmeier U.[1]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Division of Rheumatology, Frankfurt/ Main, Germany, [3]Psoriasis Rehabilitation Hospital, Bad Bentheim, Germany, [4]Institute of Epidemiology, Helmholtz Center Munich, Germany

Whole genome analysis using high-density oligonucleotide arrays allows identification of copy number variants (CNVs) in monogenic and complex diseases. Here we analysed arrays containing 1.8 million probes (Affymetrix 6.0) in 609 psoriasis arthritic (PsA) patients for CNVs. We assessed quality with MAPD, measuring pairwise differences between single intensities of neighbouring markers within one array; this criterion was fulfilled in 599 of these arrays (MAPD <0.35, 98.4%). In order to validate detection limits, we studied a 32 kb deletion of two LCE genes (LCE3C, LCE3B) on chromosome 1q, which is detected by 35 probes. The deletion is known to be associated with psoriasis vulgaris, but not with PsA. We independently genotyped the same cohort using a PCR-based fragment analysis. Genotypes of oligonucleotide arrays were determined using two different algorithms: Affymetrix algorithm (Canary, Waviness correction) and Birdsuite algorithm (Birdseye, Fawkes). Concordance of the PCR-based fragment analysis method with the Birdsuite-analysis was 98.9%, but only 88.6% for the Affymetrix algorithm, indicating that CNV analysis is highly dependent on the algorithm used and that the Birdsuite algorithm is more reliable. We are currently using this algorithm in a case-control association study for PsA versus a population based KORA cohort in order to identify possible CNVs contributing to PsA susceptibility.

P-Compl-112

VANGL1 gene mutations identified in 100 patients with neural tube defects from Slovakia, Romania, and Germany

Bartsch O.[1], Thiede A.[1], Lechno S.[1], Kutschke G.[2], Florian S.[3], Stan H.[3], Percsi P.[1], Zechner U.[1], Haaf T.[4], Sabova L.[5], Horn F.[6]
[1]Institute of Human Genetics, University of Mainz, Germany, [2]Department of Pediatrics, University of Mainz, Germany, [3]Section of Neurosurgery, University of Cluj-Napoca, Romania, [4]Institute of Human Genetics, University of Würzburg, Germany, [52]nd Department of Pediatrics, University of Bratislava, Slovakia, [6]Department of Pediatric Surgery, University of Bratislava, Slovakia

Neural tube defects (NTDs) are the second most common congenital malformations after cardiac defects, with a prevalence of 1-2 per 1.000 newborns and an approximately equal frequency of spina bifida and anencephalus. Their etiology includes environmental and genetic factors. Following the identification of mutations causing NTDs in mice, Kibar et al. (N Engl J Med 2007;356:1432-1437) reported three muta-

tions in the planar cell polarity gene VANGL1 associated with sporadic and familial cases of human NTDs. Recently, Kibar et al. (Hum Mutat 2009;7:E706-715) described five further missense mutations affecting evolutionary conserved residues and absent from all controls analysed. To advance the understanding of the role of VANGL1 in patients with NTDs, we performed direct sequencing of all coding exons of this gene in a cohort of 100 patients. The cohort included 41 patients from the Spina Bifida Center in Mainz, Germany, 50 patients from the Spina Bifida Center in Bratislava, Slovakia, and 9 patients from different hospitals in Cluj-Napoca, Romania. Patients were born from 1972-2009 (85%: 1991-2009) and were recruited from 2004-2009. The folic acid status is only known for 21 mothers of the Slovakian patients, and for the mothers of the Romanian patients, who all lacked periconceptional folic acid supplementation. The coding exons of VANGL1 (GenBank NM_138959.2) were amplified from genomic DNA using PCR and primers flanking the exon-intron junctions. The sequencing was performed on a Beckman CEQ8000 Genetic Analysis System. We identified two missense variants in VANGL1 that were absent from controls, both novel, c.518G>A (predicting p.R173H) and c.613G>A (predicting p.G205R). The p.Arg173His variant was identified in a 1987 born male from Slovakia. Arginine to histidine is a conservative substitution, as both amino acids are hydrophilic and basic, but arginine is much more strongly charged (pKa 12.5, vs. pKa 7.6 in histidine), and Arg173 is absolutely conserved in all Vangl/Stbm sequences analysed, including pufferfish (tetraodon), fruit fly (drosophila), and nematodes (C.elegans). Kibar et al. (2009) reported two very similar mutations, p.Arg175Gln and p.Arg181Gln, in NTD patients and absent in controls. The p.Gly205Arg variant was found in a 1991 born Slovakian male with sporadic open lumbosacral myelomeningocele. This variant changes a highly conserved glycin residue in the protein into an arginine, which is a non-conservative substitution dramatically affecting a residue that is conserved and unpolar in all species studied, including pufferfish, drosophila, and C. elegans. Therefore, both sequence variants most likely represent pathogenic mutations. Our findings confirm VANGL1 gene mutations as a risk factor in approximately 2% of human neural tube defects.

P-Compl-113

The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels

Berulava T., Horsthemke B.
Institut für Humangenetik, Universitätsklinikum Essen, Germany

Objectives: As shown by genome-wide association studies single nucleotide polymorphisms (SNPs) within intron 1 of the FTO gene are associated with the body mass index (BMI) and type 2 diabetes, although the functional significance of these SNPs has remained unclear.
Research design and methods: Using primer extension assays, we have determined the ratio of allelic FTO transcript levels in unspliced heterogeneous nuclear RNA (hnRNA) preparations from individuals heterozygous for SNP rs9939609. Allelic expression ratios of the neighbourig RPGRIP1L gene were investigated in individuals who were heterozygous for SNP rs4784319 and heterozygous or homozygous for rs9939609.
Results: In each of five individuals, the A (risk) allele of the FTO gene was more abundant than the T allele (mean 1.21; 95% confidence interval 1.15 to 1.27). We also observed skewed allelic expression of the RPGRIP1L gene but skewness was independent from the FTO genotype.
Conclusions: Our data suggest that increased expression of FTO is associated with increased body mass. The obesity-associated SNPs in the FTO gene did not have any influence on the expression of the neighbouring RPGRIP1L gene.

P-Compl-114

Fine mapping of the human AR/EDA2R locus in androgenetic alopecia

Brockschmidt F. F.[1,6], Hillmer A. M.[2], Heilmann S.[1,6], Eigelshoven S.[3], Hanneken S.[3], Barth S.[1,6], Herold C.[4], Becker T.[4], Kruse R.[5], Nöthen M. M.[1,6]
[1]Department of Genomics - Life & Brain Center - University of Bonn, Bonn, Germany, [2]Genome Technology & Biology Group - Genome Institute of Singapore, Singapore , Singapore, [3]Department of Dermatology - University of Düsseldorf, Düsseldorf, Germany, [4]Institute of Medical Biometry, Informatics and Epidemiology - University of Bonn, Bonn, Germany, [5]Gemeinschaftspraxis für Dermatologie, Paderborn, Germany, [6]Institute of Human Genetics, University of Bonn, Germany

Several studies have identified the androgen receptor (AR)/ectodysplasin A2 receptor (EDA2R) locus as the major genetic determinant of male-pattern baldness (androgenetic alopecia, AGA), but the reported association signals are inconsistent and the causative variant or gene has not yet been unequivocally identified. To resolve the inconsistent association data for AGA we performed a detailed fine mapping analysis of the AR/EDA2R locus in the largest androgenetic alopecia patient sample investigated to date. The case-control sample comprised 673 AGA cases and 248 unaffected controls. It was used to test 25 tagging single nucleotide polymorphisms (SNPs) and one short tandem repeat. Several previously reported association signals were among the selected genetic markers. Family members (N=855) were used to perform a family-based association test. Data were stratified to look for independent effects of investigated markers. Neither of the two previously proposed coding variants in AR (GGN-repeat) and EDA2R (R57K) is likely to be the true causative variant. We found the strongest association for rs12558842 (located between AR and EDA2R) ($p = 2.03 \times 10^{-25}$). Analysis of the family-based data suggested an additional, independent effect for rs5918801 (located >250kb telomeric of AR). Inspection of haplotypes suggested that a single haplotype defined by rs12558842 and rs5918801 is responsible for the association. These findings suggest that the true (as yet unknown) causative variant is likely to reside on a haplotype defined by rs12558842 and rs5918801. The causative variant probably confers its functional effect through the modulation of gene expression. Both flanking genes, AR and EDA2R, are plausible candidates for such an effect.

P-Compl-115

Recurrent microduplications at 22q11.21 are associated with classic bladder exstrophy

Draaken M.[1], Reutter H.[2], Schramm C.[3], Bartels E.[3], Ebert AK.[4], Rösch W.[4], Boemers T.[5], Schröder A.[6], Stein R.[6], Boyadjiev SA.[7], Nöthen MM.[1], Ludwig M.[8]
[1]Institute of Human Genetics and Department of Genomics - Life & Brain Center, Bonn, Germany, [2]Institute of Human Genetics and Department of Neonatology, Bonn, Germany, [3]Institute of Human Genetics, Bonn, Germany, [4]Department of Pediatric Urology, Regensburg, Germany, [5]Department of Pediatric Surgery and Pediatric Urology, Cologne, Germany, [6]Department of Urology, Mainz, Germany, [7]Department of Pediatrics - Section of Genetics, Sacramento, USA, [8]Department of Clinical Chemistry and Pharmacology, Bonn, Germany

Classic exstrophy of the bladder (CBE) is part of the exstrophy-epispadias complex (EEC), a spectrum of urogenital anomalies in which part or all of the distal urinary tract fails to close. Familial occurrence has been observed, and previous studies have suggested an underlying multifactorial mode of inheritance. To date, no causative genetic or non-genetic factor has been unequivocally identified in humans.
The present study aimed to identify microaberrations characterized by loss or gain of genomic material that contribute to the EEC at a genome-wide level. Molecular karyotyping, utilizing 549,839 SNPs, was performed to screen an initial cohort of 16 EEC patients. A de novo microduplication involving chromosomal region 22q11.21 was

identified in one CBE patient. Subsequent multiplex ligation-dependent probe amplification (MLPA) analysis was performed in a further 200 EEC cases. This approach identified similar 22q11.21 duplications in three further CBE patients. The size of the duplicated region in all four cases was about 3 Mb. Paternity was confirmed in all families. In one patient the duplication had been transmitted from the unaffected mother.
Chromosomal region 22q11 is well known for its susceptibility to genomic rearrangements being associated with various syndromes including the velo-cardio-facial/DiGeorge syndrome (VCFS/DGS), der(22) syndrome and cat-eye syndrome. Duplications in this region result in a wide and variable spectrum of clinical presentations including features of the VCFS/DGS, while some carriers present with a completely normal phenotype.
Our findings extent the phenotypic spectrum of the 22q11.2 duplication syndrome, and indicate that this aberration predisposes to CBE with incomplete penetrance.

P-Compl-116

Imputation and analysis of a genome-wide association study of age-related macular degeneration (AMD)

Fritsche L.G.[1], Keilhauer C.N.[2], Olden M.[3], Heid I.[3], Gieger C.[4], Wichmann H.-E.[4], Lichtner P.[5], Meitinger T.[5], Weber B.H.F.[1]
[1]Institute of Human Genetics, Regensburg, Germany, [2]Department of Ophthalmology, Wuerzburg, Germany, [3]Institute of Epidemiology, Regensburg, Germany, [4]Institute of Epidemiology, Neuherberg, Germany, [5]Institute of Human Genetics, Neuherberg, Germany

Introduction: Age-related macular degeneration (AMD) is a complex disorder of the retina/choroid and the most common cause of legal blindness in the industrialized countries. Two major susceptibility loci have been identified including CFH on 1q31 and ARMS2/HTRA1 on 10q26. Furthermore, two additional complement genes (C3 and C2/CFB) have also been shown to play a role in AMD pathogenesis. Other susceptibility genes with moderate to minor contributions to AMD risk are likely but difficult to detect in genome-wide association studies (GWAS). In particular, polymorphic marker density and statistical power are two major hurdles in these efforts. Imputation methods have recently been introduced which facilitate (1) a reliable increase of marker density without additional genotyping and (2) a meta-analysis of GWAS even in cases where different SNP panels were used for genotyping. The aim of our study was to perform an imputation of an available GWAS data set and to initially analyse the resulting genotypes.
Methods: The GWAS consisted of 335 late-stage AMD patients which were genotyped with the Affymetrix 250K StyI array and of 1636 population-based controls (KORA study, Augsburg) genotyped with the Affymetrix 500k array set (250k Sty I and 250k Nsp I). Imputation was performed using the "BEAGLE Genetic Analysis Software Package" which employes the phased haplotype data from 90 unrelated, Caucasian individuals (HapMap CEU, Release 22) as reference sample to impute the genotypes of up to 2.5 Mio SNPs. The association tests were performed using "SNPTEST" which incorporates the genotype probabilities and takes account of the imputation uncertainty.
Results: Before imputation we tested 138,353 common SNPs meeting our quality criteria (minor allele frequency [MAF] >= 10 %) for their association with AMD. This revealed four SNPs localized within the two known susceptibility loci at 1q31 and at 10q26 which obtained genome-wide significance. After imputation with a total runtime of approximately 26 days, a 6.7 fold increase in the number of common SNPs (MAF >= 10 %; N = 928,086) was obtained. As before imputation, statistically significant association signals were only found at loci 1q31 and 10q26. Notably, their association signals markedly increased in number and strength. An increase of the overall background noise was not noticed indicating the reliability of the imputation.
Conclusions: As imputation methods are based on phased haplotypes, the testing of imputed SNPs represents a method to indirectly assess haplotypic associations which otherwise would be computationally demanding and error-prone on a genome-wide level. Nevertheless, no additional frequent susceptibility locus for AMD was identified indicating that not marker density alone, but statistical power is indispensable for their detection. Further progress in imputation methods, e.g. the completion of the 1000 genomes project, will enhance the reliable estimation of even lower frequent SNPs and will have the potential to search for moderate to minor risk factors in complex diseases.

P-Compl-117

Pathway analysis of genome-wide association data for major depressive disorder

Haenisch B.[17], Zapatka M.[2], Mattheisen M.[1,7,8], Herms S.[1,7], Degenhardt F.[1,7], Frank J.[3], Treutlein J.[3], Breuer R.[3], Lucae S.[4], Zimmer A.[5], Bettecken T.[4], Müller-Myhsok B.[4], Maier W.[6], Brors B.[2], Eils R.[2], Nöthen MM.[1,7], Rietschel M.[3], Cichon S.[1,7]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Division Theoretical Bioinformatics B[080] at German Cancer Research Center, Heidelberg, Germany, [3]Department of Genetic Epidemiology in Psychiatry at Central Institute of Mental Health, Mannheim, Germany, [4]Max Planck Institute of Psychiatry, Munich, Germany, [5]Department of Molecular Psychiatry, Bonn, Germany, [6]Department of Psychiatry, University of Bonn, Germany, [7]Department of Genomics at the Life and Brain Center, University of Bonn, Germany, [8]Institute of Medical Biometry, Informatics, and Epidemiology, University of Bonn, Germany

Genome-wide association studies using Single Nucleotide Polymorphisms (SNPs) have proven to be a successful tool to identify common susceptibility variants for complex disorders such as neuropsychiatric disorders. However, due to the high degree of multiple testing, risk factors with small genetic effects are difficult to detect. One way to reduce the complexity of the genetic tests is to include biological pathway information. The analysis of SNPs in groups predefined by biological knowledge as the unit of analysis can increase the power to detect association between genes and disease.
We implemented a method analyzing data from genome-wide association screens in the context of biological pathways. The global test was originally developed for gene expression data and can be applied to categorical variables. After mapping the tested SNPs to genes we used the global test to evaluate the influence of the allele status of cases and controls based on pathway groups of genes. A data set on major depressive disorder comparing the genome wide SNP status of 597 affected and 1295 control samples is currently being analyzed using pathway definitions from several databases. Results of this analysis will be presented.

P-Compl-118

Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicentre study

Hammer C.[1], Haenisch H.[2,4], Mühleisen T.W.[2,4], Degenhardt F.[2,4], Breuer R.[3], Witt S.[3], Oruc L.[6], Rivas F.[7], Babadjanova G.[8], Grigoroiu-Serbanescu M.[9], Hauser J.[10], Rietschel M.[3], Nöthen M.M.[2,4], Cichon S.[2,4], Rappold G.[5], Niesler B.[5]
[1]Institute of Human Genetics, Heidelberg,Germany, [2]Life and Brain Center, University of Bonn, Germany, [3]Central Institute of Mental Health, Mannheim, Germany, [4]Institute of Human Genetics, University of Bonn, Germany, [5]Institute of Human Genetics, Heidelberg, Germany, [6]Psychiatric Clinic at the Clinical Center, Sarajevo, Bosnia and Herzegovina, [7]Civil Hospital Carlos Haya, Malaga, Spain, [8]Russian State Medical University, Moscow, Russia, [9]Biometric Psychiatric Genetics Research Unit at the Alexandru Obregia Clinical Psychiatric Hospital, Bucharest, Romania, [10]Department of Psychiatry, Poznan, Poland

Serotonin 3 receptors (5-HT3Rs) are involved in cognition and mood and have been implicated in psychiatric disorders such as anxiety and depression. 5-HT3 receptor antagonists are beneficial in the treat-

ment of these conditions. In a small pilot study, the SNP c.-42 C>T (rs1062613) within HTR3A as well as the deletion c.-104-102delAGA (rs3831455) and the SNP c.386A>C (rs1176744, p.Y129S) in HTR3B were found to be associated with BPAD (1, 2). All variants have been shown to be functionally relevant and may influence the susceptibility of the respective condition (3-6).
To confirm previous association findings and to provide further evidence for the assumed relevance of functional HTR3 variants in the aetiology of psychiatric disorders, we performed a European multicenter study including individuals from Germany (378 patients/768 controls), Poland (446 patients/558 controls), Romania (237 patients/235 controls), Spain (297 patients/401 controls), Russia (331 patients/331 controls) and Bosnia-Herzegowina (124 patients/115 controls). All patients received a lifetime diagnosis of BD type I or type II based on DSM-IV criteria. Genotyping was performed using the MALDI-TOF-based MassARRAY system and iPLEX Gold assays. Association analysis was carried out using the SNPassoc package of R and PLINK.
In this study, the HTR3B variants c.-104_-102delAGA and c.386A>C (p.Y129S) were confirmed to be associated with BPAD overall (P=0.04, OR=1.7 CI [1.02-2.83] and P=0.0038, OR=0.83 CI [0.74-0.94]). Yet, the HTR3A SNP c.-42C>T was not found to be associated overall. This is the first replication study investigating HTR3 SNPs in BPAD. We conclude from this data that the HTR3 system is indeed relevant in the aetiology of bipolar disorder. Future studies focusing on disease and pharmacogenetic approaches will help to unravel the particular role of 5-HT3 receptors in neuropsychiatric disorders.

References

1. B. Frank et al., Am J Med Genet B Neuropsychiatr Genet 131B, 1 (Nov 15, 2004).
2. B. Niesler et al., Pharmacogenetics 11, 471 (Aug, 2001).
3. K. Krzywkowski, P. A. Davies, P. L. Feinberg-Zadek, H. Brauner-Osborne, A. A. Jensen, Proc Natl Acad Sci U S A 105, 722 (Jan 15, 2008).
4. C. Meineke et al., Pharmacogenet Genomics 18, 219 (Mar, 2008).
5. J. Walstab, Hammer, C, Bönisch, H, Rappold, G, Niesler, B, Pharmacogenetics and Genomics (2008).
6. T. Iidaka et al., J Neurosci 25, 6460 (Jul 6, 2005).

P-Compl-119

Allele and haplotype specific expression analysis of the X-chromosomal androgen receptor (AR) and ectodysplasin A2 receptor (EDA2R) locus, the major genetic determinant for the development of androgenetic alopecia

Heilmann S.[1,4], Brockschmidt F.F.[1,4], Hillmer A.M.[2], Wehner G.[3], Tessmann P.[1,4], Lippke B.[1,4], Schweikert H.U.[3], Nöthen M.M.[1,4]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Genome Institute of Singapore, Singapore, Singapore, [3]Med. Klinik III für Hämatologie und Onkologie des Universitätsklinikums Bonn, Bonn, Germany, [4]Department of Genomics, Life & Brain Center, University of Bonn, Germany

Androgenetic alopecia (AGA) or male pattern baldness is the most common form of hair loss in humans and predominantly affects men. The pathogenesis of AGA is driven by androgens based on a genetic predisposition as the major precondition. The androgen receptor (AR)/ectodysplasin A2 receptor (EDA2R)-locus has been repeatedly reported as the main genetic determinant for AGA. Our group recently conducted a fine mapping analysis of the AR/EDA2R-locus that revealed two predominant risk variants for AGA. rs12558842 is located upstream of the two neighboring genes AR and EDA2R and rs5918801 which is located telomeric of AR. Haplotype analysis showed that the overtransmission of the AGA risk allele "A" of rs12558842 is carried out more frequently in combination with the "C"-allele of rs5918801. This "AC"-haplotype is strongly associated with AGA and suggests that the causative variant for AGA resides on a haplotype characterized by these two SNPs. We thus conducted an allele and haplotype specific expression analysis for AR and EDA2R dependent on rs12558842 and rs5918801. The analysis was carried out in 80 hair shaft samples and 46 foreskin fibroblasts. DNAs were genotyped for rs12558842 and rs5918801 and RNA and protein expression levels were determined using real-time PCR and ELISA respectively. Our data indicate an allele specific effect for rs12558842 on the RNA and protein expression of AR and the RNA expression of EDA2R, with higher expression levels in risk allele ("A") carriers. rs5918801 seemed to have only modulatory effects, if at all on AR or EDA2R expression. The haplotype specific expression analysis supported these findings. The present study suggests that the association of AGA with the AR/EDA2R-locus is conferred through a regulatory effect on AR as well as on EDA2R expression.

P-Compl-120

Multisomatoform disorder (MSD) is not associated with single polymorphisms of the serotonin system in a German case-control sample

Holm L.[1], Bernateck M.[2], Jakobi J.[1], Tran A.-T.[2], Volkmann L.[2], Buers D.[2], Lehmann U.[3], Karst M.[2], Stuhrmann M.[1]
[1]Institute of Human Genetics, Hannover, Germany, [2]Department of Anesthesiology, Hannover, Germany, [3]Institute of Pathology, Hannover, Germany

Serotonin is an important molecule in pain processing. Therefore, the receptors interacting with serotonin as well as the enzyme tryptophanhydroxylase, that catalyses the speed-limiting step of the serotonin synthesis, are strong candidates for exerting influence on developing a multisomatoform disorder (MSD). MSD is defined as 3 or more medically unexplained, currently bothersome physical symptomes plus a history of somatization of at least 2 years.
To investigate a possible association between MSD and variations in the serotonin system, we genotyped 14 single nucleotide polymorphisms (SNPs) within 5 serotonin receptor genes and 2 serotonin enzyme genes (rs6295, rs1364043 (HT1A), rs1213371, rs6296 (HT1B), rs6300, rs604030 (HT1D), rs6318 (HT2C), rs1062613, rs948983 (HT3A), rs7130929, rs1800532 (TPH1), rs4570625, rs2171363, rs4565946 (TPH2)) in a German case-control sample consisting of 149 healthy individuals (mean age 52.1 + 9,9 years, 73% women, 27% men) and 149 MSD patients (mean age 54.4 + 10,1 years, 82% women, 18% men). Lifetime best estimate diagnoses are based on a structured interview according to the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM IV). With the exception of rs6296, none of the SNPs deviated from Hardy-Weinberg equilibrium. Neither the marker analyses nor the haplotype analyses revealed a significant association with the disorder. But notably, considering the influence of the two genes HT1B und HT1D in interaction, the haplotype TTT (rs1213371, rs604030, rs6300) showed a lower risk compared to the reference haplotype CTT (OR 0.65 with CI [0.42; 1.00]) with a p-value near to the significance level (p = 0.051).
In conclusion, the results do not support an involvement of single polymorphisms of the serotonin system in the etiology of MSD. However, further studies considering interactions of the serotonin system with the dopaminergic system as well as possible interactions of multiple components of the serotonergic system are warranted. Together with a thorough investigation of sensorical, mental and serological parameters of all patients and controls that allows to evaluate possible influences of confounding variations like those of HT1B und HT1D on different types of pain sensitivity.

P-Compl-121

Genome-wide association study with DNA pooling identifies variants at CNTNAP2 associated with pseudoexfoliation syndrome and pseudoexfoliation glaucoma

Krumbiegel M.[1], Pasutto F.[1], Schlötzer-Schrehardt U.[2], Uebe S.[1], Zenkel M.[2], Mardin C.Y.[2], Weisschuh N.[3], Paoli D.[4], Gramer E.[5], Becker C.[6], Ekici A.B.[1], Weber B.H.F.[7], Nürnberg P.[6], Kruse F.E.[2], Reis A.[1]

[1]Institute of Human Genetics, Erlangen, Germany, [2]Department of Ophthalmology, Erlangen, Germany, [3]Molecular Genetics Laboratory, Tuebingen, Germany, [4]Department of Ophthalmology, Monfalcone, Italy, [5]University Eye Hospital, Wuerzburg, Germany, [6]Cologne Center for Genomics, Cologne, Germany, [7]Institute of Human Genetics, Regensburg, Germany

PURPOSE: Genetic and non-genetic factors contribute to the development of pseudoexfoliation (PEX) syndrome, a complex, age-related, generalized matrix process which is mainly manifest in eyes and frequently associated with glaucoma. In order to identify specific genetic variants underlying its aetiology, we performed a genome-wide association study (GWAS) using DNA pooling approach.
METHODS: Equimolar amounts of DNA samples of 80 subjects with PEX syndrome, 80 with PEX glaucoma and 80 controls, respectively, were combined into three separate pools and hybridized to 500K SNP arrays (Affymetrix). Array probe intensity data was analyzed and visualized with specially developed software tools GPFrontend and GPGraphics in combination with the GenePool program. For replication, independent German cohorts of 610 unrelated patients with PEX/PEXG and 364 controls as well as Italian cohorts of 249 patients and 190 controls were used.
RESULTS: Genotyping of selected SNPs in the CNTNAP2 locus revealed significant association between PEX/PEXG and two SNPs as well as their haplotype. The association found was confirmed in an independent German cohort but not in an Italian cohort. In the combined German cohorts the two SNPs remained significant after correction with permutation test (rs2107856: Pc=0.0044, rs2141388: Pc=0.0029). Ubiquitously expression pattern in human ocular tissues and a clearly localization to cell membranes of epithelial, endothelial, smooth muscle, glial and neuronal cells revealed CNTNAP2 protein as an interesting candidate for PEX disease.
CONCLUSION: Confirming efficiency of GWAS with DNA pooling approach, our data show compelling evidence for the association of CNTNAP2 with PEX syndrome and PEXG in German patients.

P-Compl-122

Alopecia areata - Genetic fine mapping in Dundee Experimental Bald Rats (DEBR) and in Humans

Kuhn JK.[1], McElwee K.[2], Blech H.[3], Fürst R.[4], Hoffmann R.[5], Nuernberg P.[1], Hennies HC.[1]

[1]Cologne Center for Genomics, Cologne, Germany, [2]University of British Columbia, Vancouver, Canada, [3]Praxis für Dermatologie, Rödental, Germany, [4]Institut of Genetics, University of Cologne, Germany, [5]Praxis für Dermatologie, Freiburg, Germany

Alopecia areata (AA) is a chronic inflammatory disorder of the hair follicles in the actively growing anagen stage. It is characterized by circular regions of hair loss on the head. Isolated hair loss on other parts of the body can occur as well.
Alopecia areata has been suggested as a tissue-specific autoimmune disease, considering a potential role of loss of immune privilege. Frequent familial occurrence of the disease demonstrates a strong genetic susceptibility with a multifactorial background in humans.
We are using the Dundee Experimental Bald Rat (DEBR) as a rodent model of AA. An intercross of DEBR with PVG rats gave an F2 population with which we performed a whole genome-scan for linkage with microsatellite markers. Amongst others this analysis resulted in one highly significant locus on chromosome 19 with a lod-score of 20. Further fine mapping of the locus on chromosome 19 identified a candidate interval in the region between 35cM to 40cM. All exons within this region were sequenced. This did not give clues to the causes of hair loss but showed polymorphisms in the DEBR and PVG rat genomes. These were used for saturation mapping and further minimized the region of interest, which is now being sequenced with Next Generation Sequencing (NGS).
In addition to the sequencing an expression analysis was done using the GeneChip® Rat Gene 1.0 ST Array from Affymetrix. This gave interesting results for 7 keratin genes, Trat1, Mmp12 and Tchh and a few others. These results were then confirmed in rat skin and fibroblasts with the LightCycler 480 System from Roche, and further tests to show organ specificity are under way.
Furthermore, a total of 330 human families were used for finemapping and tested for linkage and association using SNPstream, Taqman and Pyrosequencing techniques in a number of candidate regions including the HLA region on chromosome 6p. The statistical evaluation of a genome wide SNP Genotyping analysis is ongoing.

P-Compl-123

Systematic, Hypothesis-free Analysis of Epistatic Interaction (GWIA) in Schizophrenia

Mattheisen M.[1,8,9], Degenhardt F.[1,9], Steffens M.[8], Mühleisen TW.[1,9], Breuer R.[3], Basmanav B.[1,9], Forstner A.[1,9], Herold C.[8], Herms S.[1,9], Strengman E.[4], GROUP I.[5], Becker T.[8], Cantor R.[6], Rujescu D.[7], Baur MP.[8,10], Wienker TF.[8], Rietschel M.[3], Nöthen MN.[1,9], Ophoff R.[4,6], Cichon S.[1,9]

[1]Institute of Human Genetics, University of Bonn, Germany, [2]IMBIE, Bonn, Germany, [3]Central Institute of Mental Health, Mannheim, Germany, [4]The Netherlands Department of Medical Genetics and Rudolf Magnus Institute of Neuroscience, Utrecht, The Netherlands, [5]GROUP Investigators: Maastricht UMC, AMC Uni of Amsterdam, UMC Groningen, UMC Utrecht, The Netherlands, [6]UCLA Center for Neurobehavioral Genetics, Los Angeles, USA, [7]Department of Psychiatry, Munich, Germany, [8]Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Germany, [9]Department of Genomics, Life & Brain Center, University of Bonn, Germany, [10]German Center for Neurodegenerative Diseases, Bonn, Germany,

Schizophrenia is a common neuropsychiatric disorder with a life-time prevalence of approximately 1% in all populations world-wide. Genome-wide association studies using SNP data have started to identify common susceptibility variants as well as rare structural variants that are involved in disease development. Still, a substantial part of the assumed heritability awaits identification, and it is assumed that epistatic interactions may account for a proportion of this "missing heritability". Here we present our results and experience in a full genome-wide interaction analysis (GWIA) from a case-control study of schizophrenia. Our sample comprised of 4,747 individuals (1248 SCZ patients and 3499 population based controls) from Germany and the Netherlands, analyzed for 476,781 SNPs. This is, to our knowledge, the first systematic, hypothesis-free analysis of epistatic interaction (GWIA) in schizophrenia so far. Notably, by far the most SNPs included in our top results of the GWIA did not show strong marginal effects and thus would have been lost if SNPs had been pre-selected on the basis of detected single-marker effects in the run-up of our analysis. Guided by these observations, one should be encouraged to make use of all available SNPs in the GWIA (without pre-selection of SNPs).
Our lowest p Value of 2.73E-12 was found for two SNPs situated near to the protein coding region FAM72B and the gene sucrase-isomaltase (SI) on chromosome 11 and 3, respectively. Another two combinations for these two loci showed up among the top 5 ranked interaction partners. Interestingly two of our top 25 ranked interaction partners included SNPs that are in or near to known schizophrenia candidate genes: ERBB4 (rank 9) and MAD1L1 (rank 18). For both, the respective interaction partners (SNPs) are not situated in or near to known genes (based on RefSeq). Independent replication of our results are warranted and currently under progress.

P-Compl-124

Comparative mapping of linkage and association signals in myocardial infarction

Medack A.[1], Loley C.[1], Nahrstaedt J.[1], König I.R.[1], Mayer B.[1], Hengstenberg C.[2], Stark K.[2], Wichmann H.E.[3], Schreiber S.[4], Meitinger T.[3], Schunkert H.[1], Erdmann J.[1]

[1]University of Luebeck, Luebeck, Germany, [2]University Regensburg, Regensburg, Germany, [3]Helmholtz Zentrum, Neuherberg, Germany, [4]University of Kiel, Kiel, Germany

Background: Our group established a large collection of myocardial infarction (MI)-families with 2-5 affected first-degree relatives and large collection of unrelated MI cases and controls. Genome-wide microsatellite linkage analyses on MI sibling-pairs and three genome-wide association (GWA) studies on unrelated MI cases (n=875 [GerMIFSI]/ n=1222 [GerMIFSII]/ n=1157 [GerMIFSIII]) and controls (n=4690) were carried out. Our current approach assumes that the same genes involved in rare autosomal dominant forms of MI may also be associated with the risk for MI in the general population.
Methods: 25 families with an autosomal dominant inheritance pattern were analysed by cosegregation analyses using microsatellite markers. Genome-wide scanning was performed using high-density SNP arrays (Affymetrix 500K, 6.0 and 5.0 arrays). Data analysis was performed in a three-step procedure. First, the chromosomal regions identified by the linkage scan were reduced to so called "interesting regions", based on the fact that these regions display an association with MI in two of the three GWAS in addition. The second step comprises the condensation of these "interesting regions" to "top regions" with positive SNPs replicating in the third GWA. These "top regions" include only few genes or intergenic regions. Sequencing of these regions in the families that provide the linkage signal is the third step.
Results: With the genome-wide microsatellite linkage analysis in 25 families (including n=559 individuals) five chromosomal regions 1q42.3; 4q31.21: 4q34.1; 8q24.13 and 17q24.2 were identified for suggestive linkage. In the same chromosomal regions we found association for several SNPs tested in three GWAS. With systematic data analyses we reduced the chromosomal region to top regions encompassing only few genes or intergenic regions. For example, the chromosome 4 locus (approx. 320 genes) was reduced to four top regions, including MND1 and GALNT17.
Conclusion: The comparative analysis of linkage and GWAS data in families and case/control samples with MI allows the condensation of linkage intervals leading to a much smaller number of genes to be sequenced in order to identify the underlying mutations in familial forms of the disease.

P-Compl-125

RET+3:T allele in medullary thyroid cancer

Podralska M.[1], Lipinski D.[1], Ziemnicka K.[2], Sowinski J.[2], Plawski A.[3]

[1]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland, [2]Department of Endocrinology Metabolism and Internal Diseases University of Medical Sciences, Poznan, Poland, [3]Institute of Human Genetics Polish Academy of Sciences, Pozna, Poland

Sporadic medullary thyroid cancer is a non-hereditary type of medullary cancer. Familial type is caused by mutation in the RET gene. The RET protooncogene is one of the receptor tyrosine kinases, cell-surface molecules that transduce signals for cell growth and differentiation. Medullary thyroid cancer arises from specialized cells named parafollicular cells. The present study concerns RET+3:T polymorphism localized in enhancer region. We investigated only sporadic cases of medullary thyroid cancer. Patients are delivered from Department of Endocrinology, Metabolism and Internal Diseases, University of Medical Sciences in Poznan. In our studies, we compared the frequency of the occurrence of the RET+3:T allele in our group of 48 non-familial MTC patients with the frequency of occurrence of the allele in the Polish population. The frequency of the occurrence of the heterozygote variant of the RET+3:T for the Polish population reached almost 12% (18/152) of heterozygotes but in the group of patients with MTC, we did not find even a single RET+3:T allele. The frequency difference is statistically significant and in the Fisher's Exact Test, the two-sided P value is 0.0080. This observation allows assuming that the occurrence of the RET+3:T in the heterozygotic state may lead to the inhibition of the disease phenotype in the cases of the medullary thyroid carcinoma.

P-Compl-126

The role of SNPs in TGF-b1 at codon 10 and 25 and the occurrence of severe periodontitis

Schulz S.[1], Altermann W.[2], Klapproth J.[1], Zimmermann U.[1], Gläser C.[3], Stein J.M.[4], Schaller H.G.[1], Reichert S.[1]

[1]University School of Dental Medicine Department of Operative Dentistry and Periodontology Martin-Luther University Halle-Wittenberg, Halle, Germany, [2]Interbranch HLA Laboratory / Department GHATT Martin-Luther University Halle-Wittenberg, Halle, Germany, [3]Institute of Human Genetics and Medical Biology Martin-Luther University Halle-Wittenberg, Halle, Germany, [4]Department of Operative Dentistry and Periodontology University Hospital Aachen, Aachen, Germany

TGF-b1 is a pleiotropic cytokine that exerts its effects on bone and connective tissue metabolism which are of great importance in periodontal diseases. The expression of TGF-b1 has been shown to be under genetic control. Two SNPs at codon 10 (L10P) and codon 25 (R25P) represent functionally important genetic variants. Therefore, the aim of this study was to evaluate links between genetic variants of TGF-b1 and chronic/aggressive periodontitis and its clinical features. Patients and methods: One hundred and forty nine periodontitis patients (chronic: n=68, mean age=48.9+9.6y, 64.2% females; aggressive: n=81, mean age=40+9.5y, 63% females) and 82 healthy controls (mean age=46.6+10.7y, 53.7% females) without periodontitis were included in the study. TGF-b1 polymorphisms and haplotypes were determined using PCR-SSP (CTS-Kit, Heidelberg, Germany). The clinical investigation included smoking status, plaque (API) and bleeding indexes (BOP), pocket depth (PD) and clinical attachment loss (CAL). Subgingival bacterial colonization was evaluated molecularbiologically using the micro-Ident®test (HAIN-Diagnostik, Nehren, Germany).
Results: Hardy-Weinberg criteria were fulfilled for both SNPs. Comparing TGF-b1 genotype and haplotype distribution no significant association with the occurrence of aggressive and chronic periodontitis could be proven in our study. However, there was a trend for a higher occurrence of the CC-genotype L10P among periodontitis-free controls compared to patients suffering from aggressive periodontitis (18.3% vs. 11.1%, n.s.). Furthermore, the genetic background of TGF-b1 was not significantly associated with the subgingival colonization of periodontopathogens. However, among patients with chronic periodontitis bacteria of the red complex (P. gingivalis+T. forsythia. denticola) occurred less frequently in carriers of the TG haplotype (TG: 77.5% vs. CG+CC: 91.5%, p=0.042, pkorr.=0.073). Conclusions: Although, associations of the genetic background of TGF-b1 and periodontitis and periodontopathogens could be shown in bivariate analyses in binary logistic regression analyses the SNPs L10P and R25P and corresponding haplotypes could not be proved as independent risk factors for chronic or aggressive periodontitis.

P-CYTOGENETICS

P-CytoG-127

Oocyte Segregation Pattern in Translocation Carriers

Bartsch T.[1], Klehr-Martinelli M.[1], Buchholz T.[1]
[1]Centre for Polarbody analysis, Munich, Germany

Introduction: In translocation carriers during meiosis I segregation forms so called quadrivalents (in reciprocal translocation) or trivalents (in Robertsonian translocation) of the translocated chromosomes besides the regular homologue chromosomal pairing. Theoretically there could arise 16 different or 6 different chromosomal constitutions in secondary oocytes whereas only two of them are balanced.
Aim: The aim of this evaluation is to determine the rate of balanced and unbalanced constellations as well as the proportions of the different segregation patterns in oocytes of translocation carriers.
Methods: We performed polar body analysis to identify unbalanced partial aneuploidies in oocytes by using FISH on first polar bodies. Here we present our result from 29 patients (27 reciprocal and 2 Robertsonian translocations).
Results: Out of 147 clearly evaluable polar bodies, 78 (53,1%) were balanced and 69 (46,9%) were unbalanced. The following segregation patterns were observed: 12 adjacent 1- (17,3%), 7 adjacent 2- (10,1%) and 24 3:1-distribution (34,8%). A 4:0 segregation was not revealed. For 26 oocytes (37,7%) a trisomy or monosomy of one or both of the translocated chromosomes was predicted (2 in Robertsonian translocations)
Conclusion: Our results show a high rate of balanced segregation. It is to note that oocytes with a balanced constellation for translocated chromosomes have a higher risk of carrying an additional aneuploidy. The segregation patterns reveal a high rate of 3:1 segregation. This is associated with the localisation of the break points, which predicts the asymmetry of the quadrivalent.

P-CytoG-128

Inversion (11)(p13p15.3) in a patient with Silver-Russell syndrome

Begemann M.[1], Mau-Holzmann U.[2], Spengler S.[1], Eggermann K.[1], Eggermann T.[1], Binder G.[3]
[1]Institute of Human Genetics, Aachen, Germany, [2]Department of Medical Genetics, Tübingen, Germany, [3]University-Children's Hospital, Tübigen, Germany

Silver-Russell syndrome (SRS) is a heterogeneous disease which is associated with intrauterine and postnatal growth restriction. Other features like feeding difficulties and morphologic abnormalities including a small triangular face, a prominent forehead, relative macrocephaly and skeletal asymmetries are helpful for clinical diagnosis. In about 50% of SRS-patient (epi)genetic mutations can be found. The most often observed change in >38% of the patients is a hypomethylation of the ICR1 in 11p15, controlling the expression of H19 and IGF2. In 7-10% a maternal uniparental disomy 7 can be found. We here present a SRS patient who was born SGA after 40 gestational weeks per caesarean section. He showed a prominent forehead and the typical SRS facial gestalt. He developed cerebral spasms at the age of 11 months which have been treated successfully. Psychomotoric development was normal.
Cytogenetic analysis revealed a paternally inherited paracentric inversion of chromosome 11 [46,XY,inv(11)(p13p15.3)]. Subsequent microarray analysis did not show any imbalances neither with the 500K nor with the Affymetrix 6.0 SNP-Array. Methylation in 11p15 was normal. To verify the exact cytogenetic breakpoints of the inversion we are currently performing FISH analysis. The functional relevance of this inversion is unclear and the rearrangement can of course be a coincidental finding. However, we are aware of a second SRS patient with a similar aberration. It might therefore be assumed that the structural anomaly affects a gene involved in the aetiology of SRS or that the aberration itself affects the regulation of another gene in 11p by a position effect.

P-CytoG-129

Complex phenotype of male patient with a large duplication of Xp22

Bettecken Th.[1], Nanda I.[2], Schellenberger E.[3], Kress W.[2]
[1]Max Planck Institute of Psychiatry, Munich, Germany, [2]University of Würzburg Institute of Human Genetics, Würzburg, Germany, [3]Pediatric Hospital Am Mönchberg, Würzburg, Germany

Microscopically visible segmental duplications of the short arm of the X chromosome are relatively rare events and only a few reports exist. All male patients show mental retardation combined with various additional dysmorphic features. Most cases are inherited from a heterozygous mother. On the other hand, submicroscopic deletions or duplications occur very often within the dystrophin gene in Xp21, causing Duchenne or Becker muscular dystrophy.
Here, we report on a now 16 year old severly retarded patient in whom cytogenetically a duplication of band Xp22.1 on the X chromosome was observed. His healthy mother carried the same duplication. The patient presents with microcephaly, suffers from seizures, speaks a few words only and has an autistic-like, at times aggresssive, behaviour. His height and weight are below the 3rd percentile, and there are small down set ears.
The duplication seen in the microscope could recently be confirmed by molecular karyotyping on Illumina SNP chips. Furthermore, the limits of the duplication could be determined to within several kilobases. The duplicated region comprises about 10.5 Mb, containing more than 200 genes. From the genes in this region, known to be involved in mental retardation syndromes, none has been shown to be duplicated in an MR patient. On the other hand, it seems likely, that the overexpression of one or several of the genes in the duplicated region is causing the phenotype, since there should be no X inactivation. Work on finding the duplication breakpoints and orientation of the duplication is in progress.

P-CytoG-130

Identification and characterisation of small supernumerary marker chromosomes by molecular karyotyping

Caliebe A.[1], Martin Subero J.I.[1], Gesk S.[1], von Spiczak S.[2], Bruhn K.[2], Eckmann-Scholz C.[3], Kautza M.[4], Weimer J.[3], Liehr T.[5], Siebert R.[1], Tönnies H.[1]
[1]Institut für Humangenetik, Kiel, Germany, [2]Klinik für Neuropädiatrie, Kiel, Germany, [3]Klinik für Gynäkologie und Geburtshilfe, Kiel, Germany, [4]Praxis für Humangenetik, Kiel, Germany, [5]Institut für Humangenetik und Anthropologie, Jena, Germany

Identification and analysis of marker chromosomes can be cumbersome. Array CGH and SNP array analyses are useful high-resolution tools especially if the aberrant cell line appears with a high frequency. Here we describe findings in the identification of three different small supernumerary marker chromosomes (sSMC).
Patient 1: In a boy presenting with developmental delay, especially of expressive language and autistic features, chromosome analysis revealed in about 40% of the metaphases an additional ring chromosome. In the phenotypically normal mother the additional ring chromosome was found in peripheral blood cells in a similar percentage as in her son. SNP array analysis (Affymetrix 6.0) was performed in order to identify the euchromatic composition of the additional ring chromosome. A duplication of 0.25 Mb in 21p11.2p11.1 and 4.16 Mb in 21q11.2 was detected. The region contains 7 genes. The whole region of 21p11.2q11.2 is a known CNV. Therefore the ring chromosome is probably not causative for the phenotype.
Patient 2: In a male infant presenting with developmental delay, hyperexcitability, and growth retardation chromosome analysis revealed in about 50% of the metaphases an additional ring chromosome. FISH analysis could show that it contained the centromere of chromosome 19. It was described as der(19)(p12q12). As the child's features were suggestive of an unbalanced chromosome abnormality Array CGH using only DNA of the micro-dissected ring chromosome was performed.

It could be demonstrated that it was composed of three euchromatic parts of chromosome 19: 19p13, 19q11, and 19q13. This finding was confirmed by reverse and targeted FISH analysis. The delineation of this complex nature of the derivative chromosome 19 shows the power of molecular karyotyping.
Patient 3: In a prenatal case (amniocentesis) in about 40% of metaphases an additional de novo sSMC derived of chromosome 18 was detected. According to FISH analyses using locus specific probes it contained euchromatin (probes used: 18p: RP11-411B10 (13,988,748-14,143,336 Mb), 18q: RP11-10G8 (17,274,439-17,431,001Mb)). As the ultrasound was normal Array CGH using the 105K Agilent platform was performed on DNA isolated from cultivated amniotic cells. Using this platform no euchromatin of any chromosome was detectable. The parents decided to continue the pregnancy. The child is now 4 months old and healthy. We conclude that molecular karyotyping techniques are fast and useful in identifying the composition of marker chromosomes. This can be performed on whole genomic DNA if the percentage of aberrant cells is high enough; otherwise following microdissection of the marker chromosome, DOP-PCR and hybridization are recommended.

P-CytoG-131
Asymmetric supernumerary inv dup 15 with secondary mosaic formation in one of two developmentally retarded twins
Eggermann T.[1], Gamerdinger U.[2], Schubert R.[3], Spengler S.[1], Baudis M.[4], Scholz M.[5], Hansmann D.[5], Tariverdian G.[5], Schwanitz G.[6]
[1]Institute of Human Genetics, Aachen, Germany, [2]Institute of Pathology, Gießen, Germany, [3]Praxis für Humangenetik, Köln, Germany, [4]Institute of Molecular Biology, Zürich, CH, [5]Institute of Prenatal Medicine, Bonn-Meckenheim, Germany, [6]Institute of Human Genetics, Bonn, Germany

Two male twins born after IVF therapy were investigated over a period of 9 years. One of them (twin 1) showed a supernumerary inv dup (15), which was present in all cells after lymphocyte culture, but in buccal mucosa the derivate 15 was lost in 12% of the cells. The marker was maternal in origin and could be determined as a meiosis I abnormality by microsatellite typing. By GTG banding, microarray analysis (6.0 SNP array, Affymetrix) and FISH the asymmetric structure of the marker could be demonstrated. The karyotypes of the parents and the second twin (twin 2) were normal. The developmental retardation of twin 2 was obviously caused by an infection at the age of 4 weeks followed by multiple organ failure. Twin 1 was specifically supported from birth up to the age of 9 years and shows a better development than it could be expected by findings from the literature while his twin brother (twin 2) failed to show a progression in development. The structure of the marker chromosome and its behaviour in mitoses were compared to findings from the literature.

P-CytoG-132
A small supernumerary marker chromosome present in a Turner syndrome patient not derived from X- or Y-chromosome - evidence for an underestimated entity?
Ewers E.[1], Sheth F.[2], Kosyakova N.[1], Weise A.[1], Sheth J.[3], Desai M.[3], Andrieux J.[4], Vermeesch J.[5], Hamid A.B.[1], Ziegler M.[1], Liehr T.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Institute of Human Genetics - Foundation for Research in Genetics and Endocrinology, Ahmedabad, India, [3]Institute of Human Genetics - Foundation for Research In Genetics and Endocrinology, Ahmedabad, India, [4]Laboratory of Medical Genetics - Jeanne de Flandre Hospital CHRU de Lille, Lille, France, [5]Center for Human Genetics, Leuven, Belgium

Small supernumerary marker chromosomes (sSMC) can be present in numerically abnormal karyotypes like in a ‚Turner-syndrome karyotype' mos 45,X/46,X,+mar. Here we report the first case of an sSMC found in Turner syndrome karyotypes (sSMCT) derived from chromosome 14 in a Turner syndrome patient. According to cytogenetic and molecular cytogenetic characterization the karyotype was 46,X,+del(14)(q11.1). The present case is the third Turner syndrome case with an sSMCT not derived from the X- or the Y-chromosome. More comprehensive characterization of such sSMCT might identify them to be more frequent than only ~0.6% in Turner syndrome cases according to available data.
Supported in parts by the DAAD (D07/00070 and fellowship for ABH) and Prochance 2008 of the Friedrich Schiller University Jena 21007091 and Dept of Biotechnology (DBT) - BT/PR9111/MED/12/337/2007, India.

P-CytoG-133
Simple Terminal Deletions of the X Chromosome are Frequently Complex Rearrangements
Fritz B.[1], Jung A.[2], Heinrich S.[3], Kornmann E.[1], Köhler A.[3]
[1]Zentrum für Humangenetik, Marburg, Germany, [2]Institut für Medizinische Genetik und Molekulare Medizin, Köln, Germany, [3]Institut für Humangenetik, Gießen, Germany

Short stature is a common feature of Turner syndrome (45,X). About 10-20% of patients with Turner syndrome have structural X chromosome abnormalities involving a deletion of a part of or the complete short arm of the only or the second X chromosome. However, rearranged X chromosomes with terminal deletions of Xp and partial duplication of Xq are rarely reported.
Here we present two cases with a recombinant X chromosome that were misinterpreted as simple terminal deletions of Xp on the basis of conventional karyotyping only.
Patient 1 was a 13 year old girl with short stature suspicious of Turner syndrome. Chromosome analysis revealed the karyotype 46,X,del(X)(p?22.3). Parental karyotypes were not available.
Patient 2 was a 5 year old girl with short stature. Her karyotype was reported as 46, X,del(X)(p22.?1). The same karyotype was identified in her mother and in her one-year-old sister who also had short stature.
In both patients a deletion of the SHOX gene in the PAR was recognized by FISH. FISH analyses with the Xp subtelomeric probe confirmed the deletion while the Xq subtelomeric probe detected signals on both ends of the abnormal X chromosome consistent with a duplication of Xq in this chromosome. Hybridisation with the X chromosome painting probe confirmed that no other chromosome was involved.
SNP array investigation using the Illumina beadchip system (human 610-Quad beadchip) demonstrated in patient 1 a terminal deletion of 10 Mb in Xp (breakpoint in Xp22.2) and a terminal duplication of 8.7 Mb in Xq (breakpoint in Xq27). In patient 2 a terminal deletion of 22 Mb (breakpoint in Xp22.12) and a terminal duplication of 13.4 Mb (breakpoint in Xq26.3) were identified.
The most probable explanation for the recombinant X chromosome is a pericentric inversion in one of the ancestors, presumably in a female. One or an uneven number of crossing over events within the relatively large inversion loop might have produced these duplication deficiencies. It is well known that clinical manifestation is different in males and in females. While males are usually profoundly affected, phenotypes in females are usually normal or near normal except for short stature and some fertility problems like secondary amenorrhea. Skewed X-inactivation is responsible for the normal X chromosome being active in the majority of cells.
Our results indicate that putative simple deletions of the X chromosome that are often associated with only subtle clinical signs except for short stature in female patients might be in fact part of a rearranged chromosome. Besides FISH SNP array analysis can clarify the true chromosomal abnormality. Parents of patients should be investigated for the existence of a pericentric inversion. A large inversion with distally located breakpoints might be interpreted as a normal X chromosome by conventional cytogenetics only.

P-CytoG-134

Y chromosome diversity in great apes

Greve G.[1], Hodler C.[1], Schempp W.[1]
[1]Institute of Human Genetics, Freiburg, Germany

Recent comparative FISH analysis using probes from the human Y-specific fertility gene families CDY (chromodomain Y) and DAZ (deleted in azospermia) disclosed highly diverse signal copy numbers and gene location among chimpanzee Y chromosomes. In contrast no variation was seen among the bonobo Y chromosomes. Here we investigated the Y-chromosomal signal pattern for DAZ and CDY in the other three great ape species gorilla (Gorilla gorilla), Bornean orangutan (Pongo pymaeus) and Sumatran orangutan (Pongo abelii). Our FISH results revealed a great stability of the signal pattern on Y chromosomes of 18 individuals of Western lowland gorilla (G. g. gorilla) and one individual of Eastern lowland gorilla (G. g. graueri), the only difference that could be recognised may be due to the length of alphoid sequences in the Y centromere: there was a clear variation in the resolution of pericentric CDY-signals among gorilla Y chromosomes. While no variation in DAZ and CDY signal pattern was seen in four Bornean orangutans, the analysis of 11 Sumatran Y chromosomal lines indeed showed a clear variation in signal pattern: in four out of the 11 Sumatran Y-lines in addition to the long arm CDY and DAZ signal clusters signals were also detected in the short arm. This variation is easily explained by a single pericentric inversion having occurred in these Sumatran lines. Thus, we can conclude that the high variability of DAZ and CDY signal pattern is only seen in chimpanzee Y chromosome lines while no variation in signal pattern could be detected in the investigated individuals of bonobo, gorilla and Bornean orangutan. The only variation seen in Sumatran orangutan is the single pericentric inversion described above.

P-CytoG-135

A unique case of free trisomy 21 combined with a complex rearranged small supernumerary marker chromosome derived from chromosome 13/21 and 18

Hamid A.B.[1], Brankovic Niksic S.[2], Ivanovic Deretic V.[3], Radivojevic Pilic R.[2], Ewers E.[1], Merkas M.[1], Ziegler M.[1], Liehr T.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Institute of Mental Health - Department of Medical Genetics, Belgrade, Serbia, [3]University Children's Hospital, Belgrade, Serbia

Here the first case of free trisomy 21 combined with a complex rearranged small supernumerary marker chromosomes (sSMC) derived from chromosome 13/21 and 18 is reported. The karyotype of the patient was 48,XY,+der(13 or 21)t(13 or 21;18)(13 or 21pter->13q11 or 21q11.1::18p11.21->18pter),+21. Including the here reported case, 35 single case reports are available in the literature for a karyotype 48,XN,+21,+mar. In only 12 of the reported cases the chromosomal origin of the sSMC was determined by FISH, only one was a der(13 or 21) and non of them consisted material of two different chromosomes. In the present case influence of the partial trisomy 18p on the clinical outcome of the patient is hard to determine. Influence of the trisomy 21 on phenotype is known to be very variable, and for partial trisomy 18p there are several papers reporting on clinically healthy subjects with that chromosomal imbalance. Supported in parts by the DFG (LI 820/22-1) and DAAD (D07/00070).

P-CytoG-136

Complex interstitial deletion Xp22.11 and Xp21.3p21.2 in a boy with mental retardation and behavioural problems

Heinrich U.[1], Gabert M.[1], Rechenberg A.[1], Rost I.[1], Wahl D.[2]
[1]Centre for Human Genetics and Laboratory Medicine Dr. Klein and Dr. Rost, Martinsried, Germany, [2]Medical Practice for Genetic Counselling and Psychotherapy, Augsburg, Germany

Approximately 10% of all cases of mental retardation are caused by mutations in X-linked genes. Here we describe a boy with two separate deletions on the X-chromosome, a 146 kb deletion in Xp22.11 and a 562 deletion in Xp21.3p21.2, both inherited from his mother. The boy is the first child of his German mother and the third child of his Nigerian father and was born after an uneventful pregnancy after 37 weeks of gestation. at the age of six months he presented an asymmetric shape of the head, and at the age of one year, psychomotor developmental delay became obvious. With the age of four years he showed profound speech delay expressing only single distinct words. With advancing years behavioural problems became very onerous presenting as restlessness and tendency to throw things into disorder. Stereotypical movements of the hands appeared recently. There is a normal growth pattern, absence of malformations, and there are only moderate dysmorhpic features. The mother presented her son at the beginning of her fourth pregnancy after two miscarriages in the first trimester. A formerly accomplished chromosome analysis had shown an unconspicuous male karyotype. Analysis of the FMR1 gene had shown a normal CGG repeat extension. BAC-based array CGH using BlueGnome's cytoChip v3 revealed a deletion of clone RP11-40P7 in band Xp22.11 encompassing at least two genes as well as a deletion of clones RP11-37E19, RP6-27C10 and RP4-662N3 in band Xp21.3p21.2 encompassing the IL1RAPL1 gene. FISH analysis with the clones RP11-40P7 and RP11-37E19 confirmed the deletions. Analysis of the mother disclosed her as the carrier of both deletions. Prenatal diagnosis was practiced by FISh in the 15th week of gestation excluding the maternal deletions in the male fetus. This case will be discussed with the literature cases.

P-CytoG-137

WHO grade specific comparative genomic hybridization pattern of diffuse astrocytoma - a meta-analysis

Holland H.[1], Koschny Th.[2], Ahnert P.[3], Meixensberger J.[4], Koschny R.[5]
[1]Translational Centre for Regenerative Medicine, Leipzig, Germany, [2]Ames Laboratory and Department of Physics and Astronomy, Ames, USA, [3]IMISE, Leipzig, Germany, [4]Clinic of Neurosurgery, Leipzig, Germany, [5]Department of Internal Medicine, Heidelberg, Germany

To identify novel genetic alterations, many astrocytomas have been investigated by the genome wide screening of comparative genomic hybridization (CGH). To identify aberration profiles characteristic for WHO grade I, II, III, and IV astrocytoma we performed a meta-analysis of the detailed CGH results of all 467 cases published so far. After expansion of all given aberrations to the maximum of 850 GTG-band resolution the frequencies of genetic imbalances were calculated for each chromosomal band for all four WHO grades, separately.
In contrast to pilocytic astrocytoma which did not show a characteristic aberration pattern, low-grade astrocytoma already demonstrated the prevailing hallmark of glioblastoma multiforme, gain of chromosome 7, but not loss of chromosome 10. The most abundant aberration in WHO grade I astrocytoma is still gain of 7q32. In anaplastic astrocytoma a more complex pattern of genomic alteration with +1q32, +7q32-q36, +12p13, +17q23-q25, -9p21-p24, -10q25-q26, -13q24.2-q22 and -19q13 emerge from rather diffuse genetic imbalances, reflecting a higher tumor genome instability. In parallel to +7q32-q36, gain of 7p12 becomes the most frequent aberration at chromosome 7. In glioblastoma multiforme +7, -9p, -10, and -13 represent the by far most frequent aberrations, forming a characteristic aberration pattern. In contrast to

lower tumor grades glioblastoma multiforme demonstrate +7p12 as the most frequently affected band on chromosome 7.
To better quantify the gradual alterations from WHO grades I to IV astrocytoma we calculated the relative increase and decrease in frequency for each detected aberration of the tumor genome. The most pronounced and diverse changes of genetic material occur at the virtual transition from low-grade to anaplastic astrocytoma. Then transition to glioblastoma multiforme is characterized by gain of 1p, chromosome 7, and loss of chromosome 10.
Summing up, the expansion of the CGH results to the 850 GTG-band resolution enabled a meta-analysis to visualize WHO grade-specific aberration profiles in astrocytoma.

P-CytoG-138

Parental origin and functional relevance of a de novo UBE3A variant

Horsthemke B.[1], Wawrzik M.[1], Groß S.[1], Lich C.[1], Buiting K.[1], Krasemann E.[2], Kosyakova N.[3], Liehr T.[3], Weise A.[3], Dybowski J.N.[4], Hoffmann D.[4], Wieczorek D.[1]
[1]Institut für Humangenetik, Essen, Germany, [2]Labor Dr. Fenner und Kollegen, Hamburg, Germany, [3]Institut für Humangenetik, Jena, Germany, [4]Bioinformatik UDE, Essen, Germany

Sequence analysis of the imprinted UBE3A gene in a 3-year-old girl with microcephaly, developmental delay, absence of speech, ataxic gait, increased salivation, but no epilepsy had revealed a de novo 3 bp in frame deletion predicted to result in a protein lacking one amino acid (p.Gly538del, NM_130838). In order to assess the clinical relevance of this hitherto unknown variant, we have investigated the parental origin and the functional consequences of the deletion. For determining the parental origin of the deletion, we decided to physically separate the two homologues, determine their parental origin with the help of a cytogenetic or molecular marker segregating in the family, and identify the deletion bearing chromosome by PCR and sequencing. Cytogenetic studies revealed normal chromosomes 15. In order to distinguish the two homologues, we applied a chromosome 15 specific parent-of-origin dependent-(pod)-FISH probe set. A CEP15 sat III probe (Spectrum Green, Abbott) revealed a strong and a weak signal, respectively, on the two 15s in each metaphase. Comparison of these signals with those on the parental chromosomes suggested that the chromosome with the strong signal was of maternal origin. Guided by this polymorphism, we collected by microdissection seventeen and eighteen copies, respectively, of each homologue and amplified the DNA by DOP-PCR. Analysis of the microdissected DNA and parental DNA samples at the D15S822 microsatellite locus confirmed that the chromosome with the strong CEP15 sat III signal was of maternal origin. UBE3A sequence analysis revealed that this chromosome carried the 3 bp deletion. For determining the functional consequences of the deletion, we modelled the structure of the UBE3A deletion mutant p.Gly538del based on the wild type X-ray structure and simulated the molecular dynamics of the wildtype and mutant protein in complex with UcbH7 in aqueous solution at a temperature of 300 K. Our simulations showed that deletion of Gly538 destroys a network of salt bridges between highly conserved residues in the catalytic cleft of UBE3A. In conclusion, our results strongly suggest that the 3 bp deletion is a loss-of-function mutation of the maternal UBE3A allele that has caused Angelman syndrome in our patient. Our study may serve as a paradigm to determine the parental origin of a de novo mutation.

P-CytoG-139

Accurate characterization of an sSMC derived from chromosome 2 in a child with multiple congenital malformations confirms the adverse clinical effects of partial trisomies distal from 2q11.2

Kosyakova N.[1], Santos M.[2], Mrasek K.[1], Plaja A.[3], Vendrell T.[3], Fuster C.[2], Liehr T.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Unitat de Biologia Cel lular i Genètica Mèdica - Facultat de Medicina - Universitat Autònoma, Barcelona, Spain, [3]Unitat de Genètica - Hospital Materno Infantil de la Vall d'Hebron, Barcelona, Spain

Small supernumerary marker chromosomes (sSMC) are reported in 0.43% in newborn and 0.41% in patients with congenital malformations and/or mental retardation. The most common origin of an sSMC is chromosome 15, for over 30%-40% of all sSMC; however, there have been described sSMC for all human chromosomes. At present, only 9 of in summary 24 reported cases with an sSMC derived from chromosome 2 were characterized in detail, and almost all of them presented with ring morphology. Here we present the accurate characterization of a de novo sSMC, in mosaic and with no ring morphology, as min(2)(:p11.1->q12.1:) by means of subcentromere specific multicolor-FISH in a child with multiple malformations. The present case, as others previously described in the literature, confirms the negative effect of partial trisomies distal to 2q11.2. Supported by Ministerio de Ciencia y Tecnología (SAF 2003-03894), CIRIT (SGR05-00495) and Generalitat de Catalunya (grant 2002FI00281 and 2005BE00172) and the Evangelische Studienwerk e.V. Villigst.

P-CytoG-140

A functional assay for the classification of patients with BRCA2 mutations of unclear pathogenicity

Landwehr C.[1], Becker A.[2], Gräser M.K.[2], Hilger T.[3], Baus W.W.[3], Schmutzler R.K.[2], Weber R.G.[1]
[1]Institute of Human Genetics, Bonn, Germany, [2]Center of Familial Breast and Ovarian Cancer, Cologne, Germany, [3]Department of Radiation Therapy, Cologne, Germany

According to the German consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC) 10% of families at risk carry an unclassified variant (UCV) in the BRCA1 or BRCA2 gene the pathogenic impact of which is unclear. Many studies have suggested that the BRCA proteins play an important role in DNA repair and there is evidence that human lymphocytes with monoallelic pathogenic BRCA1 mutations show an increased level of chromosomal damage after irradiation. Thus, it was the aim of this study to investigate whether a functional assay assessing the DNA repair capacity would allow classification of patients with BRCA2 mutations of unknown pathogenicity.
We induced DNA breaks by irradiating fibroblasts and lymphocytes from patients with (a) known pathogenic BRCA2 mutations or (b) UCVs in BRCA2, and (c) sex and age matched controls without BRCA2 mutations and determined an intensity of 4Gy to be optimal in inducing chromosomal aberrations while still allowing cells to cycle and divide. After irradiation, cells were cultured for a standardized time to allow DNA repair to take place before metaphase chromosomes were prepared and 24-color-FISH experiments were performed. By analyzing 20 metaphases per case, we determined the number of translocations and double strand breaks to measure the influence of irradiation on the capacity of cells to repair DNA double strand breaks.
So far, we investigated fibroblasts from four patients with pathogenic BRCA2 mutations, four patients with UCVs in BRCA2 and four controls . The average number of aberrations per metaphase was significantly higher in fibroblasts from BRCA2 mutation carriers than from controls (0.33 vs. 0.16, p=0.032, Fisher's Exact Test). The aberration frequency in three patients with UCVs was comparable to the carriers of pathogenic mutations and in one patient lower than the lowest control.

The assay was also performed on lymphocytes from eight patients with pathogenic BRCA2 mutations, five patients with UCVs in BRCA2 and nine controls . In lymphocytes, the difference between controls and BRCA2 mutation carriers was even more significant (average number of aberrations per metaphase: 1.4 vs. 2.51, p<0.001, Fisher's Exact Test). The aberration frequency in one patient with UCV was as high as in the carriers of pathogenic mutations and in the other four patients within the range of the controls.
In summary, fibroblasts and lymphocytes with monoallelic BRCA2 mutations showed a significantly increased level of chromosomal damage after irradiation. Because this assay allows the discrimination of patients with pathogenic BRCA2 mutations and controls, the UCVs could be classified into two groups with normal and diminished DNA repair capacity.

P-CytoG-141

Centromere repositioning can be easily mixed up with a pericentric inversion

Liehr T.[1], Kosyakova N.[2], Ziegler M.[1], Schröder J.[1], Raabe-Meyer G.[3], Wagner H.[4], Stibbe W.[4], Weise A.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Jena University Hospital -Institute of Human Genetics and Anthropology, Jena, Germany, [3]Praxis für Humangenetik Dr. Schulze, Hannover, Germany, [4]Partnerschaft - Praxis für Humangenetik, Hannover, Germany

In 1993 the first ectopic or neocentromere was reported in a derivative chromosome 10. Since then 97 cases with neocentric small supernumerary marker chromosomes (sSMC), 6 cases with centromere repositioning and 13 cases with other rearrangements were reported.

Here we report on two new case of a prenatally diagnosed neocentromere on chromosome 7q32.1 and 18q22.1, respectively. Amniocentesis was done due to advanced maternal age and sonography was normal in both cases. A derivative chromosome 7 or 18 was detected in GTG-banding, respecitively. FISH analysis using the corresponding centromere specific probes revealed that the primary incision was not in concordance with the alphoid DNA on the derivative chromosomes. By array-proven multicolor banding (aMCB) we confirmed that the derivatives had the identical banding pattern as the homologous normal chromosomes, but the incision at the wrong places, i.e. in 7q32.1 or 18q22.1. In case of neocentric #7 the chromosomes of the parents were normal. After genetic counseling the parents decided for continuation of the pregnancy and a healthy child was born, which develops normal up to present (4 years of age). Unfortunately the parents refused a blood acquisition for further studies like immunohistochemistry with CENP antibodies. In case of neocentric #18 the pregnancy is still ongoing and no parental chromosome analysis was done yet. However, here we were able to use CENP antibodies proving the pseudodicentric nature of the derivative chromosome 18.
Overall, here we report two more cases of a centromere repositioning, both are the first ones for chromosome 7 and/or 18. Similar observations of centromere repositioning were made already for chromosomes 3 (2 cases), 4, 8 and Y. Interestingly, in both cases it was initially suggested that an inversion might have been the reason for the aberrant banding pattern of the derivative chromosomes. Thus, such neocentric cases with intact chromosomes and a centromere repositioning might be more frequent than up to now suggested. They are easily overlooked, as the carriers are clinically normal, an observation which fits well with the fact that genes in neocentromeric regions are normally expressed irrespective of the neocentric incision. Supported in parts by DAAD (D07/00070).

P-CytoG-142

De novo complex chromosome rearrangement (CCR) involving chromosomes 3,5 and 10 in a boy with generalized developmental delay and dysmorphic craniofacial features

Lüke Y.[1], Siebers-Renelt U.[1], Exeler-Telker R.[1], Röpke A.[1], Ledig S.[1], Wieacker P.[1]
[1]Institute of Human Genetics, Münster, Germany

Complex chromosome rearrangements (CCRs) are rare structural abnormalities involving more than two chromosome breaks and the reciprocal exchange of segments between at least two chromosomes. Here we describe a two year old patient with generalized developmental delay and muscular hypotonia and dystonia. Craniofacial features include frontal bossing, low set ears and microphthalmia. A congenital bilateral cataract was diagnosed at the age of two months and demanded recurrent surgery. Malformations of the brain were not detected. G-banded chromosomal study (resolution 550 bands) revealed a balanced de novo rearrangement involving chromosomes 3, 5 and 10 with three breakpoints (46,XY,t(3;5;10)(q13.3;q14;q21)) which was further confirmed by FISH analysis. Array –CGH using a 244A chip was performed but revealed no evidence of enhancement or diminishment of any chromosomal region involved in the complex rearrangement. Translocation breakpoint analysis must be performed to identify candidate genes which might be responsible for the patient`s phenotype. This case illustrates the importance of combining traditional and molecular cytogenetic techniques for a more comprehensive analysis of the rearrangements observed.

P-CytoG-143

Complex karyotype defined by FISH and M-FISH studies in an infant with acute megakarioblastic leukemia and neurofibromatosis.

Manvelyan M.[1], de Jesus Marques-Salles T.[2], Pereira Leite E.[3], Maria Soares-Ventura E.[3], Cartaxo-Muniz M.T.[3], Ferreira E.[3], Liehr T.[1], Macedo Silva M.L.[4], Santos N.[5], Junker S.[1], Mkrtchyan H.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Pediatric Oncohematology Center - Hospital Oswaldo Cruz and Genetic Department - Pernambuco University, Recife, Brazil, [3]Pediatric Oncohematology Center - Hospital Oswaldo Cruz -Pernambuco University, Recife, Brazil, [4]Cytogenetic Department of the National Center for Bone Marrow Transplant - National Cancer Institute, Rio de Janeiro, Brazil, [5]Genetic Department - Pernambuco University, Recife, Br

Acute leukemia in childhood is a heterogeneous group of diseases and different epidemiologic factors are related to its etopathogenesis. Genetic syndromes are one of the predisposing factors of acute myeloid leukemia (AML), such as Down syndrome (DS), Bloom syndrome and neurofibromatosis. Acute megakarioblastic leukemia (AMKL) is the main subtype in DS infants and acquired chromosomal anomalies are closely related to the physiopathology of the illness. The main chromosomal anomalies in the AMKL are structural such as t(1;22), however, complex karyotypes are also found frequently. Here we describe an infant with neurofibromatosis developing an AMKL with a complex karyotype including 5q and 17q deletions, TPp53 deletion, and an unusual chromosomal translocation t(11;19)(q13;p13), but absence of rearrangements within the MLL gene. This work was partially supported by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), CAPES/ DAAD (D/07/09624) and Monika Kutzner-Stiftung. The authors are grateful to Programa Criança e Vida for the helpful support in the childhood cancer network care.

P-CytoG-144

Four small supernumerary marker chromosomes derived from chromosomes 6, 8, 11 and 12 in a patient with multiple hyperpigmented skin nevi and almost no other clinical signs

Merkas M.[1], Fernández-Toral J.[2], Rodríguez L.[3], Plasencia A.[4], Martínez-Frías M.L.[5], Ewers E.[1], Hamid A.B.[1], Ziegler M.[1], Liehr T.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Pediatría y jefe de sección de genética pediatrica del HUCA, Oviedo, Spain, [3]AbaCid-Genética Hospital de Madrid Norte Sanchinarro, Madrid, Spain, [4]Servicio de genética del HUCA, Oviedo, Spain, [5]Estudio Colaborativo Español de Malformaciones Congénitas del Centro de Investigación sobre Anomalías Congénitas - Instituto de Salud Carlos III - Ministerio de Sanidad y Consumo, Madrid, Spain

Here the first case with 4 small supernumerary marker chromosomes (sSMC) is reported with almost no clinical signs, apart from slight developmental retardation in youth, and non-malignant hyperpigmentation. The sSMC were derived from chromosomes 6, 8, 11 and 12, leading overall to a small partial trisomy in 12p11.1~12.1. Including the here reported case, 4 single case reports are available in the literature for a karyotype 50,XN,+4mar. The clinical relevance of the small partial trisomy in 12p and the other 3 sSMC present in this case is discussed. Supported in parts by the DFG (LI 820/22-1) and DAAD (D07/00070).

P-CytoG-145

Fanconi anemia (FA) as a model for the mapping of rarely observable FS

Mrasek K.[1], Wilhelm K.[1], Blaurock N.[1], Behr K.[1], Teichmann A.-C.[1], Liehr T.[1], Schoder C.[1], Weise A.[1]
[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany

Within cytogenetic preparations chromosomal breaks can be observed as so called fragile sites (FS). Especially in patients suffering from Fanconi anemia (FA), a recessively inherited syndrome with an extremely elevated cancer risk, but also in healthy individuals FS are present. FA is caused by different defects in proteins of the pathway responsible for DNA double strand breakage repair. Cytogenetically, FA is characterized by a high incidence of chromosomal gaps, breaks and rearrangement figures, consisting of several chromosomes or chromosome parts. So far it was known that FS cytogenetically co-localize with tumor- and evolutionary conserved chromosomal breakpoints. The co-localization of FS and FA associated breakpoints (FA-bp) was studied here for the first time systematically by molecular cytogenetics. Metaphase chromosomes were obtained from lymphocytes of two FA patients (FANC-A and FANC-C, respectively). Overall, 50.58% of the investigated FA-bp correspond to cytogenetic regions with known FS. A detailed molecular cytogenetic study applying FS-spanning probes revealed that 24/29 (= 82.8%) of analyzed FS are in concordance with FA-bp. Notably, FA-bp show a distribution pattern deviating from that of aphidicolin induced FS. Our observation that FS, which normally occur infrequently in aphidicolin induced cells, can be found frequently in FA-chromosome spreads makes FA to a model system for the fine mapping of infrequent FS, such as FRA1B, FRA1E, FRA1F and FRA1G. Supported in parts by Evangelische Studienwerk e.V. Villigst, IZKF Jena (Promotionsstipendium to CS and KW, and Start-up S16 to AW), IZKF together with the TMWFK (TP 3.7 and B307-04004), Ernst-Abbe-Stiftung, DFG (LI 820/15-1) and Deutsche Fanconi-Anämie-Hilfe e.V.

P-CytoG-146

Inherited ring chromosome 6 with deletion of 6p25.3 in a 4.5-year-old patient and her mother

Möhlendick B.[1], Gebauer H.J.[1], Rieder H.[1]
[1]Institute for human genetics, Düsseldorf, Germany

The formation of a ring chromosome, which implies breakage and subsequent fusion of both end segments of the chromosome, is a rare event. Usually this chromosome disorder arises de novo, only 5.6% patients with inherited ring chromosomes are described up to now. Maternal ring transmission is observed more often and chromosomes 14, 21 and 22 are preferentially involved. Here, we report a 4.5-year-old girl with a ring chromosome 6 and growth retardation (<3. percentile), prominent forehead, anteverted nares, small upper lip, severe hypermetropia (+11 dpt right, +10 dpt left), hypertelorism, epicanthus, brachydactyly and widely spaced nipples. The patient also showed a hypoplastic pelvic kidney with a proportionate function of 30%. Conventional chromosome banding analysis and fluorescence in situ hybridisation (FISH) revealed the karyotype 46,XX,r(6)(p25p27).ish del(6)(p25.3)(CTB-62I11-) in all analysed metaphases. Surprisingly the 39-year-old mother showed the same ring chromosome by conventional analyses. So far, no further cases with an inherited ring chromosome 6 have been reported. The mother showed a mild phenotype with a short stature, hearing impairment and hypermetropia. To delineate the breakpoints, we analysed the daughter by array comparative genomic hybridisation (aCGH) with a 44k DNA Microarray (Agilent), with an average resolution of 43 kb. The distal breakpoint ranged between 0-215 kb, with the last available deleted probe P109100. The potential proximal breakpoint of the deletion could be localised between 1.905.172-2.062.744 basepairs (bp) on chromosome 6p. No losses of material from 6q were detected. To further define the breakpoint we designed a custom array of chromosome region 6p25.3 with an average resolution of 1 kb. Mother and daughter were analysed and the breakpoint could be narrowed down to 2.046.381-2.047.169 bp. The distal breakpoint is located in the subtelomeric region starting at 0 Mb. The FOXC1 (forkhead box C1) gene, which is located to 6p25.3 has been shown to play a role in embryonic ocular development and early organogenesis of the kidney and urinary tract. Mutations in FOXC1 are associated with severe eye pathologies (Kaur et al. 2009) and kidney malformations (Kume et al. 2000). So, a deletion in this gene could be responsible for the phenotype in our patients, too. Patients with ring chromosome 6 have a wide range of phenotypes from growth delay and mild mental retardation to profound congenital malformations including the ocular, auditory, cardiac and central nervous system. The deletion size may have an impact on the phenotype. So far, the patients only have been classified by conventional cytogenetics with low resolution and a distinct correlation to a certain phenotype was not possible. So high resolution aCGH is a useful tool to determine the sizes of the deletions and to distinguish the genotypes and correlate them to the phenotype more precisely.

P-CytoG-147

Three patients with chromosome 15q11-q13 duplication syndrome

Neumann T.E.[1], Wieacker P.[1], Röpke A.[1]
[1]Institut für Humangenetik - Universitätsklinikum, Münster, Germany

Background: Chromosome 15q11-q13 duplication syndrome (MIM 608636) is an autistic disorder characterized by development delay, mental retardation, infantile autism and seizures. Most patients show delayed or absent language development and impaired social interactions. Physical abnormalities are rare, facial dysmorphism is subtle. The phenotype is caused by duplication of the chromosome 15q11-q13 region, either by interstitial duplications of 15q11-q13, or by derivative chromosomes (inv dup (15) or idic (15)). This region contains the ~4Mb segment that encompasses the Prader-Willi / Angelman syndrome critical region (PWASCR). The majority of the abnormal chromosomes appears to be derived from the mother, displaying parent-of-origin effect.

Results: We report on three patients with chromosome 15q11-q13 duplication syndrome. They all show typical delayed attainment of developmental milestones, delayed language development and mental retardation, peculiar face and impaired social interactions. One has primary microcephaly (–4.3 SD), one had congenital heart defect. Two of the children clearly do not show infantile autism, none developed seizures up to now. Our patients have apparently normal karyotype on conventional cytogenetic analyses. Array-based comparative genomic hybridization (array CGH) uncovered interstitial duplications within chromosomal region 15q11-q13. All duplications were confirmed by metaphase and interphase in situ hybridization (ish, nuc ish) and by quantitative PCR. The duplications are 4 Mb, 5 Mb and 6.6 Mb in size respectively. All duplications include the PWASCR. In one case we detected mosaicism in the mother.
Conclusion: The developmental and clinical characteristics in our three patients demonstrate the highly variable phenotype of patients due to chromosome 15q11-q13 duplications. Developmental delay and intellectual disability may affect all individuals, however infantile autism or distinct autistic-like behavior and seizures are not mandatory signs. If chromosome 15q11-q13 duplication is detected, the parents should be analysed properly, since parental mosaicism has to be considered. Furthermore, our observations highlight the usefulness of array CGH analysis in patients with delayed motor and language development and mental retardation.

P-CytoG-148

Diagnosis of Fetal Abnormalities by Ultrasound: CVS Analysis yields a Balanced De Novo Translocation $^{9;21}$ in Addition to a De Novo Micro Duplication at Xp.

Parczewska J.[1], Pleyers A.M.[1], Gebel V.[1], Lemmens M.[1]
[1]Praxis für Humangenetik Dr. med. Marta Lemmens, Aachen, Germany

Nuchal transparency of 3,0 mm, hydrops fetalis and a pes equinovarus at gestational age of 17 weeks plus two days were the indication for chorionic villus sampling in a 32 years old pregnant woman of good health conditions. Chromosomal analysis of the biopsy material revealed the balanced occurring translocation t(9;21)(q22.3;q22.3). Since the parental karyotypes were normal, e.g. 46,XX an 46,XY, the chromosomal translocation in the fetus was a de novo one. Because of the fetal abnormalities, array-CGH-analysis of CVS-DNA was performed. The 9;21 breakpoint area did not exhibit any abnormality, however, a micro duplication of 0.2 Mb at Xp22.31 was detected, containing the two genes VCX an PNPLA4 and the putative gene Q9UHT6, respectively. The respective analyses of the parent´s DNA did not reveal the above mentioned duplicated area. Thus, the child exhibited a de novo duplication at Xp22.31. Meanwhile, the child has been born and so far does to our knowledge not show any significant abnormalities. The possible involvement of VCX und PNPLA4-genes leading to the child`s prenatal abnormalities and further diagnostic steps will be discussed.

P-CytoG-149

Rubinstein-Taybi syndrome due to a 380Kb deletion on chromosome 22q13.2 including the entire EP300 gene

Röpke A.[1], Müller-Hofstede C.[1], Bohring A.[1], Wieacker P.[1], Neumann T.E.[1]
[1]Institut für Humangenetik, Universitätsklinikum Münster, Germany

Rubinstein-Taybi syndrome (RSTS; MIM 180849) is a rare congenital anomalies – mental retardation syndrome characterized by distinctive facial features, skeletal abnormalities, in particular broad and angulated thumbs and great toes, postnatal growth retardation, and moderate to severe mental retardation. RSTS is associated with mutations in the CREBBP gene found in approximately 40 – 60% of patients. Mutations were also detected in the EP300 gene, a paralog of CREBBP, located on chromosome 22q13.2. Up to now only six patients with EP300 mutations were described in the literature. Individuals reported with mutations in the EP300 gene have a milder skeletal phenotype, especially abnormalities of the thumbs and great toes may be absent.
We report on a five years old girl with typical facial features of RSTS, microcephaly, mental retardation, but mild skeletal phenotype. Her height is normal (–1.3 SD), thumbs and great toes have only slightly broadened and shortened terminal phalanges. Speech development is delayed.
Our patient showed an apparently normal female karyotype on conventional cytogenetic analysis. Array CGH analysis uncovered an interstitial deletion within chromosomal region 22q13.2. This 380 kb deletion encompasses six genes including the EP300 gene. Deletion of the entire EP300 gene was not described before. However, it is still unclear whether or not one of the other five deleted genes has any influence on the phenotype in our patient.
The morphological characteristics in our patient confirm the distinct phenotype of RSTS patients due to EP300 mutations. Consistently, all show typical facial features, microcephaly, mental retardation of variable degree, but only mild skeletal abnormalities on hands and feet. To this day, none of the patients with EP300 mutations showed the classic broad and angulated thumbs and great toes. Furthermore, this is the first description of an entire EP300 allele deletion causing RSTS.

P-CytoG-150

Analysis of abortions with array-CGH and QFPCR revealed higher efficiency and detection rate compared to conventional chromosome analysis

Schloegl S.[1], Dietze-Armana I.[1], Mehnert K.[1], Rettenberger G.[1]
[1]genetikum, Neu-Ulm, Germany

Main portion of abortions result from genetic reasons like chromosomal defects. Most aberrations are de novo, but some are also inherited unbalanced from a parent carrying a balanced structural chromosome change. Conventional chromosome analysis is the common method applied in most labs.
A big challenge however is often cell growth because nearly one third of tissues fail culturing due to already degraded, contaminated or fixed material. Another problem is the unclear fetal origin of normal female karyotype, which cannot be verified without comparison with maternal DNA by molecular techniques. A handicap is also low banding quality and resolution.
We applied array-CGH in order to compare detection rate of aberrations, material costs and hands on time.
Tissues were analyzed by conventional cytogenetic technique, 8x15k or 8x60k oligo-array-CGH and QFPCR. Results were partially verified with MLPA and FISH technique in a portion of cases.
In our hands a higher and faster throughput could be achieved with less labour. Altogether results from array-CGH testing confirm literature data: e.g. the rate of affected chromosomes in the cases with trisomy matched with distribution-curve of the most prevalent trisomies. Nearly the half of cases showed normal karyotypes. They were further analyzed with QFPCR to exclude female triploidies, which can not be detected with array-CGH, or maternal contamination by comparative STR-analysis with maternal blood.
Moreover we could detect small unbalanced aberrations, that wouldn't have been identified with conventional chromosome analysis.
With array-CGH in combination with QFPCR, it was possible to reduce the rate of unexplained cases due to culture failure significantly. Furthermore we were able to detect some small unbalanced rearrangements often depending on familiar undetected balanced changes. A big advantage is the success rate of presenting a reason for the miscarriage, which gives much better understanding and psychological support to physicians and patient. Further benefit is time saving and lower costs on hands on time. Disadvantage is the high price of material and processing, depending on individual lab organisation.

P-CytoG-151

The phenotypic spectrum of the 17q21.31 microdeletion syndrome – a case with comparatively mild clinical picture

Schüler H.M.[1], Spengler S.[1], Lott A.[2], Baudis M.[3], Bachmann N.[4], Rudnik-Schöneborn S.[1], Elbracht M.[1]

[1]Institute of Human Genetics, Aachen, Germany, [2]Centre of Medical Genetics, Osnabrück, Germany, [3]Institute for Molecular Biology, Zürich, Switzerland, [4]Bioscientia Centre for Human Genetics, Ingelheim, Germany

The microarray technology has facilitated the identification of submicroscopic chromosomal alterations and led to the characterisation of novel microaberration syndromes, which became clinically well characterised and therefore recognisable conditions, whereas the full extend of the clinical pictures is still evolving.

The typical phenotypic features of the 17q21.31 microdeletion include mental retardation from mild to severe, hypotonia and a characteristic facial appearance (long face, tubular or pear-shaped nose, bulbus tip, large and low-set ears, broad chin). Common features of medical importance are epilepsy, congenital heart defects, urologic anomalies and skeletal problems.

Till now, all of the 17q21.31 deletions described arose de novo, and NAHR (non-allelic homologous recombination) was considered to be the most likely causative mechanism in the majority of cases. Band 17q21.31 contains a 900 kb inversion polymorphism with two divergent haplotypes named H1 and H2. The H2 allele is less common (20%), but in all families studied, the parent-of-origin carried at least one H2 chromosome.

We report on the clinical and molecular characterisation of a patient with a 17q21.31 microdeletion and present the genotyping for H1/H2 and parent-of-origin analyses.

The 17-years old girl showed the typical facial phenotype, mild mental retardation (IQ 64) and body measurements within normal ranges. With the exception of scoliosis and hearing impairment, she showed no common features of the 17q21.31 microdeletion spectrum which needed special medical attention. Family history was negative.

A 17q21.31 deletion was detected by whole genome microarray analyses (Agilent 44k Oligo-array and Affymetrix 6.0 SNP-array). The size of the 17q loss could be defined as approximately 500 kb. Genotyping of the H1/H1 haplotypes showed that both parents are heterozygote carriers of the 900 kb inversion polymorphism.

However, our patient shows the facial characteristics of older patients, but she also depicts a considerable phenotypic variability coming along with the 17q21.31 microdeletion.

P-CytoG-152

No evidence of copy number changes at the breakpoints of a familial paracentric inversion of 9q using molecular karyotyping with a resolution of 500 bp

Sotke A.[1], Möhlendick B.[1], Bachmann I.[1], Schulze B.[2], Raabe-Meyer G.[2], Fritz B.[3], Rieder H.[1]

[1]Institut fuer Humangenetik und Anthropologie, Universitaetsklinikum Duesseldorf, Germany, [2]Praxis fuer Humangenetik, Hannover, Germany, [3]Institut fuer Humangenetik, Universitaetsklinikum Marburg, Germany

Background: Chromosome aberrations may be found in 5.5%-8% of infertile couples. In the female partners, balanced chromosomal changes may account for 27% of the abnormalities. Among these, reciprocal translocations represent 51% and paracentric or pericentric inversions 22% of the cases. Several studies in reciprocal translocations carriers showed, that phenotypically normal patients lack imbalances at the breakpoints, whereas cryptic imbalances could be identified at the breakpoints in several patients with phenotypical abnormalities. However, so far, no patient with an inherited, cytogenetically balanced inversion has been investigated for cryptic genome alterations.

Case report: Chromosome analysis aimed at the investigation of genetic factors for infertility in a 44 years old female patient revealed a paracentric inversion of chromosome 9, inv(9)(q21.3q34.1). The abnormality was inherited from her mother. No clinical abnormality except infertility was found.

Method: Two-colour fluorescence in situ hybridisation (FISH) with BAC probes was used to narrow down the proximal and distal breakpoints to an interval of 200-400 kb. Oligonucleotide array comparative genomic hybridisation (oligo-aCGH) with an average resolution of 500 bp was performed to detect cryptic genomic changes within the respective intervals.

Results: By using breakpoint spanning BAC probes, the proximal breakpoint was assigned to 9q21.31 at 87.5-87.7 Mb, where the MAK10 gene is located, and the distal breakpoint to 9q34.12 at 132.6-132.8 Mb, which contains the FIBCD1 gene. A custom array was designed with oligonucleotides covering the respective regions at an average distance of 500 bp. No genomic changes were detected within the breakpoint regions.

Discussion and conclusion: So far, no imbalances have been found in healthy carriers of balanced chromosome aberrations in aCGH analyses at an average resolution of down to 150 kb. Using breakpoint mapping with BAC probes and custom oligo-aCGH we extended the resolution down to 500 bp in a case with inv(9) without evidence of genomic imbalances. Obligatory or potential gene disruption has been demonstrated in 32% or 41%, respectively, of phenotypically normal translocation carriers using array painting of flow sorted translocation chromosomes. In our case, chromosome microdissection of the part of the long arm of abnormal chromosome 9, which contains the proximal or distal chromosome breakpoint, may help to clarify, if the MAK10 and/or the FIBCD1 gene have been disrupted by the inversion.

P-CytoG-153

Breakpoint Characterization in a Familial Balanced and Unbalanced ins(7;6)(p14.2;q16.1q21)

Spreiz A.[1], Müller D.[1], Zotter S.[2], Albrecht U.[2], Baumann M.[2], Fauth C.[1], Erdel M.[1], Utermann G.[1], Zschocke J.[1], Kotzot D.[1]

[1]Department of Medical Genetics Molecular and Clinical Pharmacology, Innsbruck, Austria, [2]Division of Pediatric Neurology and Inherited Metabolic Disorders, Innsbruck, Austria

Constitutional insertional translocations are rare findings in clinical cytogenetics. Here, we report on the breakpoint characterization in a family with unbalanced segregation of a balanced paternal insertional translocation ins(7;6)(p14.2;q16.1q21). A 17-year-old daughter affected by severe mental retardation, obesity, and minor dysmorphisms inherited the der(6) chromosome and so confirmed the previously reported phenotype of severe mental retardation and obesity in patients with del(6)(q16.2). A daughter with an almost unremarkable phenotype and only minor features in neuropsychological testing at 19 years of age as well as her 14-years-old half-brother with minor dysmorphisms and a mild delay in cognitive development, most likely caused jointly by the chromosomal rearrangement and asphyxia during delivery, inherited the der(7) chromosome.

So far, investigations by conventional karyotyping, FISH with locus-specific probes, and SNP-array based copy number analysis (HumanCytoSNP-12, Illumina®) revealed a size of the inserted segment of 11.3 Mb and localized the breakpoints between rs6931706 and rs794669 and between rs9398069 and rs7772706 on 6q16.1 and 6q21, respectively, as well as within BAC clone RP11-182J23 on 7p14.2. Fosmid clones on 7p14.2 and the high resolution Human Omni1-Quad (Illumina®) array will help to narrow the breakpoints further down, so that standard primer PCRs will allow an exact characterization, where even smallest microrearrangements down to the level of basepair changes around the breakpoints arisen during paternal meiosis will be visible. As a whole the results will improve the understanding of the molecular behaviour of such rearrangements in meiosis.

P-CytoG-154

Detailed characterization of small supernumerary marker chromosomes reveals breakpoint hot spots and narrows down the critical regions of clinical impact

Weise A.[1], Kosyakova N.[1], Mrasek K.[1], Ewers E.[1], Bacino C.[2], Patel A.[2], Cheung S.W.[2], Cai W.W.[2], Senger G.[3], Melo J.B.[4], Carreira I.M.[5], Dufke A.[6], Mehnert K.[7], Yardin C.[8], Wegner R.D.[9], Stumm M.[9], Kistner G.[10], Leipoldt M.[11], Thiel G.[12], Petersen M.B.[13], Junge A.[14], Fritz B.[15], Joksic G.[16], Heilbronner H.[17], Ujfalusi A.[18], Brecevic L.[19], Hamid A.B.[1], Merkas M.[1], Liehr T.[1]

[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Baylor College of Medicine, Houston, USA, [3]Practice for Medical Genetics and Gynaecology, Regensburg, Germany, [4]Laboratório de Citogenética - Instituto de Biologia Médica - Faculdade de Medicina and CIMAGO - Universidade de Coimbra, Coimbra, Portugal, [5]Laboratório de Citogenética - Instituto de Biologia Médica - Faculdade de Medicina and CIMAGO and - Centro de Neurociências e Biologia Celular - Universidade de Coimbra, Coimbra, Portugal, [6]Institute of Human Genetics, Tübingen, Germany, [7]Zentrum für Humangenetik, Neu-Ulm, Germany, [8]Centre Hospitalier Universitaire de Limoges - Hospital de la Mère et de l'enfant, Limonges, France, [9]Partnerschaft, Berlin, Germany, [10]Labor für humangenetische Diagnostik, Rampe, Germany, [11]Institut für Humangenetik und Anthropologie, Freiburg, Germany, [12]Praxis für Humangenetik, Berlin, Germany, [13]Department of Genetics - Institute of Child Health, Athens, Greece, [14]Institut für Medizinische Genetik, Dresden, Germany, [15]Zentrum für Humangenetik, Marburg, Germany, [16]Institue of Nuclear Sciences Vinca and Clinic of Gynecology and Obstatrics Larodui Front, Belgrade, Serbia, [17]Klinikum Stuttgart - Institut für Klinische Genetik, Stuttgart, Germany, [18]Genetic Lab - Department of Pediatrics - University of Medicine and Health - Science Center, Debrecen, Hungary, [19]School of Medicine Zagreb University - Croatian Inst. of Brain Research, Zagreb, Croatia

Array-comparative genomic hybridization (aCGH) was done in 64 small supernumerary marker chromosomes (sSMC). The studied sSMC-specific DNA was derived from glass-needle based microdissection, avoiding by that the problem of mosaicism as present in ~40% of corresponding patients. Furthermore, a detailed analysis of overall 128 characterized breakpoints in non-heterochromatic chromosomal regions of sSMC was done. It turned out that 82.8% and 53.1% of the breakpoints are located within copy number variant regions and regions with segmental duplications, respectively. 6.3% of the breakpoints locate within sequence gaps, making an overall of 90.6% of association of non heterochromatic sSMC-related breakpoints with 'critical genome structure'. Moreover, approximately three quarters of the breakpoints were concordant with fragile sites. Still, there was a 7.0% overlap of the observed breakpoints and interspersed telomeric sequences (ITS), but only two out of 128 breaks were within an olfactory receptor gene family region. Overall, we present the largest ever done aCGH study in sSMC and provide evidence for hotspots involved in sSMC formation. Deduced from that data it was also possible to characterize regions causing clinical problems if present additionally, for all human chromosomes except for chromosomes 6, 13, X and Y. Supported in parts by the Dr. Robert Pfleger-Stiftung, DLR/BMBF ARM 08/001 and BLR 08/004, the DFG (LI820/17-1, LI 820/15-1, WE 3617/2-1), the Boehringer Ingelheim Fonds, the Erwin-Riesch Stiftung, the Evangelische Studienwerk e.V. Villigst, DAAD (D07/00070 and A0896742) and Prochance 2008 of the Friedrich Schiller University Jena 21007091.

P-CytoG-155

Structural chromosomal aberrations under the light of microarray analysis

Wimmer R.[1], Stuke-Sontheimer A.[1], Nürnberg P.[2], Heling K.-S.[3], Chaoui R.[3], Thiel G.[1]

[1]Praxis für Humangenetik, Berlin Friedrichstraße, Germany, [2]ATLAS Biolabs GmbH, Berlin Friedrichstraße, Germany, [3]Praxis für Pränataldiagnostik, Berlin Friedrichstraße, Germany

Chromosomal analysis sometimes reveal unexpected structural aberrations that urge finer analysis. In former times this analysis was limited by banding resolution and/or a restricted panel of FISH-probes that often could not characterize the karyotyp exactly. Due to the rise of microarray analysis in genetic diagnosis we are now able to gain deeper insights into these structural abnormalities. Here, we want to show some selected pre- and postnatal cases with derivative chromosomes that could be investigated in detail. Finally, we could clarify the underlying chromosomal changes as simple deletions or duplications or as highly complex rearrangements with multiple gains and losses. Microarray analysis is thus an apropriate technique to study the index patients and to define breakpoints and gene content. It helps to assess the recurrance risk in prenatal cases or to establish of easier and cheaper tests for other family members in cases of inherited aberrations.

P-CytoG-156

Molecular and cytogenetic characterization of human interferon transgenic rabbit fibroblasts as a source of nuclei in the somatic cloning.

Wozniak A.[1], Nowak A.[2], Zeyland J.[2], Lipinski D.[2], Slomski R.[2]

[1]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland, [2]Poznan University of Life Sciences, Poznan, Poland

The production of pharmaceutically important human proteins in the mammary gland of transgenic animals constitutes an important field of biotechnology.

In this study rabbits' fibroblasts were transfected by lipofection with transgene, which contained human gene encoding interferon under the control of the tissue specific WAP promoter. Transfected fibroblasts were cultured with selective medium with blasticidine.

Transgene integration was examined by PCR method. Chromosomal aberrations were examined using the GTG-binding pattern.

Transgenic cells are going to be used as a source of nuclei in the experiments of obtaining transgenic goats by somatic cloning technique. The application of tissue specific WAP promoter allows to reduce the expression of the trangene to mammary gland. Human interferon is going to be found only in the milk of animals being accurately in the period of lactation. After the separation of interferon from milk components it would be applied in medical treatment.

P-CytoG-157

Three new cases with small supernumerary marker chromosomes 1 and normal phenotype

Ziegler M.[1], Wegner R.-D.[2], Stumm M.[2], Martin T.[3], Gillessen-Kaesbach G.[4], Kosyakova N.[1], Ewers E.[1], Hamid A.B.[1], von Eggeling F.[1], Hentschel J.[1], Weise A.[1], Liehr T.[1]

[1]Jena University Hospital - Institute of Human Genetics and Anthropology, Jena, Germany, [2]Zentrum für Pränataldiagnostik, Berlin, Germany, [3]Genetische Beratung und klinische Genetik Biomedizinisches Zentrum, Homburg, Germany, [4]Institute of Human Genetics, Lübeck, Germany

Small supernumerary marker chromosomes (sSMC) are still a major problem in prenatal cytogenetic diagnostics. According to the literature, over two third of cases carrying an sSMC derived from chromosome 1 are associated with clinical abnormalities. Here we report three further cases of such sSMC, which did not show any clinical abnormal-

ities. All 3 studied sSMC were detected prenatally and characterized comprehensively for their genetic content by molecular cytogenetics using (sub)centromere-specific multicolor fluorescence in situ hybridization, and for a possibly associated uniparental disomy (UPD). After exclusion of additional euchromatin due to sSMC presence and a UPD, parents opted for continuation of the pregnancies and healthy children were born in all 3 cases. Here the importance of quick and straightforward prenatal sSMC characterization is shown, together with the necessity to collect all available sSMC cases in the sSMC homepage (http://www.med.uni-jena.de/fish/sSMC/00START.htm) hosting now over 3,600 cases. Supported in parts by the DAAD (D07/00070) and the Prochance 2008 of the Friedrich Schiller University Jena 21007091.

P-CytoG-158

Two patients with psychomotor retardation and overlapping de novo microdeletions in 2p14-p15

Zink A.[1], Wohlleber E.[1], Kreiß-Nachtsheim M.[1], Kirchhoff K.[2], Jepsen B.[3], Kjaergaard S.[2], Engels H.[1]
[1]Institute of Human Genetics - Rheinische Friedrich-Wilhelms-University, Bonn, Germany, [2]Department of Clinical Genetics - Rigshospitalet, Copenhagen, Denmark, [3]Department of Pediatrics - Holbæk Sygehus, Holbæk, Denmark

The interpretation of a submicroscopic copy number change detected by molecular karyotyping e.g. in a patient with idiopathic mental retardation can be complicated if no other patient with a similar copy number change and a similar clinical presentation is available for a more reliable genotype–phenotype correlation. International databases such as DECIPHER (http://decipher.sanger.ac.uk/) help to find such similar patients.
Here, we present a boy with a de novo microdeletion in 2p14-p15 with mild to moderate mental retardation concerning especially the language development, and only mild facial dysmorphisms. At the age of 5 11/12 years, he showed relative microcephaly (3rd centile) in relation to his height (75-90th centile) as well as smooth fetal finger pads. At the age of 8 3/12 years, he presented with hypermetropia, mild funnel chest, a slight syndactyly of the 2nd and 3rd toes of the right foot and bilateral atypical simian creases. After two generalized seizures, he received an anticonvulsant therapy. Since ending the anticonvulsant therapy at the age of 6 2/12 years, he has had only one seizure. At the age of 8 8/12 years, sensorineural hearing loss was detected by BERA (Brainstem Evoked Response Audiometry). No other malformations or abnormalities were reported.
Conventional karyotyping revealed a Robertsonian translocation 13/14 inherited from the patients´ healthy mother. Uniparental disomy 14 was excluded. Molecular karyotyping using an Illumina 610k SNP chip detected a 2.23 Mb deletion in 2p14-p15 including 13 genes (Ensembl / Havana genes, Ensembl release 54 May 2009). qPCR confirmed the deletion and demonstrated a de novo occurrence.
A search of the literature yielded only overlapping deletions which were microscopically visible and without additional molecular-cytogenetic work-up. A comparable microdeletion was then located in the DECIPHER database (http://decipher.sanger.ac.uk/). Clinical similarities between the two patients include mild to moderate mental retardation concerning especially the language development and the absence of congenital malformations and severe dysmorphisms. The second patient's 2.84 Mb de novo microdeletion was detected using an Agilent 244k oligo-array and contained 16 genes. The overlapping deleted region of the two patients had a genomic size of 1.6 Mb and contained 11 genes. All known genes contained in the common deleted region show ubiquitous expression. One of the genes (SLC1A4) encodes the solute carrier 1A4 (SLC1A4) which showed a changed expression in various psychiatric phenotypes.
This report illustrates the power of databases like DECIPHER in enabling clinical geneticists and molecular cytogeneticists from different departments to share information about patients with rare microdeletions and thus determine the phenotypic consequences of genomic array findings.

P-PRENATAL DIAGNOSIS, REPRODUCTIVE MEDICINE

P-Prenat-159

Methylation analysis of NDN

Beygo J.[1], Lich C.[1], Kanber D.[1], Horsthemke B.[1], Buiting K.[1]
[1]Institut für Humangenetik, Essen, Germany

The Prader-Willi-Syndrome (PWS) is a neurogenetic disorder caused by loss of function of paternally expressed genes in 15q11q13. These are MKRN3, MAGEL2, NECDIN (NDN), C15orf2, SNRPN and approximately 70 genes coding for small nucleolar RNAs (snoRNAs). The paternal expression of SNRPN and NDN is regulated by allele-specific DNA methylation resulting in an active, unmethylated paternal allele and an inactive, methylated maternal allele.
The parent-of-origin specific methylation of SNRPN is very robust and is used for the molecular diagnostic of PWS. For NDN methylation only few data are available so far. Thus methylation analysis of 39 CpG dinucleotides at the CpG island of the NDN gene was conducted by amplification, cloning and sequence analysis of bisulfite-treated genomic DNA. The methylation analysis of blood DNA from a normal control, a patient with PWS and a paternal deletion of 15q11q13 and a patient with AS and a maternal deletion of 15q11q13 revealed that the paternal chromosome is almost completely unmethylated and the maternal chromosome is partially methylated.
In previous studies it has been shown that methylation at the SNRPN locus is already established in chorion villi and amniotic cells. Since no data for the NDN locus are available for extraembryonic and fetal tissues, methylation analyses of these tissues were performed. For (extraembryonal) chorionic tissue a significant hypomethylation with completely unmethylated and slightly methylated sequences was observed. Analyses by MS-MLPA (methylation-specific multiplex ligation-depended probe amplification) in 16 different chorionic samples also showed a hypomethylation at the NDN locus. Methylation analyses of amniotic cells showed a slight tendency to hypomethylation compared to blood DNA from a normal control. However, such a hypomethylation could not be detected by MS-MLPA of 19 samples tested. These results suggest that the methylation of the NDN locus is not fully established in chorionic tissue. We therefore recommend to interpret only the methylation status of the SNRPN locus in cases of prenatal diagnosis.

P-Prenat-160

Blood chimerism in a girl with Down syndrome and possible freemartin effect leading to aplasia of the Müllerian derivatives

Bogdanova N.[1], Kelsch R.[2], Siebers U.[1], Röpke A.[1], Markoff A.[3], Exeler R.[1], Tsokas J.[4], Wieacker P.[1]
[1]Institute of Human Genetics, Münster, Germany, [2]Insitute of Transfusion Medicine and Transplantation Immunology, Münster, Germany, [3]Institute of Medical Biochemistry, Münster, Germany, [4]St. Franziskus Hospital, Münster, Germany

Cytogenetic and molecular genetic analysis in a case of sex-discordant dizygotic twins revealed blood chimerism in the girl (46,XY in blood and 47,XX,+21 in fibroblasts) caused by feto-fetal transfusion from her healthy brother. The girl presented with Down syndrome, aplasia of the uterus and the Fallopian tubes and normal female external genitalia. We propose that the lack of Müllerian structures is caused by the effect of the Müllerian Inhibiting Substance transferred from the male to the female twin in early pregnancy. This disorder of sex development is known as Freemartin Phenomenon in female cattle from sex-discordant twin pairs.

P-Prenat-161

An add-on of 5 STR-Marker for the standard rapid prenatal QF-PCR to increase time and cost efficiency

Henning K., Glaser M., Hentschel J., Busch A., Weise A.
Institut für Humangenetik und Anthropologie, Jena, Germany

Since 2004 the quantitative fluorescence PCR for rapid diagnosis of common chromosome aneuploidies is a well established procedure. One drawback of the commonly used STR marker set is, that copy number changes of the X chromosome can not be detected if the fetus is female. An additional gap of the currently used sets is that markers for the Down Syndrome Critical Region 2 (DSCR2) of chromosome 21 are not included.
To integrate the additional five STR markers in the existing set, the length standard was moved from ROX to LIZ channel and new markers were stained with ROX.
Two of the new markers are located on the X chromosome and one within the SRY region of the Y chromosome The SRY-Region and two X-chromosomal markers allow, together with the existing amelogenin system, a more thorough sex chromosome differentiation.
Two markers for the phenotypic important Down Syndrome Critical Region 2 (DSCR2) are added to fill the above mentioned diagnostic gap. We tested over 100 prenatal samples (amniotic fluid, chorionic villi or, more rarely, fetal blood) including samples for trisomy X, 13, 18 and 21, Turner-Syndrome (45,X), Klinefelter-Syndrome (47,XXY) and triploidy of cytogenetic confirmed karyotypes respectivly.. All Samples were diagnosed correctly. We achieved for a small additional cost (less than 1 € /sample) a higher reliability for gonosomal aneuploidies and trisomy 21.

P-Prenat-162

Walker-Warburg-Syndrome: the role of haplotype analysis before sequencing in a family with three aborted fetuses

Friedrich-Freksa A.[1], Hehr U.[2], Coerdt W.[3], Seidmann L.[3], Mueller H.[4], Macchiella D.[5], Merz E.[6], Huebner A.[7]
[1]Institute for Clinical Genetics, Mainz, Germany, [2]University of Regensburg, Institute of Human Genetics, Germany, [3]University Medical Center Mainz, Institute of Pathology, Germany, [4]University Medical Center Mainz, Department of Neuropathology, Germany, [5]University Medical Center Mainz, Department of Obstetrics and Gynecology, Germany, [6]Hospital Nordwest, Frankfurt/M, Germany, [7]Children's Hospital, Technical University Dresden, Germany

Walker-Warburg-Syndrome (WWS) is one of the severe autosomal recessively inherited congenital muscular dystrophies accompanied by brain malformations, eye abnormalities and abnormal glycosylation of alpha-Dystroglycan. WWS is genetically heterogeneous in that six causative genes are known to date. Therefore, the search for mutations may be very labor- and cost-intensive.
Here we describe the gynecological, pathological, immunohistochemical findings in a case of WWS diagnosed by ultrasound in the 16. week of pregnancy. This was the third pregnancy terminated due to severe brain malformations with hydrocephalus and hypoplasia of the cerebellum of healthy, not related parents. With haplotype analysis from chorionic villi DNA of the three pregnancies we excluded the genes for MDC1C (FKRP), MDC1D (LARGE), LGMD2O (POMGNT1), LGMD2N (POMT2) and LGMD2M (FCMD) as candidate genes for WWS in this family. However, using markers flanking the POMT1 gene, which maps to chromosome 9q34.13, we found identical parental alleles in all three affected fetuses. Subsequent sequencing of the POMT1 gene revealed a compound heterozygous mutation c.842_844delTCT and c.2168insG in all affected siblings, whereas the parents are healthy heterozygous carriers of one mutation each. Thus a prenatal diagnosis is now possible in this family.
We conclude that in informative families haplotype analysis is still an economic and powerful tool for selecting the appropriate candidate gene(s) for rapid sequencing. Collection of fetal DNA with informed consent of the parents might be helpful for establishing the genetic diagnosis and for genetic counselling of the family.

P-Prenat-163

Prenatal High Resolution Array CGH in Pregnancies with Ultrasound Abnormalities and a normal Karyotype

Kähler C., Auber B., Kerber S., Arps S., Held K.
MVZ genteQ, Hamburg, Germany

Background: Genome-wide high resolution array analysis for the detection of submicroscopic copy number variations (CNVs) is now an adjunct to chromosomal investigations in individuals with mental retardation and congenital anomalies. The use of high resolution array genomic hybridisation analysis in routine prenatal testing remains to be systematically assessed.
Methods: We studied 46 prenatal samples with abnormal ultrasound and a normal karyotype using the Agilent 105 K chip. Detected CNVs were classified using database resources (e.g. Database of Genomic Variants, Decipher, Ecaruca).
Results: A total of 4 pathogenic CNVs (8,7%) were identified: (1) A male fetus with severe IUGR presented with a typical Prader Willi-/Angelman deletion on chromosome 15q11q13. Further investigations showed that the deletion was de novo and occurred on the paternal allele (Prader Willi-Syndrome). (2) A male fetus with microcephaly, holoprosencephaly and midface hypoplasia had a deletion of 16,73 Mb on chromosome 13q32.2q34 and a duplication of 9,9 Mb on chromosome 20p11.23 (unbalanced translocation t(13;20)). (3) In a female fetus with severe IUGR and facial dysmorphisms, a terminal deletion of 17,5 Mb on chromosome 4p was found. (4) In a male fetus with semilobar holoprosencephaly, a deletion of 1,593 Mb on chromosome Xp22.32 was identified. Deletions in this region comprise the STS gene and are associated with X-linked ichthyosis, but semilobar holoprosencephaly was not described until now.
Conclusions: Prenatal array testing in pregnancies with abnormal ultrasound findings and a normal karyotype result identifies (potentially) pathologic CNVs accurately. Sometimes, testing of parents is necessary, and the average detection rate of pathologic CNVs by array CGH in pregnancies with abnormal ultrasound findings remains to be assessed.

P-Prenat-164

Identification of mutation p.S128Y in the FSHR gene in a patient with spontaneous ovarian hyperstimulation syndrome

Ledig S.[1], Steinhard J.[2], Wieacker P.[1]
[1]Institute of Human Genetics, Münster, Germany, [2]Department of Obstetrics and Gynecology, Münster, Germany

Ovarian hyperstimulation syndrome (OHSS) is a serious iatrogenic side effect of hormone stimulation in assisted reproduction. In its most severe form this syndrome involves massive ovarian enlargement and the formation of multiple ovarian cysts, capillary leak with fluid sequestration, renal failure, hypovolemic shock, and in some cases death. Moderate to severe OHSS is rare and is thought to occur in 0,5% to 5% of all ovarian stimulation cycles. However, OHSS rarely can occur in the absence of exogenous gonadotropins during the course of a normal spontaneous conception. Lately, heterozygous mutations in the follicle-stimulating hormone receptor (FSHR) gene have been implicated in the pathophysiology of spontaneous OHSS (sOHSS) by inducing a promiscuous stimulation of FSHR by human chorionic gonadotropin (hCG). To date only few FSHR mutations causing sOHSS have been described. Surprisingly, the majority of these mutations affects the carboxyterminal region of FSHR responsible for signal transduction and not the aminoterminal hormone binding domain.

We performed sequential analysis of the FSHR gene in a turkish patient with sOHSS.
By direct sequencing we were able to identify in this patient heterozygosity for a transversion from C to A at base pair position 383 (mutation c.383C>A). This transversion causes an aminoacid exchange from serin to tyrosin at aminoacid position 128 (mutation p.S128Y) affecting the aminoterminal extracellular domain of the FSHR protein which is implicated in high-affinity hormone binding. Recently, the same mutation has been described in a patient also of turkish descent and with sOHSS. Functional analyses have shown that the mutated protein displays increased affinity toward hCG in contrast to mutations affecting the carboxyterminal region of the FSHR which cause increased sensitivity to both hCG and TSH and furthermore constitutive activity.

P-Prenat-165

Detection of DNA damage in human spermatozoa with the Halosperm®Test (Fa. Gynemed) - Establishment of a new diagnostic tool for routine application in a human genetic laboratory

Mathwig A.[1], Töpfer F.[1], Pfeiffer L.[1], Buske A.[1], Kinzel M.[1], Belitz B.[1]
[1]Praxis für Medizinische Genetik, Berlin, Germany

Approximately 15-20% of males in the western industrial countries are affected by idiopathic infertility. Determining a distinct genetic cause is not possible in those cases and the conventional sperm parameters (motility, morphology, concentration) simply offer an estimation of the male fertility potential. In fact, probably 10-15% of males with normal semen parameters are sterile.
The sperm DNA integrity plays a crucial role for the male fertility. During spermiogenesis a highly compact and complex structure of the chromatin is achieved by protamination of the DNA. The proper condensation of the chromatin permits the transfer of the male genetic information to the oocyte and its contribution onto the next generation. On the other hand it acts as a protection against DNA damage. Abnormalities of the DNA structure or DNA damage in terms of single and double strand breaks (DNA fragmentation) are suggested to negatively influence the male fertility. Based on different studies infertile men possess substantially more spermatozoa with fragmented DNA compared to fertile controls. As a potential risk factor for poor embryo development, reduced embryo implantation and recurrent miscarriages the percentage of DNA fragmentation (DNA fragmentation index, DFI) in an ejaculate is used as an additional and independent marker of sperm quality. The evaluation of damaged sperm DNA in combination with the conventional semen analysis may improve the diagnostic and prognostic approaches in the prediction of pregnancy for couples with male infertility.
Several tests like the Sperm Chromatin Structure Assay (SCSA), the TdT-mediated-dUTP nick end labeling Test (TUNEL) and the Sperm Chromatin Dispersion Test (SCD) are used to investigate DNA fragmentation in spermatozoa. Although each method determines different kinds of DNA damage the prediction according to the different levels of DNA fragmentation is comparable. In our laboratory we established the commercial available Halosperm(r)Test (Fa. Gynemed) an improved SCD test. The method includes an acid denaturation to generate single-stranded DNA segments from DNA breaks and a deproteinization of nuclear proteins. The preparation is stained with 4´6-Diamidin-2´-phenylindol-dihydrochlorid (DAPI). Using fluorescence microscopy the DFI value is estimated by counting 500 spermatozoa for each sample. Spermatozoa with intact DNA show big or medium-sized DNA loops (halos) dispersing from a central nuclear core. Sperms with fragmented DNA show a small halo or exhibit no halo with a solid or a faint staining of the core. The DNA fragmentation index is classified into three categories. DFI values =15% are consistent with a healthy male fertility. DFI values >15%-<30% correspond to a restricted male fertility indicating the performance of an intrauterine insemination (IUI). DFI values =30% are pathologic. The initiation of a pregnancy by natural conditions is hardly expected and assisted reproductive treatment (ART) including in-vitro-fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) is suggested.
In cooperation with „Praxisklinik für Fertilität am Gendarmenmarkt" in Berlin and „Berliner Samenbank" we have investigated more than 30 ejaculates of males in preparation for ART since May 2009. Predominantly we expect an enhanced selection of patients with normal semen parameters and low DNA fragmentation for an effective application of the IUI. Our prospect is to avoid hopeless therapies as well as to explain failed infertility treatment.

P-Prenat-166

Identification of two active choline-esterases in human ovary: Elevated choline-esterase activity in follicular fluid of women suffering from polycystic ovary syndrome

Schmidt T.[1], Kunz L.[2], Dissen G.A.[3], Ojeda S.[3], Berg D.[4], Berg U.[4], Wessler I.[5], Mayerhofer A.[2]
[1]Department of Human Genetics, Essen, Germany, [2]Department of Cell Biology, University of Munich, Germany, [3]Division of Neurosciences, Oregon National Primate Research Center, U.S.A., [4]Assisted Reproductive Technologies Bogenhausen, Munich, Germany, [5]Department of Pathology, University of Mainz, Germany

Locally produced factors in the ovarian follicular compartment coordinate follicular development. Acetylcholine (ACh) may be such a local factor produced by granulosa cells of the human ovary. ACh regulates granulosa cells via muscarinic receptors thereby altering ion channels, gap junctions and transcription factors. How the action of ACh is terminated is not known, but in neuronal and non-neuronal tissues two prinicipal types of cholinesterases degrade ACh. We studied expression and activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in human and monkey ovary, in cultivated human granulosa cells (GCs) and follicular fluid of patients suffering from PCOS (Polycystic ovary syndrome) and non PCOS patients. AChE and BChE proteins and mRNAs were present in granulosa cells of growing follicles of human and monkey ovaries in distinct patterns and in isolated cultivated human GCs. The activities of AChE and BChE were higher in PCOS-derived follicular fluids and in cultivated GCs from PCOS patients than in non PCOS samples. Levels of AChE in GCs obtained from PCOS-patients and in GCs stimulated with hCG, respectively, were elevated over values in normal or untreated samples, implying regulation by LH/hCG. Finally, ACh levels in follicular fluid of PCOS patients tended to be lower than in non-PCOS controls.
We conclude that the cholinesterases BChE-and AChE are present and active in human ovarian follicles and follicular fluid, where they most likely control the level of ACh. Other explored actions are possible, but higher activities in PCOS results in lower ACh levels and suggest that downstream actions could differ. This implies that the cholinergic system is involved in the pathogenesis or maintenance of the PCOS phenotype in women. (Support Friedrich-Baur Stiftung; DFG)

P-Prenat-167

Gaucher's disease with a hydropic phenotype – differentialdiagnosis and mutation analysis

Schoner K.[1], Bald R.[2], Meyer-Kleine C.[3], Rehder H.[4]
[1]Institut für Pathologie; Universitätsklinikum Giessen und Marburg GmbH, Marburg, Germany, [2]Klinikum Leverkusen GmbH; Frauenklinik; Pränatalmedizin, Leverkusen, Germany, [3]Centrum für Humangenetik, Linden, Germany, [4]Institut für Pathologie; Universitätsklinikum Giessen und Marburg GmbH; Marburg; Germany und Department für Medizinische Genetik, Med. Universität Wien, Austria

Aims: Gaucher's disease is an autosomal recessive lysosomal storage disorder caused by a deficiency in the enzyme glucocerebrosidase. This deficiency results in accumulation of monocytes and macrophages and thus produces multiple organ and skeletal complications. Presentation

and age at symptom onset are highly variable. Three disease states have been classified based on presence and severity of neurological involvement. We present an extreme phenotype in a perinatal lethal hydrops and give an overview of differential diagnosis concerning nonimmune hydrops fetalis (NIHF).
Methods: Fetal sonography revealed severe NIHF of unknown origin in a male fetus of consanguineous Turkish parents. Prenatal serological analysis did not confirm suspected CDG syndrome. Because of developing preeclampsia the pregnancy was terminated at 33 gestational weeks and autopsy was performed.
All exonic and intronic sequences were selectively amplified. Cycle sequencing was accomplished using the Dye Terminator Cycle Sequencing kit (ABI) and all mutation were confirmed by sequencing using both forward and reverse primers.
Results: Postmortem examination revealed a thick, collodion like skin and pronounced hepatosplenomegaly. Histology evidenced abundant PAS-positive storage cells of wrinkled tissue paper appearance in multiple organs, compatible with Gaucher cells. Thus we suspected severe Type 2 Gaucher disease resulting in a collodion baby phenotype. Mutation analysis of the glucocerebrosidase gene in 1q21 (GBA, 606463) confirmed our diagnosis. The RecNciI gene conversion (c.1389 -68 T>C; c.*92 G>A) was detected homozygot.
Conclusions: Acute neuronopathic Gaucher's disease (type 2) is classically considered to be a disease of late infancy, but also includes a spectrum of variant phenotypes such as perinatal lethal hydrops, or the collodian baby phenotype in the newborn period. Post mortem examination including goal directed application of molecular techniques are required for the final diagnosis. Homozygosity for a recombinant allele was always associated with prerinatal lethality. The clinical diagnosis of a severe Type 2 Gaucher disease was confirmed by molecular techniques.
This case exemplifies the manifestation of a storage disease in early fetal period due to lack of maternal compensation of the fetal enzyme deficiency. On the other hand it underlines the need for an interdisciplinary cooperation and thereby the interdisciplinary status of the fetal pathologist.

P-Prenat-168
Polar body aneuploidy screening in IVF; 12 hours from single cell to arrayCGH result.
Shelley P.[1], Brown A.[1], Haan N.[1], Snudden G.[1], Gordon T.[1]
[1]BlueGnome, Cambridge, UK

Advanced maternal age (over 37yrs) severely reduces pregnancy rates from natural conception and IVF. It is believed a major cause of reduced pregnancy rates at advanced maternal age are increased rates of oocyte and embryo aneuploidy (Hassold et al 2007). A reliable test for aneuploidy in this group is desirable, since it is logical that pregnancy rates will be increased by only fertilising euploid oocytes (Sher et al 2007). The traditional method of pre-implantation genetic screening (PGS) utilises fluorescence in-situ hybridisation (FISH). However this method targets selected chromosomes and can maximally test only half the chromosomes of the cell's complement. In contrast array comparative genomic hybridisation (CGH) measures copy number across the whole genome; however single cell arrayCGH has been hindered by technical difficulties. Here we present a robust technique for single cell aneuploidy screening in polar bodies, blastomeres and trophectoderm biopsies which can be performed in 12 hours from receipt of sample.
Single cells are collected into PBS and transported at 4C. On receipt the cell is lysed and its DNA amplified using the SurePlex amplification system (2.5 hours). Amplified DNA is labelled with fluorescent dyes; sample with cyanine 3 and SureRef control with cyanine 5 (3 hours). Labelled DNA is hybridised on a 24sure BAC microarray (3 hours), hybridised slides are washed, scanned and analysed for copy number change (3 hours).
Data from BlueGnome's 24Sure service lab produced by analysis of first polar body (n=291) collected at a number of IVF centres across the UK shows an amplification success rate and QC pass of 90% (261 polar bodies). Assay failure is caused by absence of amplification products, which is presumed to be the result of insufficient or poor quality genomic DNA in the first polar body (e.g. from fragmented polar bodies). Of the 261 first polar body samples that passed QC; 192 were aneuploid and 69 were euploid (26%).
The subjects in this cohort have an average age of 41 years and a history of failed IVF cycles. Correspondingly they have a predicted IVF success rate of only 5-7%, which could be accounted for in part by the high levels of aneuploidy observed. On-going pregnancy rates and birth rates in this cohort will be discussed. Polar bodies screened in this way have lead to the first child to a couple with an exceptionally high IVF failure rate in 2009 (Fishel et al 2009).

References
Hassold T. et al. 2007 The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet. Oct 15.
Sher G. et al. 2007. Oocyte karyotyping by comparative genomic hybridization provides a highly reliable method for selecting „competent" embryos, markedly improving in vitro fertilization outcome. Fertil Steril. May 87.
Fishel S. et al. 2009. Live birth after polar body array comparative genomic hybridization prediction of embryo ploidy-the future of IVF? Fertil Steril. Nov 23.

P-Prenat-169
Do copy number variants play a role in spermatogenic failure and male infertility?
Tüttelmann F.[1], Kliesch S.[2], Gromoll J.[2], Simoni M.[3], Dworniczak B.[1], Wieacker P.[1], Röpke A.[1]
[1]Institute of Human Genetics, University of Münster, Germany, [2]Centre of Reproductive Medicine and Andrology, University of Münster, Germany, [3]Department of Medicine, Endocrinology and Metabolism, University of Modena and Reggio Emilia, Italy

Introduction: Male infertility accounts for about 50% of couple infertility with the most prevalent phenotype of reduced sperm production. The causes for the azoo- or severe oligozoospermia remain frequently obscure with around 30% of idiopathic infertility and 40% with not sufficient/uncertain causes (e.g. varicocele). We hypothesise that the number or specific patterns of CNVs may disturb chromosome synapsis during meiosis and cause meiotic incompetence responsible for reduced sperm output. On the other hand, specific CNVs might de-mask mutations in individual genes important for spermatogenesis.
Subjects and Methods: Men with idiopathic infertility (where known causes had been ruled out) were selected retrospectively from the patient clientele of the Centre of Reproductive Medicine and Andrology. So far, DNA extracted from peripheral blood of 64 controls with normal spermatogenesis and 43 patients with severely reduced sperm concentration/count have been analysed by array CGH using the 244A Array Set (Agilent Technologies).
Results: The mean number of CNVs of 9.9±2.7 in patients and 10.6±3.0 in controls was comparable. Neither was the mean quantity of DNA gains and losses different between patients and controls. The distribution of CNVs according to their size was equivalent in both groups. When only patient specific CNVs (not found in controls) were analysed, an overrepresentation of X-chromosomal variants, mostly duplications, became obvious. Presently, no recurring patients-specific CNVs have been found.
Conclusions: To date, no indication for a substantial involvement of CNVs in the pathogenesis of male infertility could be established. The overrepresentation of X-chromosomal CNVs requires further investigation. Currently, the numbers of patients and controls are being increased to allow more in-depth analyses (e.g. cluster analysis of semen and hormone parameters).
The study was supported by the Deutsche Forschungsgemeinschaft Research Unit ‚Germ Cell Potential , (FOR 1041/TU 298/1-1).

P-MOLECULAR AND BIOCHEMICAL BASIS OF DISEASE

P-MoleG-170

Gene expression profiling and immunohistochemical analysis of 3D skin models mimicking a syndrome of ichthyosis, follicular atrophoderma, and hypotrichosis

Alef T.[1], Eckl K. M.[1], Metze D.[2], Hausser I.[3], Türsen Ü.[4], Lestringant G. G.[5], Hennies H. C.[1]

[1]Univ. of Cologne; Cologne Center for Genomics; Div. of Dermatogenetics, Cologne, Germany, [2]Univ. Hospital Münster; Dept. of Dermatology, Münster, Germany, [3]Univ. Hospital Heidelberg; Dept. of Dermatology, Heidelberg, Germany, [4]Mersin University; Dept. of Dermatology, Mersin, Turkey, [5]Tawam Hospital, Al Ain, United Arab Emirates

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 phenotype of ichthyosis, follicular atrophoderma, and hypotrichosis from the United Arab Emirates and Turkey, respectively. After genome wide linkage analysis we identified mutations in the suppression of tumorigenicity 14 gene (ST14). We found a homozygous splice site mutation (c.2269+1G>A) in the Emirati patients and a 1-base deletion (c.2034delG) in the Turkish patient. Western blot analysis showed reduced proteolytic activation of prostasin (PRSS8) and loss of processing of profilaggrin (FLG). Since filaggrin monomers play a pivotal role in epidermal barrier formation, we suggest that matriptase, the product of ST14, 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 this syndrome, we have established 3D skin models with primary keratinocytes and fibroblasts from patients and healthy donors. The primary control keratinocytes were transfected with siRNA to knock down expression of ST14, PRSS8, and FLG, respectively. After eight days, the 3D skin models were harvested and the knock down efficiency was tested by quantitative RT-PCR. Samples taken from these 3D skin models were further analyzed by gene expression profiling, western blotting, and immunohistochemical methods to elucidate the enzymatic cascade downstream of matriptase, and approve potential interaction partners of matriptase such as serine proteases of the kallikrein family, urokinase-type plasminogen activator (uPa), stromelysin (MMP-3) or hepatocyte growth factor (HGF).

P-MoleG-171

LCoR acts as androgen receptor corepressor, inhibits prostate cancer growth and is functionally regulated by Src kinase in vivo

Baniahmad A.[1], Asim M.[1], Hafeez B.B.[2], Siddiqui I.A.[2], Gerlach C.[1], Patz M.[1], Mukthar H.[2]

[1]Institute of Human Genetics and Anthropology, Jena, Germany, [2]Deparment of Dermatology, Wisconsin, USA

Constitutively active androgen receptor (AR) function is a hallmark of advanced prostate cancer (PCa). Mutations or overexpressed human AR lead to therapy-resistant PCa. The activation function of AR is, however, repressed by corepressors. Here we identified the corepressor LCoR as a novel androgen-dependent corepressor for the AR, which is able to suppress human PCa growth. Both AR and LCoR are co-recruited to chromatin at the AR target gene PSA in response to agonist in vivo associated with gene repression indicative of regulation at epigenetic level. LCoR leads to decreased AR-mediated transactivation independent of the nuclear receptor interacting domain LXXLL. Interestingly, the non-receptor tyrosine kinase Src, often activated in advanced PCa, abrogates repressive function of LCoR.
In mouse xenograft model, forced expression of LCoR decreased prostate tumor growth. Interfering with endogenous Src function by coexpressing a dominant negative Src mutant, the growth inhibitory activity of LCoR could be enhanced potently in vivo. In line with this, progressive decrease in LCoR expression was observed in both TRAMP mouse model and also human PCa during increasing PCa stages. Also, a decrease of LCoR expression was associated with human PCa of tissues with increasing Gleason score. Our studies uncover LCoR as an important physiological corepressor for ligand-activated AR in vivo that inhibits PCa growth and one role of Src kinase for stimulation of PCa proliferation is the attenuation of LCoR.

P-MoleG-172

Multilocus hypomethylation in Silver-Russell syndrome: does tissue-specific distribution of epigenetic mosaicism explain the phenotype?

Begemann M.[1], Spengler S.[1], Baudis M.[2], Kanber D.[3], Markus S.[4], Fricke-Otto S.[5], Siebert R.[6], Buiting K.[3], Eggermann T.[1]

[1]Institute of Human Genetics, Aachen, Germany, [2]Institute of Molecular Biology, Zurich, Switzerland, [3]Institute of Human Genetics, Essen, Germany, [4]Praxis für Humangenetik, Regensburg, Germany, [5]Helios-Klinikum, Krefeld, Germany, [6]Institute of Human Genetics, Kiel, Germany

In all known congenital imprinting disorders an association with aberrant methylation or mutations at specific loci was well established. It was therefore amazing that patients with transient neonatal diabetes mellitus and Beckwith-Wiedemann syndrome (BWS) can exhibit multilocus hypomethylation (MLH). Recently, single BWS and Silver-Russell syndrome patients with the same MLH pattern affecting paternally as well maternally imprinted genes in blood lymphocytes have been reported. Phenotypically, these patients exhibit the characteristic syndrome-specific features. However, the reason why patients with similar epigenetic alterations in leukocytes either show the SRS or the BWS phenotype is currently unknown. We now report on the molecular findings in blood and buccal swab DNA in three SRS patients with hypomethylation of both imprinting center regions (ICRs) in 11p15. Whereas this aberrant methylation affected both ICRs in leukocytes, in buccal swab DNA only ICR1 hypomethylation was visible in two patients. In the non-affected monozygotic twin of one of these patients aberrant methylation was also present in leukocytes but not in buccal swab DNA. Despite screening of several factors involved in establishment and maintenance of methylation marks including ZFP57, the molecular clue for the MLH in our patients remained unclear. However, our data provide evidence that in case of MLH the epimutation which is predominant in tissues others than blood is causative for the phenotype and therefore explains the clinical outcome.

P-MoleG-173

Identification of a novel recurrent type of NF1 microdeletion spanning 1Mb and with breakpoints located in the LRRC37B duplicons (NF1-REPs) at 17q11.2

Bengesser K.[1], Tinschert S.[2], Wimmer K.[3], Steinmann K.[1], Kluwe L.[4], Mautner V.M.[4], Kehrer-Sawatzki H.[1]

[1]Institute of Human Genetics, Ulm, Germany, [2]Institute of Clinical Genetics, Dresden, Germany, [3]Department of Medical Genetics, Innsbruck, Austria, [4]Department of Maxillofacial Surgery, Hamburg, Germany

Microdeletions encompassing the Neurofibromatosis type-1 (NF1) gene and its flanking regions belong to the group of genomic disorders that is caused by aberrant recombination between segmental duplications. The most common NF1 microdeletions span 1.4 Mb and have breakpoints located within the NF1-REPs A and C, which are large duplicons (or low-copy repeats) located within 17q11.2. These duplicons encompass the LRRC37B gene and its pseudogene sequences. NF1-REP A spans 128kb and is located 366kb proximal to the NF1 gene, whereas NF1-REP C is 75kb in length and located 643kb distal to the NF1 gene. During our ongoing attempt to characterize NF1 microdeletions, we have discovered a new type of recurrent NF1 microdeletion. The proximal breakpoints of these 1Mb deletions occur within another copy of

the NF1-REPs, termed NF1-REP B, which spans 45kb and is located 44kb proximal to the NF1 gene. The distal deletion breakpoints of these 1Mb NF1 microdeletions are located within NF1-REP C. Three examples of this 1Mb NF1 deletion have been found to occur de novo in our cohort of NF1 patients. We characterized the breakpoints of all three microdeletions at the highest possible resolution and established that the recombination breakpoints lie within the LRRC37B sequences in a region characterized by several hundred base-pairs of absolute sequence identity. Our analysis indicates that non-allelic homologous recombination (NAHR) between the highly homologous low-copy repeats NF1-REP B and NF1-REP C is the mechanism underlying this novel type of recurrent NF1 microdeletion (which leads to the loss of 9 genes including the NF1 gene).
Our findings also established the highly recombinogenic potential of the LRRC37B sequences located at 17q11.2. These sequences are members of the LRRC37 gene family that includes the LRRC37 core duplicons belonging to one of the most abundant and rapidly evolving low-copy repeat sequence families in the human genome. LRRC37 core duplicon sequences also flank the breakpoints of the 970-kb inversion of the MAPT (microtubule-associated protein tau) locus at 17q21.3. This locus occurs in humans as two distinct haplotypes, H1 (direct orientation) and H2 (inverted orientation), which display no recombination between them over a distance of 1.5 Mb. The two haplotypes differ in terms of their functional impact: whereas the H1 haplotype has been associated with neurological disorders including Alzheimer disease and amyotrophic lateral sclerosis, the H2 haplotype appears to predispose to recurrent microdeletions associated with the 17q21.31 microdeletion syndrome. The involvement of LRRC37 duplicons in NAHR events leading to different types of recurrent NF1 microdeletion demonstrated in the current study confirms the potential of LRRC37 sequences to promote instability in the human genome.

P-MoleG-174

Additional hypomorphic mutations in different PKD genes may explain early and severe polycystic kidney disease

Bergmann C.[1], Ortiz Brüchle N.[2], von Bothmer J.[2], Venghaus A.[2], Zerres K.[2]
[1]Bioscientia Center for Human Genetics Ingelheim and Department of Human Genetics, RWTH Aachen University, Germany, [2]Department of Human Genetics, RWTH Aachen University, Germany

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian human disorders with a frequency of 1/400-1000 and one of the leading causes for end-stage renal disease. Typically, ADPKD is a late-onset disease caused by germline mutations in PKD1 or PKD2. About 2% of ADPKD patients show an early and severe phenotype with considerable perinatal morbidity and mortality that can be clinically indistinguishable from the recessive form of polycystic kidney disease (ARPKD) with PKHD1 mutations. In addition, we and others could recently show that patients with HNF1ß mutations can mimic polycystic kidney disease. Underlying mechanisms for early and severe clinical courses are still unknown, reflected by gross phenotypic variability among affected family members (thought to carry the same mutational load). The high recurrence risk for further children with early and severe disease expression strongly suggests a common familial modifying background. Here, we present unpublished data of patients with early and severe polycystic kidney disease who carry mutations in different PKD genes (PKD1, PKD2, HNF1ß, PKHD1) in trans and de novo in addition to their expected germline mutations. We postulate that these alterations aggravate the phenotype and contribute to the early and severe clinical course of these individuals. Our findings provide further insight into the pathogenesis of polycystic kidney disease and suggest a general concept for the modification of disease expression in other so-called monogenic disorders.

P-MoleG-175

New mutations in the CXORF5 (OFD1) gene, including the deletion of the whole gene, in patients with Type I Orofaciodigital Syndrome

Bisschoff I.J.[1], Zeschnigk C.[1], Horn D.[2], Wellek B.[3], Kunstmann E.[4], Tyshchenko N.[5], Linné M.[6], Lechner H.[7], Evers C.[8], Wolff G.[1], Morris-Rosendahl D.J.[1]
[1]Institut für Humangenetik, Universitätsklinik Freiburg, Germany, [2]Institut für Medizinische Genetik Charité, Berlin, Germany, [3]Institut für Humangenetik, Johannes-Gutenberg-Universität Mainz Klinikum, Germany, [4]Zentrum für Medizinische Genetik, Würzburg, Germany, [5]Institut für Humangenetik und Anthropologie, Technische Universität Dresden, Germany, [6]Gemeinschaftspraxis Humangenetik, Dresden, Germany, [7]Praxis für Kinderheilkunde, Prien am Chiemsee, Germany, [8]Genetische Poliklinik, Heidelberg, Germany

Thirteen forms of Orofaciodigital Syndrome (OFDS) have been described, however CXORF5 (OFD1, Xp22.3-p22.2) is currently the only known causative gene, in which mutations cause OFD type I (OFD1, OMIM: 311200). Mutations in OFD1 also cause X-linked Joubert Syndrome and Type 2 Simpson-Golabi-Behmel Syndrome. OFD1, which is now recognised as a ciliopathy, is characterized by malformations of the face, oral cavity, and digits and is transmitted as an X-linked dominant condition with lethality in males. Polycystic kidney disease appears to be specific to OFD1, with 37% of patients affected. There may be central nervous system involvement in as many as 48% of cases. We have studied 42 sporadic and four familial cases of suspected OFD1. Mutation analysis was performed using DNA sequencing and quantitive PCR (qPCR). Mutations were found in 20 female patients, 13 of the mutations have not been previously described. Twelve mothers of patients were tested for their carrier status, only four mothers were found to carry the mutation in their daughter. One female patient is heterozygous for a deletion spanning the entire OFD1 gene. The deletion includes genes 5' of OFD1 and is a maximum of 600 kb in size. The latter patient is severely mentally retarded and presented with self-mutilation. The OFD1 gene apparently escapes X-inactivation, however it is not clear whether the escape from inactivation is complete. X-inactivation studies were performed in three families in order to investigate the extensive intrafamilial variation between females carrying OFD1 mutations. Despite the observation of substantially skewed X-inactivation, the direction of skewing did not correlate with disease severity. Therefore X-inactivation can be excluded as the main cause of clinical variability in OFD1.

P-MoleG-176

Fine-tuning of insulin secretion

Brixel LR.[1], Ingenbrandt CS.[1], Fleig A.[2], Penner R.[2], Enklaar T.[1], Zabel BU.[3], Prawitt D.[1]
[1]Center for Pediatrics and Adolescent Medicine, Mainz, Germany, [2]Center for Biomedical Research at The Queen's Medical Center and John A. Burns School of Medicine, Honolulu, USA, [3]Center for Pediatrics and Adolescent Medicine - Pediatric Genetics Section, Freiburg, Germany

Elevated plasma glucose levels induce the secretion of insulin from beta-pancreatic cells via the coordinated alteration of cellular ion concentrations and a substantial membrane depolarization. Although known in principle, the exact mechanisms underlying the cycles of glucose induced insulin secretion remain mostly elusive. Current knowledge argues for the involvement of additional ion channels in this process.
One ion channel fulfilling the requirements for participating in this scenario is TRPM5: it is active in pancreatic beta-cells, forms a functional calcium-activated non-selective cation (CAN) channel, that conducts mainly Na+- and K+- without significant permeation for Ca2+-ions and upon activation leads to membrane depolarization. We show that suppression of TRPM5-function in beta-pancreatic cells leads to impaired insulin secretion during hyperglycemia. This effect can be detected both in pancreatic islets and the whole organism. Our data sug-

gest, that the task of TRPM5 is not only membrane depolarization, but seems to be an indispensable part of the insulin secretion process. This makes TRPM5 an attractive novel candidate for diagnosis and therapy of particular types of insulin secretion defects.

P-MoleG-177

Differential Allelic Expression of genes associated with Asthma

Burkhardt J.[1], Kirsten H.[2], Wolfram G.[3], Quente E.[3], Ahnert P.[4]
[1]Universität Leipzig, Leipzig, Germany, [2]Fraunhofer IZI and TRM Leipzig, Leipzig, Germany, [3]TRM Leipzig, Leipzig, Germany, [4]IMISE Leipzig, Leipzig, Germany

Objective: Research on genetic factors defining individual susceptibility to complex disease traits is an ongoing issue across many fields of clinical medicine. An important question is the functional relevance of an identified association of a genetic factor with a disease. In humans, differential allelic expression (DAE) has been estimated to affect 20–50% of genes. DAE is found when the expression level of a gene is different in respect to the allele of a certain genetic polymorphism. Cis-regulated DAE is characterized by the observation that the same allele always has the same, either increasing or decreasing, effect on the expression level of the gene. DAE can be measured directly by genotyping coding SNPs in cDNA samples of heterozygous individuals e.g. by mass spectrometry. If cis-regulation is found, the observed allele might have an effect on efficiency of transcription, mRNA stability and/or processing.
Previously, genetic polymorphisms (SNPs) in the coding region of IL13 and CSF2, two genes located in a 5q31 cytokine cluster, were found to be associated with asthma. We investigated whether those cSNPs are correlated with expression of a specific allele and whether this allele is differentially expressed in a cis directed manner. Results could provide a functional link between a specific genetic variants and a specific pathological mechanism related to asthma.
Methods: One coding SNP in IL13 and one in CSF2 were investigated. About 50 immortalized B cell lines were screened for heterozygous genotypes of these SNPs. Genomic DNA (gDNA), RNA and subsequently complementary DNA (cDNA) was isolated from those cells. To identify DAE, a mass-based genotyping system was used for quantitative genotyping of both alleles within a heterozygous sample. Significant derivation of allelic ratios between cDNA and gDNA samples indicated allelic regulation. Similar direction of intra-sample effects for a given SNP indicated cis-regulation.
Results: Both SNPs showed significant DAE (p[IL13] = 0.002, p[CSF2] = 0.01).
For the Il13 SNP 6 of 11 heterozygous samples showed significant differential allelic expression. Within those 6 samples, the minor A allele was constantly overexpressed, outside of fluctuation of allelic ratios in genomic DNA. On average expression of the A allele was about 22% above expression of the major G allele. Thus, the effect clearly was cis-directed. We could also verify our findings in two regulated samples via fluorescence based sequencing.
For CSF2 of the 8 heterozygous samples 5 were significantly differentially expressed with the major T allele being constantly overexpressed. On average the T allele was expressed about 38% more then the C allele. Again, the effect could be verified by sequencing of two samples.
Discussion: Expression of a cis regulated gene might for example be influenced by a sequence change in a regulatory DNA binding site, altering the affinity with which a regulatory protein (transcription factor) is recruited. In our study we could detect pathological relevant cis-directed DAE in genes associated with asthma. For IL13, higher expression in the presence of the minor allele of the investigated SNP might contribute to the known increase in functional activity of this IL13 isoform. For CSF2 the minor allele of the investigated SNP previously showed a genetic risk of susceptibility to asthma. This allele was also significantly higher expressed compared to the major allele. It might be speculated that genetically entailed elevated CSF2 expression promotes macrophage and neutrophil function during inflammatory reactions which is linked to increased inflammatory events in asthma.
Overall, analysis of allele-specific gene expression of markers associated with diseases proves a efficient approach for elucidating possible functional relevance of these genetic disease markers.

P-MoleG-178

A missense variant in the MTM1 gene associated with X-linked myotubular myopathy in Labrador retrievers

Böhm J.[1], Beggs A.H.[2], Snead E.[3], Kozlowski M.[2], Minor K.[4], Tiret L.[5], Childers M.K.[6], Taylor S.M.[3], Mickelson J.R.[4], Guo L.T.[7], Mizisin A.P.[7], Shelton G.D.[7], Laporte J.[1]
[1]IGBMC, Strasbourg, France, [2]The Manton Center for Orphan Disease Research at Children's Hospital and Harvard Medical School, Boston, USA, [3]Western College of Veterinary Medicine, University of Saskatchewan, Canada, [4]School of Veterinary Medicine, University of Minnesota, USA, [5]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France, [6]Wake Forest University, Winston-Salem, USA, [7]School of Medicine University of California, La Jolla, USA

Centronuclear myopathies (CNM) are a group of rare genetic disorders characterized by muscle weakness and centralized nuclei in muscle fibers. The most severe form is linked to mutations in the phosphoinositides phosphatase myotubularin (MTM1), milder etiopathologies are associated to mutations in BIN1 and DNM2. There is no therapy to date and animal models are a prerequisite to test potential approaches. We report here the molecular identification of a canine model for X-linked centronuclear myopathy. Five young male Labrador retrievers from three litters were evaluated for generalized weakness and muscle atrophy. Cryostat sections and ultrastructural studies on muscle biopsies showed variability in fiber size, centrally placed nuclei resembling fetal myotubes, abnormal perinuclear structure and mitochondrial accumulations. Triads were infrequent with an abnormal orientation of T tubules and immunofluorescence staining using antibodies against T tubules (DHPR alpha 1) and adjacent sarcoplasmic reticulum (RYR1) confirmed an abnormal distribution of these structures. DNA analysis of the exonic sequences from the MTM1 gene in all five affected males revealed a unique variant in exon 7 causing the non-conservative missense change c.465C>A (N155K) in the linker region between the GRAM-PH and phosphatase domains of myotubularin. Immunoblot analysis using anti-myotubularin antibodies showed that myotubularin protein was absent in muscle extracts from affected dogs but present in muscle from a control littermate. Two proven carrier dams were shown to be heterozygous for the mutation. Analysis of a world-wide panel of 237 unrelated and unaffected Labrador retrievers, and 59 additional control dogs from 25 breeds, failed to identify this variant, strongly indicating it is the pathogenic mutation responsible for the canine myopathy.

P-MoleG-179

Functional analysis of two novel BIN1-mutations in autosomal recessive centronuclear myopathy

Böhm J.[1], Tosch V.[1], Yis U.[2], Claeys K.[3], Stojkovic T.[3], Nicot A.S.[1], Toussaint A.[1], Mandel J.L.[1], Laporte J.[1]
[1]IGBMC, Strasbourg, France, [2]Dokuz Eylül University School of Medicine, Izmir, Turkey, [3]Institut de Myologie Groupe Hospitalier Pitié-Salpêtrière, Paris, France

Centronuclear myopathies are a group of congenital disorders characterized by hypotonia and skeletal muscle biopsies typically showing atrophic fibers with central nuclei. Associated disabilities are delayed motor milestones and respiratory complications. Other clinical features as high arched palate, long digits, bell shaped chest and long face have also been observed. Mutations in MTM1 cause the X-linked neonatal form, which involves a very severe and generalized muscle weakness.

Although a few milder cases were described, the affected newborn males have a strongly reduced life expectancy due to an increased risk of respiratory failure. The autosomal recessive (ARCNM, childhood onset) and dominant (ADCNM, adult onset) forms are less common and less severe and result from mutations in BIN1 and DNM2, respectively. All three genes are implicated in membrane trafficking: Myotubularin (encoded by MTM1) is a phosphoinositide phosphatase, Amphyphisin 2 (BIN1) induces membrane curvature via its BAR-domain and recruits Dynamin 2, essential for tubulation and endocytosis. To date, only 3 BIN1 mutations have been reported and here we present the functional analysis of two novel BIN1 mutations and the clinical description of the respective index patients. The p.R154Q missense and the p.Q573X nonsense mutations are in direct spatial proximity to the previously described p.D151N and p.K575X mutations, indicating putative hot spots prone to mutations which might be correlated with particular phenotypes as the nonsense mutations seem to involve a more severe phenotype.
In the cell culture model we could demonstrate that the constructs harboring the nonsense mutations result in stable truncated proteins, but the functionally important interaction with Dynamin 2 and its recruitment to the membrane tubules is lost. In contrast, the missense mutations in the BAR domain abrogate membrane tubulation in transfected cells. This clearly shows that membrane remodeling is necessary for normal muscle function. Our results are important for genetic counselling, and downstream applications as mice models and therapeutic approaches are currently under investigation in our team.

P-MoleG-180
Disruption of the dynein heavy chain-like 1 (DNHL1) gene in a girl with 46,XX,t(2,12)(p11.2;p11.2) and hearing impairment
Damatova N.[1], Beyer V.[2], Zechner U.[2], Poot M.[3], Keilmann A.[4], Bartsch O.[2], Haaf T.[1]
[1]Institute of Human Genetics, Würzburg, Germany, [2]Institute of Human Genetics, Mainz, Germany, [3]Department of Medical Genetics, Utrecht, The Netherlands, [4]Department for ENT and Communication Disorders, Mainz, Germany

Here we report on a girl with moderate unilateral hearing impairment, single transverse palmar crease, and clinodactyly of fingers DIII/DIV. In early childhood she suffered from recurrent inflammations of the middle ear, which were treated with a ventilation tube. Because of incoordination she underwent ergotherapeutic treatment. Complete sequencing of the GJB2 gene did not yield any mutations. Cytogenetic analysis of the girl revealed a balanced translocation 46,XX,t(2,12)(p11.2;p11.2)pat, which was also found in her father and brother, who did not show phenotypic abnormalities. Genome-wide screening for chromosomal imbalances with the Infinium HumanHap370 Genotyping BeadChip SNP Array detected a 0.1 Mb duplication in the chromosome 12p11.2 breakpoint region. FISH mapping with region-specific BAC probes and long-range PCR products narrowed the chromosome 2p11.2 breakpoint down to a <100 kb interval, disrupting the dynein heavy chain-like 1 (DNHL1) gene. The chromosome 12p11.2 breakpoint lies in an intergenic DNA segment. Dynein defects are known to cause the immotile cilia syndrome (also known as primary ciliary dyskinesia). Among other symptoms, patients with immotile cilia suffer from recurrent ear infections, similar to our patient. Motility of cilia is crucial for normal assembly and localization of the otoliths in the vestibular labyrinth of the cochlea, which are responsible for coordination and balance. We propose that biallelic inactivation of DNHL1 may cause hearing impairment. It is conceivable that the second copy of the DNHL1 gene in our patient is inactivated by a classic genetic or epigenetic mutation, whereas the healthy translocation carriers in the family are endowed with one disrupted and one wildtype allele. Sequencing of DNHL1 in the patient, her brother and her father is underway.

P-MoleG-181
Evaluation of the HEXB gene in patients with Morbus Sandhoff
Dekomien G.[1], Akkad AD.[1], Hoffjan S.[1], Lefeber DJ.[2], Epplen JT.[1]
[1]RUB Human Genetics, Bochum, Germany, [2]Radboud University Medical Centre, Nijmegen, Nederland

Background: Sandhoff disease (GM2 gangliosidosis, type 0) is an autosomal recessively transmitted lysosomal storage disease resulting from deficiency of hexosaminidase activity. The disease shows a wide spectrum of clinical expression from severe infantile forms fatal before age 4 to late-onset courses with survival into adult age. Sandhoff disease is caused by mutations in the HEXB gene, encoding the beta-subunit of hexosaminidase B.
Methods: For mutation analysis, the 14 coding exons of the HEXB gene and flanking intronic regions were sequenced directly in 8 families and isolated patients. RNA analyses were performed when not yet published splice site variations were identified.
Results: 16 mutations were detected in the HEXB gene presumably causing Sandhoff disease, ten of which had not been published before. These 16 changes included five missense and three stop mutations, three deletions/insertions, one published splice variation as well as four putative splice site changes. The splice variations were not observed in 200 controls. Yet, the interpretation of RNA data and their pathogenetic relevance remain problematic due to common differential splicing of the gene.
Conclusion: Cloning and functional analyses will be performed in order to further evaluate the functional consequences of the splice site variations in Sandhoff disease.

P-MoleG-182
Maternal de novo triple mosaicism for two single OCRL nucleotide substitutions (c.1736A>T, c.1736A>G) in a Lowe syndrome family
Draaken M.[1], Giesen CA.[2], Kesselheim AL.[2], Rami Abou Jamra RA.[3], Chrzanowska KH.[4], Krajewska-Walasek M.[4], Ludwig M.[2]
[1]Institute of Human Genetics and Department of Genomics - Life & Brain Center, Bonn, Germany, [2]Department of Clinical Chemistry and Pharmacology, Bonn, Germany, [3]Institute of Human Genetics, Bonn, Germany, [4]Department of Medical Genetics Children's Memorial Health Institute, Warsaw, Poland

Since the identification of the Lowe's oculocerebrorenal syndrome gene more than 100 distinct OCRL mutations have been observed. Germline mosaicism has been detected in two Lowe families yet, however, the significance of mosaic mutations, in particular triple mosaicism, may be underestimated, because they usually remain undetected during sequence analysis.
In the course of OCRL analysis in a Polish family, the index case showed a hemizygous nucleotide transition, c.1736A>G, in exon 15, leading to a p.His507Arg substitution. Surprisingly, gene analysis in the patient's mother not only provided evidence that she is a carrier of the mutant allele transmitted to her son, but also showed an additional c.1736A>T (p.His507Leu) transversion affecting the same base position. Replicated experiments performed after repeated blood sampling and with different automated sequencing systems gave identical results and ruled out a PCR artefact or an error in sample handling. Moreover, DNA from a mouthwash sample obtained from the mother showed a similar fluorescence intensity pattern at the affected nucleotide position.
This data obtained from mesoderm-derived leukocytes and ectodermal tissue implied a germline mosaicism in the mother and led us to perform further studies. First, maternal grandparents were analyzed for the presence of the mutations however, their respective PCR products solely showed the wildtype sequence. Also, paternity testing revealed no incompatibility with the genotypes in this family indicating a de novo event. The possibility that the triple nucleotide pattern was due to triple X syndrome in the mother was ruled out by karyotype analysis. A partial or complete gene duplication could also be excluded as

qPCR data obtained from leukocytes and from buccal epithelia showed no significant difference in gene-dosage when compared with female controls.
We then employed allele-specific amplification and the results of these PCR reactions confirmed the sequencing results of three alleles being present in the mother. Data from subsequent qPCR analysis verified these findings and showed similiar amounts of these alleles (A > G > T) in the tissues analyzed.
Both mutations in the carrier mother affect OCRL codon 507, however this non CpG-triplet seems to be no hotspot of mutation. The p.His507Arg substitution has been reported only once and a p.His507Gln was observed in another patient. A possible mechanism how a single nucleotide can be affected by two subsequent events is based on the assumption that an adenine residue in its imino (Aimino) form would allow mispairing since the imino form of either A or C makes for A-C pairs. Consequently, an A-to-G transition would result in the next replication round. If this Aimino residue is not recognized, it can subsequently also mispair with another adenine residue present as a syn isomer (Asyn). According to this tautomerism in the template residue the resultant Aimino -Asyn pair gives rise to an AT-to-TA transversion.
Taken together, all data suggest that the mutations observed are the result of two de novo events in early embryogenesis of the mother. To the best of our knowledge, this is the first observation of triple mosaicism at a single nucleotide position and this finding has important implications for genetic counseling.

P-MoleG-183
Enhancer elements upstream of the SHOX gene are active in the developing limb
Durand C.[1], Bangs F.[2], Signolet J.[2], Decker E.[1], Tickle C.[2], Rappold G.[1]
[1]Institute of Human Genetics, Heidelberg, Germany, [2]Department of Biology and Biochemistry, Bath, UK

Léri-Weill Dyschondrosteosis (LWD) is a dominant skeletal disorder characterized by short stature and distinct bone anomalies. SHOX gene mutations as well as deletions of regulatory elements downstream of SHOX resulting in haploinsufficiency have been found in patients with LWD. SHOX encodes a homeodomain transcription factor and is known to be expressed in the developing limb. We have now analysed the regulatory significance of the region upstream of the SHOX gene. By comparative genomic analyses, we identified several conserved non-coding elements, which subsequently were tested in an in ovo enhancer assay in both the chicken limb bud and cornea, where SHOX is also expressed. In this assay, we found three enhancers to be active in the developing chicken limb, but none were functional in the developing cornea. A screening of 60 LWD patients with an intact SHOX coding and downstream region did not yield any deletion of the upstream enhancer region.
Thus, we speculate that SHOX upstream deletions occur at a lower frequency due to structural organization of this genomic region and/or that SHOX upstream deletions may cause a phenotype that differs from the one observed in LWD.

P-MoleG-184
Homozygosity mapping and identification of a candidate gene region for the rare autosomal recessive skin disorder peeling skin syndrome in a large consanguineous family
Eckl K. M.[1], Oji V.[2], Aufenvenne K.[2], Nätebus M.[1], Walker T.[3], Ackermann K.[4], Seller N.[2], Metze D.[2], Nürnberg G.[1], Fölster-Holst R.[5], Schäfer-Korting M.[4], Hausser I.[6], Traupe H.[2], Hennies H. C.[1]
[1]Cologne Center for Genomics, Cologne, Germany, [2]Department of Dermatology, Münster, Germany, [3]Department of Dermatology, Münster, Germany, [4]Institute for Pharmacy, Free University of Berlin, Germany, [5]University Clinic of Dermatology, Kiel, Germany, [6]Department of Dermatology, Heidelberg, Germany

Generalized peeling skin disease (PSD) is an unusual autosomal recessive ichthyosiform erythroderma characterized by lifelong patchy peeling of skin on the the entire body. Up to date only 12 to 15 individuals have been reported who showed generalized PSD with a strong association of pruritus and high IgE-levels, also known as peeling skin syndrome type B. Affected individuals show first symptoms at birth or shortly thereafter, which encompass erythematous, peeling lesions and pruritus as well as hypohidrosis and periodic mild to moderated onychodystrophy. In early infancy, some affected children suffer from moderate failure to thrive. High IgE-levels are not necessarily accompanied by the clinical manifestation of allergic rhinitis or asthma.
We have studied a large consanguineous family with four affected individuals with PSD with severe pruritus and food allergies with a history of urticaria and angioedema. Due to the phenotypic similarities of PSD with Netherton syndrome, as well as the acral type of peeling skin syndrome, we performed mutation analyses in the genes SPINK5 and TGM5, respectively. Additionally we analysed the genes FLG, ALOX12B, ALOXE3, TGM1, NIPAL4, CYP4F22, and ABCA12 for pathogenic mutationsbut no mutation was detected.
We have therefore recruited the 5-generation pedigree and carried out a whole-genome linkage scan using chip-based SNP analysis, which identified a 3.3 cM candidate region with a maximum lod score of 5.4, corresponding to 5.7 Mb in length. All four patients originate from the same Roma tribe but from two different core families. The detailed connection between both families remained unclear. For candidate gene identification we then searched for identical homozygous regions between all four patients using SNPs and microsatellites in the linked region. The largest homozygous interval was 3.0 Mb in length and contained 195 genes. Candidate gene analyses are now under way. In parallel we developed a 3D skin model with keratinocytes from one of the patients to further analyse the biochemical and physiological findings in an in-vitro setting, which are supposed to represent an epidermal barrier defect accounting for the predisposition to atopic diseases.

P-MoleG-185
Search for subtelomeric aberrations in patients with Marinesco-Sjögren-like syndrome
Eggermann K.[1], Senderek J.[2], Schwanitz G.[3], Roos A.[1]
[1]Institute of Human Genetics, Aachen, Germany, [2]Institute of Cell Biology, Zürich, CH, [3]Institute of Human Genetics, Bonn, Germany

Marinesco-Sjögren syndrome (MSS) is an autosomal recessive multiorgan disorder featuring cerebellar atrophy, early bilateral cataracts, progressive myopathy, and a varying degree of mental retardation. SIL1 mutations have been shown to cause MSS in part of cases, however, the primary pathology has remained unknown in other cases with MSS and conditions resembling MSS. Clinical features found in MSS and MSS-like conditions may also be associated with chromosomal abnormalities eventually including cryptic rearrangements occuring in subtelomeric regions. We performed subtelomere screening in a series of 25 patients with MSS-like conditions presenting with at least three features like early cataracts, mental retadation, brain malformations, growth retardation, skeletal abnormalities and muscular

hypotonia. Karyotype and the SIL1 gene were normal in all cases. Subtelomere screening by multiplex ligation-dependent probe amplification (MLPA) did not identify any subtelomeric imbalances. Therefore, a causative role for these regions in manifesting an MSS-like phenotype is unlikely, and subtelomere screening may not be efficient to increase the diagnostic yield in MSS-like cases.

P-MoleG-186

First recessive mutation in the coiled-coil region of myosin-6 (MYO6) in a DFNB37 family suggests impaired dimerization as cause of deafness

Elsayed S.M.[1], Becirovic E.[2], Elsobky E.[3], Bolz H.J.[4]
[1]Medical Genetics Center, Cairo, Egypt, [2]Institute of Human Genetics, University Hospital of Cologne, Germany, [3]Children's Hospital, Ain Shams University Cairo, Egypt, [4]Bioscientia Center for Human Genetics Ingelheim and Institute of Human Genetics, University Hospital of Cologne, Germany

Non-syndromic hearing loss (NSHL) affects about 1/500 newborns and shows extensive allelic and non-allelic heterogeneity. Defects in approximately one hundred different genes can cause autosomal recessive (ARNSHL), autosomal dominant (ADNSHL), X-linked or mitochondrial hearing loss. Different mutations in the same genes can cause either NSHL or syndromic hearing loss, and the trait can be dominantly or recessively inherited depending on the causative mutation. Among the proteins involved in the hearing transduction process, unconventional myosins play an important role, with mutations in seven members of this motor protein family known to be implicated in deafness. MYO6 functions as a minus end-directed actin motor and fulfils several functions in cochlear hair cells, such as maintenance of stereocilia/hair bundle integrity, endocytosis, vesicle transport and Golgi complex maintenance and secretion. MYO6 exists in both monomeric and dimeric forms. Here, we have conducted genomewide linkage analysis in a consanguineous Egyptian family with congenital sensorineural deafness which tested negative for the most prevalent recessive deafness gene, GJB2. We found linkage to a 60 Mb region on chromosome 6p21.2-q21 and subsequently identified a homozygous missense mutation, p.L926Q (c.2777T>A), in the DFNB37/DFNA22 gene, MYO6. So far, (heterozygous) missense mutations in MYO6 have only been described in patients with autosomal dominant hearing impairment, whereas truncating alterations result in autosomal recessive deafness (DFNB37). Expression of c.2777T>A in a pSPL3 minigene construct excluded a mutation-associated splicing error. The p.L926Q mutation affects a highly conserved residue within the coiled-coil domain (CC), a region mediating dimerization of the MYO6 motor. p.L926 represents the core ("a") position of an alpha helix' heptad repeat that is crucial for CC formation. The mutation shifts the heptad repeat, resulting in lower CC prediction scores. In silico prediction suggests a reduction of the molecule's ability to form dimers. We propose a novel mechanism for DFNB37: Not loss of function, but a shifted monomer/dimer ratio towards monomeric MYO6 protein may cause deafness.

P-MoleG-187

Blood cell mosaicism in Fanconi anemia

Endt D.[1], Steiner B.[2], Neveling K.[3], Eirich K.[3], Schinzel A.[4], Hoehn H.[3], Hanenberg H.[5], Schindler D.[3]
[1]Department of Human Genetics University of Würzburg, Würzburg, Germany, [2]Department of Medical Genetics University of Zürich, Zürich, Switzerland, [3]Department of Human Genetics, Würzburg, Germany, [4]Department of Medical Genetics, Zürich, Switzerland, [5]Department of Pediatric Oncology Hematology and Clinical Immunology, Düsseldorf, Germany

Somatic mosaicism due to cell type-specific reversion of constitutional mutations has been interpreted as a manifestation of "natural gene therapy". In Fanconi anemia (FA), overt mosaicism in the hematopoietic system has been associated with hematological improvements and long-term stability of blood parameters. However, it is not clear how many mosaics remain unrecognized. Their detection could help us to arrive at an unbiased view on the natural course of revertant mosaicism. Here we report on a 36 year-old patient who came to our attention at age 33 because of squamous cell carcinoma (SCC) of the oral cavity and infertility. Because of SCC at relatively young age and typical malformations, we considered the diagnosis of FA despite normal blood cell counts and no history of prior hematological problems. FA was confirmed by proof of cellular hypersensitivity (blood lymphocytes and cultured fibroblasts) towards mitomycin C. Assignment to complementation group FA-D2 was achieved by standard FANCD2 immunoblotting revealing lack of either FANCD2 isoform. However, traces of both FANCD2 isoforms were readily detected on overexposure of X-ray films, consistent with the presence of residual protein. FANCD2 direct sequencing of fibroblast gDNA and cDNA disclosed compound heterozygous mutations consisting of the maternal missense substitution in exon 26, c.2444G>A (p.R815Q) and the paternal splice site mutation c.3467-2A>G (intron 34). The splice site change resulted in skipping of exon 35 in mature RNA, leading to a frameshift and premature termination of translation (p.A1156VfsX10). The possibility of revertant mosaicism accrued from the lack of hematological problems, in-depth evaluation of chromosome breakage distributions in blood lymphocytes, and from the fact that an EBV-transformed lymphoblast cell line from the patient proved MMC-resistant. When MACS-sorted cells from individual blood cell lineages were separately analyzed, T lymphocytes showed about 2%, B lymphocytes approximately 30%, granulocytes 2-10%, and monocytes 20-30% reversion back to normal of the paternal splicing mutation c.3467-2A>G. These findings are conceptually instructive in several ways: (1) the conventional study predominantly of T lymphocytes in human genetic diagnosis may result in failure to detect low-level hematopoietic mosaicism in FA; (2) the level of mosaicism may vary considerably among different blood cell lineages; (3) such "variegated" type of mosaicism may sustain normal hematopoiesis into adulthood, but may not necessarily involve all cell lineages, nor reach completion in any of them, notwithstanding the postulated selective advantage of self-corrected hematopoietic cells. Clinically, despite the presence of typical congenital malformations, the diagnosis of FA may not be a primary consideration in adult patients without hematological manifestations. However, minor or even masked mosaicism may be relevant to conditioning regimens prior to hematopoietic stem cell transplantation.

P-MoleG-188

Loss of ATP6V0A2 impairs trafficking in several intracellular compartments and results in apoptotic cell death.

Fischer B.[1], Chan H.W.L.[2], Dimopoulou A.[1], Egerer J.[3], Chan D.[2], Mundlos S.[1], Kornak U.[1]
[1]Institute für Medizinische Genetik, Charité Universitätsmedizin Berlin, Germany, [2]Department of Biochemistry, University of Hong Kong, China, [3]Max-Planck-Institut für Molekulare Genetik, Berlin, Germany

The ATP6V0A2 gene encodes the V-type H+-ATPase a2 subunit, a membrane protein which is localised in the Golgi apparatus and in endosomal compartments. The a subunits are a pivotal part of the V-ATPase domain Vo and beyond proton translocation a direct role in membrane fusion was suggested. We could recently show that loss of the a2 subunit results in autosomal recessive cutis laxa type 2, Debré type (ARCL2; MIM 219200) and wrinkly skin syndrome (WSS; MIM 278250). Both disorders are characterized by a congenital glycosylation defect on the level of the Golgi apparatus (CDG type II). Cultured patient skin fibroblasts showed a defective retrograde Golgi-ER trafficking upon brefeldin A treatment and an altered tropoelastin secretion. To analyse the consequences of a loss of function of the a2 subunit in more detail we investigated HeLa cells after RNAi-mediated knockdown of a2 and patients skin fibroblasts. This intervention lead not only to the known impairment of retrograde Golgi-ER trafficking, but

also to a disruption of the Golgi network as well as a mislocalization of endosomal vesicles. Furthermore, receptor-mediated endocytosis of marker proteins was delayed. Glycosylation in a2-deficient cells was tested and reflected an alteration in highly glycosylated marker proteins. We also detected strikingly increased apoptosis rates in a2-deficient cells. In skin fibroblasts from patients with other forms of ARCL we did not observe a delay in Golgi-ER trafficking after brefeldin A treatment.
In summary, we detected multiple changes in vesicular trafficking after loss of the a2 V-ATPase subunit affecting the endosomal and the Golgi compartment. The highly reproducible brefeldin A effect appears to be specific for the loss of the a2 subunit and thus allows to differentiate between the different forms of ARCL if skin fibroblasts are available. Apoptosis-mediated cell loss in affected tissues could be a key event in the pathogenesis of ARCL2.

P-MoleG-189

Functional analysis of variants in 10q26 strongly associated with age-related macular degeneration (AMD)

Friedrich U.[1], Milenkovich A.[1], Corbo JC.[2], Weber BHF.[1]
[1]Insitute of Human Genetics, Regensburg, Germany, [2]Department of Pathology and Immunology, St. Louis, USA

Introduction: AMD is a multifactorial disease of the central retina and a leading cause of irreversible vision loss in developed countries. A total of 15 genetic variants in chromosomal region 10q26 have been strongly associated with the disease and are located within a 23.3 kb region of strong linkage disequilibrium (LD). These polymorphisms center over two nearby genes, ARMS2 (age-related maculopathy susceptibility 2) and HTRA1 (HtrA serine peptidase 1), both of which are excellent candidates for the AMD susceptibility gene. With the exception of a single non-synonymous variant (rs10490924), the remaining associated polymorphisms do not reveal any obvious functional consequences but may influence expression levels of either one of the two genes in question (1, 2, 3). Thus far, several groups have reported contradictory results. Interestingly, all studies have focused solely on single polymorphisms in the 10q26 region. In an effort to identify the AMD gene in 10q26, the aim of our study was to analyze the effects of the risk-associated polymorphisms either alone or in combination with each other on the expression levels of ARMS2 and/or HTRA1.
Methods: To analyze the influence of AMD associated polymorphisms on HTRA1 expression, quantitative real-time (qRT)-PCR was performed with cDNA from human lymphocytes and placental tissue. After determining the retinal transcription start site of HTRA1 via 5' RACE, luciferase assays were performed with HTRA1 promoter constructs of non risk and risk-associated haplotypes up to 4.5 kb in size. HTRA1 promoter constructs were also electroporated into ex vivo mouse retinal tissue. To complement data on ARMS2 expression in human tissue (1), Cos-7 cells were transfected with genomic, risk and non risk associated ARMS2 variants, and ARMS2 expression was compared via qRT-PCR and immunocytochemistry. In addition, the influence of the c.* 372_815del443ins54 variant on HTRA1 expression was tested by generating chimeric constructs of the 3' untranslated regions with risk and non risk associated haplotypes.
Results: Our in vivo and in vitro data fail to show an influence of any of the risk-associated polymorphisms, including the c.* 372_815del443ins54 variant, on HTRA1 expression. In contrast, a strong influence was obtained on the mRNA level of ARMS2 transcripts further confirming previous data (1). Accordingly, the risk isoform of ARMS2, but also the chimeric non risk isoform of ARMS2 (harboring the variant c.* 372_815del443ins54), is highly unstable compared to the non risk isoform. These findings could be replicated in for ARMS2 protein expression in transfected Cos-7 cells.
Conclusions: Our data strongly indicate that the frequent ARMS2 variant c.* 372_815del443ins54 is the sought-after functional AMD associated polymorphism in 10q26, resulting in reduced ARMS2 levels in the retina. No functional effects of the remaining 14 disease-associated variants could be defined. This strongly suggests a causal involvement of ARMS2 in the pathogenesis of AMD.

Literature:
1) Fritsche et al., 2008, Nat. Genet., 40:892-6;
2) Dewan et al., 2006, Science, 314: 989-92;
3) Kanda et al., 2007, PNAS, 104: 16725-6

P-MoleG-190

Identification and characterization of human MCPH1 splice variants and NLS motifs

Gavvovidis I.[1], Trimborn M.[2], Kaiser F.J.[3], Hanenberg H.[4], Neitzel H.[5], Schindler D.[1]
[1]Institut für Humangenetik, Würzburg, Germany, [2]Institut für Medizinische Genetik, Berlin, Germany, [3]Institut für Humangenetik, Lübeck, Germany, [4]Klinik für Kinder-Onkologie -Hämatologie & -Immunologie, Düsseldorf, Germany, [5]Institut für Humangenetik, Berlin, Germany

MCPH1 encodes the nuclear protein Microcephalin containing three BRCT-domains. It is involved in the regulation of chromosome condensation and in DNA-damage repair responses. Biallelic mutations in MCPH1 cause primary autosomal recessive microcephaly (MCPH, OMIM #606858) with premature chromosome condensation (PCC, OMIM #607117) as a unique cellular phenotype. Here we demonstrate the existence of alternatively spliced isoforms of MCPH1 each of which is able to complement the PCC cellular phenotype in MCPH1-deficient cells. Expression studies of GFP-tagged MCPH1 splicing and deletion variants identify the N-terminally located BRCT-domain as the crucial for the timely condensation and decondensation of chromosomes during cell cycle in human cells. Subcellular fractionation assays indicate a nuclear localization of Microcephalin. In silico analysis of the MCPH1 protein revealed three putative nuclear localization signals (NLS): KKKRK (NLS1), KRKRVSHGSHSPPKEKCKRKR (NLS2) and PYSGKKK (NLS3). By PCR-based in vitro mutagenesis we generated MCPH1 variants with different combinations of deleted NLSs as GFP-fusion constructs. Analysis of the cellular localization of these proteins reveals that NLS2 and NLS3 are sufficient to target the MCPH1 isoforms to the nucleus independently from each other. These findings contribute to better understanding and explanation of multitude of functions claimed to MCPH1.

P-MoleG-191

Allelic expression imbalance of TRPS1 in a patient with tricho-rhino-phalangeal syndrome type II caused by deletion of novel 5'-UTR exons

Gkalympoudis S.[1], Heitmann M.[1], Hennig C.[2], Horsthemke B.[1], Lüdecke H.-J.[1]
[1]Institut für Humangenetik, Essen, Germany, [2]Humangenetische Beratungsstelle, Dresden-Friedrichstadt, Germany

The tricho-rhino-phalangeal syndrome type II (TRPS II) is an autosomal dominant malformation syndrome that combines the clinical features of two conditions which also occur independently of each other, TRPS type I and multiple cartilaginous exostoses (MCE). Major clinical features are sparse scalp hair, a bulbous tip of the nose, a long flat philtrum, cone-shaped epiphyses of the phalanges, MCE and short stature. So far, all patients have been found to have a deletion on chromosome 8q, which spans at least the interval from TRPS1 through EXT1, which are responsible for TRPS I and MCE, respectively.
Here we report on a 13-year-old female patient with MCE and facial features consistent with TRPS. However, cone-shaped epiphyses were only hardly detectable at some phalanges. A SNP array analysis (GeneChip® Human Mapping 250K NspI Array, Affymetrix performed by L. Klein-Hitpass, BioChip Labor Universitätsklinikum Essen) of genomic DNA of our patient disclosed a 4-Mb deletion in 8q23.3-q24.12 includ-

ing EXT1 but surprisingly leaving the TRPS1 gene intact. The proximal breakpoint is located between 16.6 kb and 27.4 kb upstream of TRPS1. A mutation in the protein coding part of TRPS1 was excluded by direct sequencing. Analyses with polymorphic microsatellite markers showed that the deletion was a paternally derived de novo deletion.
To investigate if a deletion upstream of TRPS1 affects the transcription of this gene, we analyzed the allelic transcript ratio in RNA from fibroblasts of our patient by fluorescence-taqged single nucleotide primer extension analysis (SNaPshot™) using the 3'-UTR SNP rs800899. This revealed that the expression of the allele in phase with the deletion is reduced by 60% in contrast to equal allelic expression in normal individuals. Thus, our results indicate that a deletion of elements upstream of TRPS1 influences the allelic transcription of this gene.
By performing database searches, RT-PCR and 5'-RACE on fibroblast RNA from normal individuals, we detected four novel 5'-UTR exons of the TRPS1 gene up to 140 kb upstream of the common transcription start site leading to at least six alternative TRPS1 transcripts. Three of the four novel exons are deleted in our patient. This is the most likely reason for the allelic expression imbalance.
We conclude that far upstream elements are necessary for TRPS1 expression. Apparently, the deletion of these elements, although not completely abolishing transcription, is a new disease causing genetic alteration in TRPS.
The presence of TRPS1 transcripts with different 5'-UTRs indicates the existence of alternative promoters. We are currently cloning potential promoter regions to examine their capability of driving transcription in reporter gene assays. The characterization of different promoters may lead to the identification of factors that regulate transcription of TRPS1 in a tissue specific manner.

P-MoleG-192
Methylation analysis of the imprinted MEG3 locus supports the random parental origin of the del(14)(q24q32) in B-cell malignancies
Haake A.[1], Nagel I.[1], Richter J.[1], Ammerpohl O.[1], Martin-Subero J.-I.[1], Gillessen-Kaesbach G.[2], Siebert R.[1]
[1]Institute of Human Genetics, Kiel, Germany, [2]Institute of Human Genetics, Lübeck, Germany

Deletion del (14)(q24q32) is a recurrent chromosomal aberration in B-cell chronic lymphocytic leukemia and other B-cell lymphomas. In the vast majority of the cases, the deletion juxtaposes the IGH locus in 14q32 next to the ZFP36L1 locus in 14q24. Nevertheless, the mechanism by which this deletion leads to lymphomagenesis is currently unknown. The clustering of breakpoints in the ZFP36L1 gene and the development of IGH-ZFP36L1 fusion transcripts consequences suggests ZFP36L1 deregulation to play a pathogenetic role. Alternatively, inactivation of a tumor suppressor gene in the deleted region might be pathogenetically relevant. Indeed, biallelic inactivation of the TRAF3 gene has been documented in a subset of cases (Nagel et al., 2009). The deleted region contains the imprinted gene MEG3, also known as GTL2. MEG3 is located at 14q32 and a maternally expressed gene. The imprinted locus in 14q32 has been suggested to harbor a tumor suppressor involved for solid tumors. Therefore, we here addressed the question whether the del (14)(q24q32) is associated with preferential loss of one parental allele and might be involved in lymphomagenesis via loss of imprinting. We established a bisulfite pyrosequencing assay targeting the differentially methylated region (DMR) at the MEG3 locus. Using DNA isolated from 10 healthy male and female peripheral blood samples as controls, the range of "normal" DNA methylation for each individual CpG was determined (range 33 %-50 %, median 44 %). Pooled peripheral blood genomic DNA obtained from 20 healthy donors (10 males and 10 females) and enzymatically methylated DNA (Millipore, Germany) acted as controls. Patients with Temple Syndrome due to constitutional deletion in 14q32 or UPD14 showed hypomethylation (< 20 % methylation). The DNA methylation levels in 31 lymphomas with del(14)(q24q32) were above and below the normal range (median in controls +/- 3 SD) in 16 and 10 cases, respectively. Consequently, these results do not support the preferential deletion of one specific parental allele. We also analysed a total of 13 cell lines from classical Hodgkin lymphoma, mantle cell lymphoma, anaplastic large cell lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma. All cell lines analysed show markedly elevated methylation levels (range 83-89 %) as compared to controls. In summary, methylation analysis of the MEG3 locus lacks evidence for a preferential loss of one parental allele in lymphomas with del (14)(q24q32) but indicates that hypermethylation of this locus potentially inactivating its tumor suppressive function is present in a subset of lymphomas.

P-MoleG-193
Functional knock-down of human Scyl1 in HELA cells to provide new insides in cellular function of human Scyl1 and its variants.
Hermann I., Kraus C., Apel M., Reis A.
Humangenetisches Institut, Erlangen, Germany

In mdf-mice, a 1bp-insertion in the Scyl1 gene induces nonsense-mediated decay of the Scyl1-RNA leading to an autosomal-recessive muscle-deficiency. The full-length Scyl1-Protein is highly conserved in eukaryotes, is mainly expressed in neurons and enriched at central nervous system synapses and neuromuscular junctions. But hSCYL1 is expressed as several splice-variants, some of which show significant differences in cellular localization, function and magnitude. There is still no exact information about its function available. What has been discovered about its role in the cell comprises being (1) a component of the centriol complexes, (2) a transcription factor for hTERT and (3) part of the nucleocytoplasmic transport machinery. There, hSCYL1 interacts with hCOPBI and participates in the retrograde transport of membrane vesicles from the cis-Golgi to the ER.
In this study we used the so called esi-RNA system to knock-down the endogenous expression of human SCYL1 in Hela cells. This system utilises not just one but a pool of different si-RNAs against a target transcript, making it more feasible and in many cases more efficient. Furthermore, it enhances the chance for a complete knock-down of the SCYL1-variant repertoire, thus reproducing the effects of the RNA-decay in mdf-mice more precisely. The knock-down was confirmed on RNA level by real-time PCR with Taqman probes and by western blot analysis on protein level. We performed fluorescent microscopy on knock-down HELA cells to visualize the influence of the knock-down on the Golgi-Apparatus and the ER. Isolated RNA from knock-down cells was analysed on our Affymetrix GeneChip array platform to perform a large-scale screening for expression changes of other genes caused by the absence of hSCYL1. The resulting data included several deregulated genes of which we selected five candidates as most relevant (hCLIC3, hETV5, hGDF15, hHERC5, hKRT19). Interaction and co-localization studies are ongoing.

P-MoleG-194
Interaction profiling of the tumor suppressor protein and senescence-inducer p33ING1b
Jennek S., Riesener G., Baniahmad A.
Jena University Hospital, Institute of Human Genetics and Anthropology, Germany

The p33ING1b protein belongs to the inhibitor of growth (ING) tumor suppressor family, which is highly conserved in mammals and acts at epigenetic level. In human tumors, ING1 gene expression is deregulated or even lost. p33ING1b protein is involved in epigenetic regulation by binding to the histone mark Histone H3 K4me3. Also involvement in DNA damage repair and cell cycle regulation has been described. Notably, DNA microarray analyses revealed an induction of p33ING1b expression in senescent prostate epithelial cells. Furthermore, p33ING1b exhibits gene silencing function and is associated with

a histone methyltransferase. Our studies revealed that expression of p33ING1b inhibits cell growth in NIH 3T3 cells and induces premature cellular senescence in primary human fibroblasts.
Despite the large range of p33ING1b-mediated functions little is known about the cellular interacting factors and molecular pathways. Our aim was the identification of new specific molecular partners of p33ING1b to investigate the detailed mechanisms how p33ING1b realizes their tumour suppressive functions and induction of cellular senescence.
To detect physical protein-protein-interaction, surfaced-enhanced laser desorption ionization and mass spectrometry (SELDI-MS) combined with immunological techniques such as co-immunoprecipitation (Co-IP) and immunoblotting were used. Selected candidate interacting factors were functionally analysed using reporter gene assays. The presented data confirm a functional protein-protein interaction.

P-MoleG-195
Molecular characterization of the promoters of the X-linked mental retardation gene JARID1C and additional members of the JARID1 gene family
Jensen L.R., Schlicht M., Lipkowitz B., Ropers H.-H., Kuss A.W.
Max Planck Institute for Molecular Genetics, Berlin, Germany

X-linked mental retardation (XLMR) is genetically heterogeneous disorder affecting approximately 2 in 1000 males. Causative mutations have been found in over 100 different genes, but a significant proportion of the cases are still without molecular diagnosis. The majority of mutations were so far detected in the protein coding regions of X-chromosomal genes. However, sequence changes in regulatory regions may result in similarly detrimental effects by influencing the transcription efficiency of the respective genes.
Therefore one can assume that part of the unsolved cases of XLMR may be due to promoter mutations. Thus, it is necessary to identify functional promoter elements in known XLMR-genes in order to determine where sequence changes can have functional consequences. Furthermore, the identification of factors involved in the transcription of these genes is also a way to find additional genes with a putative role in MR. Therefore we investigated the promoter region of one of the more frequently mutated XLMR-genes, JARID1C, for functionally relevant sequences.
As JARID1C encodes a transcription factor, mutations in target promoters might also cause MR. Thus, having evidence of regulatory interplay between JARID1C and its homolog JARID1B, we included JARID1B as well as the other JARID1 family members (JARID1A and JARID1D) in our study. Using a dual-luciferase reporter assay to measure the activity of different parts of the 5'-flanking regions of these genes in HEK and SH-SY-5Y cells, we defined regions that are important for transcriptional activity in all four JARID1 genes.

P-MoleG-196
5-HT receptor diversity in the human colon: expression of novel 5-HT3 receptor subunits 5-HT3C, 5-HT3D and 5-HT3E
Kapeller J.[1], Moeller D.[1], Liu M.-T.[2], Lasitschka F.[3], Autschbach F.[3], Hovius R.[4], Rappold G.A.[5], Gershon M.D.[6], Niesler B.[5]
[1]Institue of Human Genetics,Heidelberg,Germany, [2]Columbia University New York,New York,USA, [3]Institute of Pathology,Heidelberg,Germany, [4]EPFL,Lausanne,Switzerland, [5]Institute of Human Genetics,Heidelberg,Germany, [6]Columbia University,New York,USA

Since the first description of serotonin 3 (5-HT3) receptors more than 50 years ago, there has been speculation about the molecular basis of their receptor heterogeneity. We have been able to clone novel 5-HT3 subunit candidates and show that they are capable of building functional heteromeric receptors upon co-expression with the 5-HT3A subunit, but there was still doubt that the respective proteins are expressed in human tissue. In the current study, expression of the novel subunits 5-HT3C, 5-HT3D and 5-HT3E as well as the 5-HT3A subunit were analyzed in resected human colon by immunocytochemistry, Western and Northern blots. RT-PCR was also used to study microdissected tissues. For the first time, we have been able to show that 5-HT3AC, 5-HT3AD and 5-HT3AE are co-expressed in enterocytes of the colonic mucosa as well as in cell bodies of myenteric neurons. These data provide a fundamental basis for the future exploration of the role of specific 5-HT3 receptor subtypes in functional and regulatory processes in the enteric and central nervous systems. It will furthermore elucidate the contribution of 5-HT3 receptors to the pathophysiology of neurogastrointestinal disorders such as irritable bowel syndrome, dyspepsia as well as gastroesophagal reflux disease.

P-MoleG-197
Characterization of a novel deletion in the FBN1 gene in a patient with Marfan syndrome
Kathiravel U.[1], Keyser B.[1], Steinemann D.[2], Rybczynski M.[3], Schlegelberger B.[2], Schmidtke J.[1], v. Kodolitsch Y.[3], Stuhrmann M.[1]
[1]Institute of Human Genetics, Medical School of Hannover, Germany, [2]Institute of Cell and Molecular Pathology, Medical School of Hannover, Germany, [3]Department of Cardiology, University Hospital Hamburg Eppendorf, Germany

Marfan syndrome (MFS, OMIM#154700) is an autosomal dominant inherited disorder of the connective tissue with a prevalence of 1 in 10.000 cases of all ethnic groups and is characterised by involvement of three major organ systems these are the cardiovascular, skeletal and ocular system. Marfan syndrome is mainly caused by mutations in the FBN1 gene that consists of 65 coding exons. To date numerous studies identified more than 600 mutations interspersed throughout the gene, that are listed in the FBN1 universal mutation database (http://www.umd.be). Mutations in TGFBR1 and TGFBR2 genes, respectively, were identified to be responsible for Marfan syndrome type II and Loeys-Dietz syndrome (LDS1A, OMIM# 609192; LDS1B, OMIM# 610168; LDS2A, OMIM# 608967;
LDS2B, OMIM# 610380) which show overlapping clinical features with classic MFS.
Recently, we have shown that approximately 6% of patients with clinically proven or highly suspected Marfan syndrome and no detectable mutation by sequencing the FBN1, TGFBR1 and TGFBR2 genes, were carriers of large deletions in the FBN1 gene. Therefore we included MLPA (multiplex-ligation-dependent probe amplification) in our routine mutation screening protocol.
Our current study describes a case with a large deletion in the FBN1 gene that commences in exon 64 deleting the entire exon 65 and the 3'UTR. The size of the deletion in 3' direction was ascertained by high resolution array CGH (3 probes per 1000 bp). Breakpoint spanning PCR was employed to confirm deletion and bidirectional sequencing was performed to define the exact breakpoints. The size of the deletion is 9.134bp consisting of 147bp of exon 64, intron 64, complete exon 65, the 3'UTR of the FBN1 gene and 7.484bp of the contiguous genomic region. Expression studies at the RNA and protein levels are in progress.

P-MoleG-198

Polymorphisms in genes involved in the response to cellular stress and their contribution to the pathophysiology of Nijmegen Breakage Syndrome

Kim R.[1], Radszewski J.[2], Seemanova E.[3], Chrzanowska K.H.[4], Sperling K.[2], Digweed M.[2]
[1]Institut für Humangenetik, Charité- Universitätsmedizin Berlin, Germany, [2]Institut für Humangenetik, Charité - Universitätsmedizin Berlin, Germany, [3]Medical School of Charles University, Prague, Czech Republic, [4]Children's Memorial Health Institute, Warsaw, Poland

Nijmegen breakage syndrome (NBS [MIM 251260]) is an autosomal recessive chromosomal instability disorder with hypersensitivity to ionizing radiation. The clinical phenotype is characterized by congenital microcephaly, mild dysmorphic facial appearance, growth retardation and immunodeficiency. Patients have a highly increased risk for lymphoreticular malignancy and a median survival of only 3 years after its diagnosis. This reflects the profound disturbance to genomic integrity and cellular homeostasis that is caused by the mutation of the essential mammalian gene, NBN. Most NBS patients are homozygous for a founder mutation, nevertheless, there is considerable clinical and cellular variability. Since nibrin, the product of the NBN gene is involved in DNA repair we hypothesised that sequence variation in other genes involved in the repair of DNA double-strand breaks or more generally in the response to DNA damage, might influence the cellular and clinical phenotype in NBS patients.
We therefore examined a set of 18 SNPs in appropriate genes in the DNA of 37 NBS patients who were clinically well characterised with regard to malignancy and immunodeficiency. We found no statistically significant correlations for immunoglobulin status or for age at onset of malignancy with any of the SNPs examined. However, there were two significant correlations between SNPs and survival with malignancy amongst the 19 patients who had developed cancer. Average survival with the XPD rs1799793 genotype AA (Asn/Asn) or AG (Asn/Asp) was 6.03 years but only 2.29 years for patients with the genotype GG (Asp/Asp), a statistically significant difference in Fisher's two-tailed test ($p = 0.037$). The second significant association was the MnSOD-SNP, rs 4880 (Ala-9Val), here homozygosity for the rarer T allele (Val) was significantly more frequent amongst patients surviving longer than 3 years ($p = 0.046$ in Fisher's two-tailed test).
Since correction for multiple testing in this small cohort negates these significant results, we sought to verify the impact of the XPD and Mn-SOD SNPs at the cellular level by investigation of selected cell lines from patients with the appropriate genotypes. Endpoints for analysis were ATM phosphorylation, chromosome breakage and apoptosis after DNA double-strand break induction. The results will be presented and discussed with respect to clinical variability in NBS.

P-MoleG-199

Testing for CMT1A and HNPP using Multiplex Ligation-Dependent Probe Amplification (MLPA) revealed an atypical Duplication/Deletion event

Kraus C., Rothkegel S., Thiel C., Reis A.
Institute of Human Genetics, Erlangen, Germany

Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are two autosomal dominant peripheral neuropathies resulting from DNA rearrangements on chromosome 17p. The abnormally decreased nerve conduction velocties observed in CMT1A patients correlate directly with the stable inheritance of a 1.5-Mb duplication including the PMP22 gene on chromosome 17p11.2-p12. In contrast, a 1.5-Mb deletion at 17p11.2-p12 occurs in multiple patients with HNPP. The size of the region which is duplicated or deleted is quite consistent in multiple unrelated ethnically diverse families and even in de novo duplication or deletion patients. Duplications/deletions are the reciprocal products of an unequal crossing over with reciprocal recombination between flanking 24-kb homologous sequences termed CMT1A-REPs on chromosome 17p.
Several techniques can be used to detect the typical 1.5-Mb duplication or deletion associated with these respective conditions. Multiplex ligation-dependent probe amplification (MLPA) is one of these techniques. We have assessed its performance by retrospectively testing of 135 patients with CMT1A (107) and HNPP (28), which have been previously analyzed by quantitative Real Time PCR. The MLPA kit (P033-B2) was obtained from MRC-Holland, Amsterdam, The Netherlands. The probe mix contains 16 probes specific for sequences present in the CMT1A region. These include 9 probes for the five exons of the PMP22 gene, two probes for the COX10 gene and two probes for the TEKT3 gene. MLPA was performed as described by the manufacturer's instructions. Four healthy controls were included as control samples. Data analysis was performed using the MLPA module of the SeqPilot Software (version 3.3.0. JSI medical systems GmbH, Kippenheim, Germany).
By the use of the MLPA assay we could confirm the PMP22 duplication in all 107 CMT1A patients, as well as the PMP22 deletion in all 28 HNPP patients tested. Furthermore, with the use of a panel of probes within and flanking the CMT1A region we were able to identify two patients with an atypical duplication and two patients with an atypical deletion, respectively.

In these samples no copy number variation was observed for the two COX10 specific probes (01468-L00925 and 01469-L00924). The COX10 gene has been mapped 10 kb centromeric to the distal CMT1A-REP element. It consists of seven exons and spans a length of approximately 135kb. Exon VI localises to a region within the distal CMT1A-REP element, while a duplicated "pseudo" exon VI is present in the proximal CMT1A-REP element. Exons I-V map telomeric to the distal CMT1A-Rep element while exon VII maps within the CMT1A/HNPP duplication/deletion region. As the two COX10 specific MLPA probes are localized in exon VII a copy number variation is anticipated in CMT1A/HNPP patients. Therefore, the results of our MLPA analysis indicate that the distal CMT1A-REP element is not involved in the recombination event in our patients and the breakpoint is localized more proximal. To further elucidate these results COX10 specific quantitative Real Time PCRs and sequencing of the breakpoint fragment will be performed.

P-MoleG-200

An unbalanced translocation unmasks a recessive mutation in the follicle stimulating hormone receptor (FSHR) gene and causes FSH resistance

Kuechler A.[1], Hauffa BP.[2], Köninger A.[3], Kleinau G.[4], Albrecht B.[1], Horsthemke B.[1], Gromoll J.[5]
[1]Institut für Humangenetik, Universitätsklinikum Essen, Germany, [2]Dept. of Paediatric Endocrinology and Diabetology, University Children's Hospital Essen, Germany, [3]Dept. of Obstetrics and Gynaecology, University Hospital Essen, Germany, [4]Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany, [5]Center for Reproductive Medicine and Andrology, University of Münster, Germany

Follicle stimulating hormone (FSH) mediated by its receptor (FSHR) is pivotal for normal gametogenesis. Inactivating FSHR mutations are known to cause hypergonadotropic hypogonadism with disturbed follicular maturation in females. So far, only very few recessive point mutations have been described. We report on a 17-year-old female with primary amenorrhoea, hypergonadotropic hypogonadism and disturbed folliculogenesis. Chromosome analysis detected a seemingly balanced translocation 46,XX,t(2;8)(p16.3or21;p23.1)mat. FSHR sequence analysis revealed a novel non-synonymous point mutation in exon 10 (c.1760C>A, p.Pro587His), but no wildtype allele. The mutation was also found in the father, but not in the mother. Further molecular-cytogenetic analyses of the breakpoint region on chromosome 2 showed the translocation to be unbalanced, containing a deletion with

one breakpoint within the FSHR gene. The deletion size was narrowed down by array analysis to approximately 163 kb, involving exons 9 and 10 of the FSHR gene. Functional studies of the mutation revealed the complete lack of signal transduction presumably caused by a changed conformational structure of transmembrane helix 6. To our knowledge, this is the first description of a compound heterozygosity of an inactivating FSHR point mutation unmasked by a partial deletion. This coincidence of two rare changes caused the clinical signs consistent with FSH resistance.

P-MoleG-201

CTS – Targets for imprinting control involved in BWS and SRS

Langer D.[1], Weber R.[1], Martiné U.[1], Zabel B.[2], Prawitt D.[1], Enklaar T.[1]
[1]University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, [2]University Medical Center, Freiburg, Germany

The Beckwith-Wiedemann (BWS) and Silver-Russell syndromes (SRS) have been shown to be associated with imprinting defects of the chromosomal region 11p15.5. Two imprinting control regions (ICR) are indispensable for proper regulation of allele specific gene expression in two clusters of imprinted genes. The distal human ICR1 is made up of 400-450bp direct repeats, harbouring six target sites (CTS) for the zinc finger protein CTCF. Binding of this epigenetic regulatory factor was shown to be crucial for reciprocal expression of the IGF2- and H19-genes and is supposed to act as an insulator. Deletions of some ICR1-repeats on the maternal chromosome are detected in BWS cases and go along with biallelic upregulation of IGF2-expression. Yet, the role of the repetitive architecture of the control region remains obscure. We analysed the insulation potential of truncated ICRs in a softagar assay to test for dispensability of parts of the repetitive elements. In order to dissect binding frequencies of CTCF to the six individual target sites of the ICR, single repeat fragments were pulled down by ChIP-experiments, cloned and identified by sequencing.
Rating of dispensability and individual binding frequencies suggests not single CTS, but the bipartite ICR-structure to be essential for proper regulation of imprinted IGF2- and H19-expression.

P-MoleG-202

RNA Analysis in Smith-Lemli-Opitz Syndrome

Lanthaler B.[1], Zschocke J.[1], Witsch-Baumgartner M.[1]
[1]Department Medical Genetics, Innsbruck, A

Introduction: The Smith-Lemli-Opitz-Syndrom (SLOS) is an autosomal recessive metabolic and malformation disorder. It is caused by mutations in the DHCR7 gene encoding the enzyme delta7-sterol reductase which catalyses the last step of cholesterol biosynthesis. More than 120 mutations have been reported. About 90% of mutations are predicted to cause amino acid alterations, however the impact on splicing or RNA stability is unknown. The aim of the study was (1) to establish a technique to analyse the transcription efficiency depending on mutations in the gene, and (2) to study the effect on transcription of two frequent SLOS causing mutations. The splice site mutation c.964-1G>C (IVS8-1G>C) accounts for nearly one third of all SLOS alleles. It disrupts the splice acceptor site of intron 8 leading to usage of an upstream alternative splice acceptor site. The result is insertion of 134 bp of intron sequence shifting the reading frame and introducing a premature stop. The other investigated mutation p.Thr93Met affects a conserved amino acid which is important for the protein structure. Analyses in additional mutations have been started.
Methods and Results: Transcriptional activity of the DHCR7 gene was assessed by real time PCR in heterozygous fibroblast cell lines from parents of SLOS patients. Because of high variability in expression of wildtype DHCR7 it became necessary to establish an approach that allows comparison of mutated versus wildtype allele in the same cell. The amount of wild type cDNA and mutant cDNA was compared after reverse transcription of mRNA using specific primers for the mutated region and specific probes for the wild type and the mutant alleles. Calculation of relative cDNA amounts was performed by deltaCt method, GAPDH and alpha-actin were used to subtract background. For both mutations investigated so far there was no difference in transcription levels compared to wildtype allele.
Conclusion: Our real time PCR technique allows the analysis of transcription levels of two different alleles separately in the same cell independently of transcription level of the investigated gene in general. Transcription and transcript stability of the alleles carrying p.Thr93Met or c.964-1G>C are not impaired compared to the normal allele. The splice site mutation c.964-1G>C leads to a premature stop codon, but does not trigger nonsense mediated decay due to the fact that it is located in the last intron of the gene. There is also no other apparent mechanism to eliminate mutated transcripts. The results on analysis of further DHCR7 mutations are pending.

P-MoleG-203

LAP2 as a candidate gene for Emery-Dreifuss muscular dystrophy

Le Thi Thanh H.[1], Braun J.[2], Pilat U.[2], Foisner R.[2], Felbor U.[1], Wehnert M.[1]
[1]Institute of Human Genetics, Greifswald, Germany, [2]Max F. Perutz Laboratories, Vienna, Austria

Laminopathies are a group of rare genetic disorders caused by mutations in genes encoding lamins or lamin-interacting proteins. LMNA, EMD and to a lower degree the FHL1, SYNE1 and SYNE2 genes have been associated with Emery Dreifuss muscular dystrophy (EDMD), characterized by contractures of the Achilles tendons, progressive skeletal muscle weakness and heart rhythm disturbances leading to dilated cardiomyopathy (DCM) and sudden cardiac death. Since ~60% of EDMD patients are not associated to known genes, we used a functional candidate-gene approach to identify additional genes involved in EDMD. Based on reported interactions of lamina associated polypeptide 2 (LAP2) with nucleoplasmic lamin A/C and the association of the LAP2alpha isoform to DCM, 111 EDMD and 87 DCM patients were investigated for DNA variations in LAP2 (encoding six LAP2 isoforms) using heteroduplex analysis and direct sequencing. Among ten variations found, four changes were unique for EDMD patients: p.P426L in LAP2alpha, p.D271E in LAP2beta, p.V423L and p.M381I in LAP2gamma. No unique amino acid exchanges were found in DCM patients. Segregation analysis indicated only p.P426L in LAP2alpha as a mutation potentially associated to EDMD. In p.P426L mutant skin fibroblasts, LAP2alpha localized to the nucleoplasm like in wild-type cells and the localization of lamin A was not altered either. However, in p.P426L mutant fibroblasts phosphorylated retinoblastoma protein (Rb) levels were reduced when compared to wild-type cultures, suggesting that the mutated protein may affect cell cycle progression. This observation is in agreement with previous studies implicating LAP2alpha-lamin A in Rb-mediated cell cycle control. The present study suggests that LAP2alpha mutations might add to the pathology of EDMD in ~1% of EDMD patients.

P-MoleG-204

Analysis for AFG3L2 mutations in german ataxia families

Löbbe A.[1], Kang S.J.[2], Stanek S.[1], Müller U.[1], Nolte D.[1]
[1]Institut für Humangenetik, Giessen, Germany, [2]Klinik für Neurologie, Frankfurt/Main, Germany

Spinocerebellar ataxias (SCA) are a heterogeneous group of autosomal dominant inheritated neurodegenerative disorders. Clinical features include gait and limb ataxia, dysarthria, and nystagmus in combination with other signs and symptoms. To date, 27 different SCA loci and 16 genes are known. Recently, mutations in the ATPase family gene 3-like (AFG3L2) gene were identified as the cause of spinocerebellar ataxia type 28 (SCA28). AFG3L2 is composed of 17 exons and the deduced

polypeptide functions, together with the SPG7 gene product, as a mitochondrial metalloprotease protein complex (m-AAA protease). Missense mutations have been identified in single families of Caucasian origin. So far, all known mutations are located in exons 15, and 16 of AFG3L2, coding for the carboxyterminal peptidase domain.
26 independent patients with a proven family history of ataxia were screened for mutations in AFG3L2. Other known SCA-loci (SCA1-3, 6-8, 10, 12-14, 17, 27) had been excluded previously. We sequenced all 17 exons including exon-intron boundaries of AFG3L2 in the patients and found a novel heterozygous T to C transition in exon 16 of the gene in one patient. The base exchange results in the substitution of tyrosine by histidine in the M41 peptidase domain of AFG3L2. Since this exchange was not detected in 210 healthy controls (420 chromosomes), we postulate a pathogenic effect for this alteration.

P-MoleG-205

Somatic mosaicism for large deletions in 8q23-q24 in two mentally normal patients with Tricho-Rhino-Phalangeal Syndrome Type II

Lüdecke H.-J.[1], Heitmann M.[1], Gkalympoudis S.[1], Albrecht B.[1], Weschke B.[2], Horn D.[3], Horsthemke B.[1]
[1]Institut für Humangenetik, Essen, Germany, [2]Otto-Heubner-Centrum für Kinder- und Jugendmedizin, Berlin, Germany, [3]Institut für Humangenetik, Berlin, Germany

Patients with the tricho-rhino-phalangeal syndrome type II (TRPS II, also known as Langer-Giedion syndrome, LGS) display the clinical features of two conditions which also occur independently of each other as dominantly inherited diseases, the tricho-rhino-phalangeal syndrome type I (TRPS I) and hereditary multiple exostoses (HME). They are caused by mutations or deletions of the TRPS1 or EXT1 gene, respectively. All patients with TRPS II have sparse scalp hair, a bulbous tip of the nose, a long flat philtrum, cone-shaped epiphyses and exostoses at multiple sites of their skeleton. And all have an interstitial deletion on chromosome 8q that leads to hemizygosity of these genes that are approximately 2.2 Mb apart from each other. The deletions are very variable in size with no breakpoints in common, and often affect not only the interval TRPS1 through EXT1 but also different sets of flanking genes. Patients with large deletions are almost always mentally retarded.
Here, we describe two female, mentally normal patients with the typical facial and skeletal signs of TRPS II. At examination, they were 9 (patient A) and 14 (patient B) years of age. We first analysed DNA isolated from blood from the patients and their healthy parents with polymorphic microsatellite markers from the Langer-Giedion syndrome chromosome region (LGCR). Unexpectedly, we found both patients heterozygous for all informative markers including the TRPS1- and EXT1-intragenic ones. This argued against a typical deletion. However, the signals of several maternal alleles in patient A and of paternal alleles in patient B appeared less intense than expected, suggesting that the patients may be mosaic for deletions in the LGCR. This was confirmed by multiplex ligation-dependent probe amplification (MLPA) with the TRPS-LGS kit P228 (MRC Holland). In order to determine the sizes of the deletions precisely, we next arranged for SNP array analyses (Affymetrix GeneChip Human Mapping 250K NspI array (patient B) or the Genome-Wide Human SNP Array 6.0 (patient A); performed by L. Klein-Hitpass, BioChip Labor Universitätsklinikum Essen). These analyses disclosed overlapping deletions in 8q23-q24.1 of 16.75 Mb (chr8: 111.44 - 128.19 Mb, patient A) and 11.35 Mb (chr8: 110.24 - 121.59 Mb, patient B), respectively. We were able to obtain fibroblasts from patient A, and found the deletion in 100 % of these cells by SNP array analysis. Absence of the maternal allele in fibroblasts was further confirmed by fluorescence-tagged single nucleotide primer extension analysis (SNaPshot™) using SNP rs800899 in the 3'-UTR of the TRPS1 gene. Unfortunately, patient B refused a skin biopsy and could not be analysed further for gene dosage changes in a tissue other than leukocytes.
The extent and gene content of the deletion in patient A is comparable to that of the mentally retarded patient reported by Riedl et al (2004). And even the deletion of patient B is larger than those found in several, so far unreported, mentally retarded patients. Thus, it appears that the processes in hair and skeletal development which are affected in TRPS and HME are extremely sensitive to dosage changes of genes in the LGCR whereas those in mental development are not. Only if additional genes are deleted like in the patient with a 19.79 Mb mosaic deletion of the LGCR reported by Shanske and colleagues (2008), mental development appears inevitably affected even in mosaics.

P-MoleG-206

Incompletely penetrant PKD1 alleles as a cause of early onset autosomal dominant polycystic kidney disease (ADPKD)

Markoff A.[1], Vujch M.[2], Heyer C.[3], Ars E.[4], Hopp K.[5], Torres V.[5], Torra R.[4], Harris P.[3], Bogdanova N.[1]
[1]University of Muenster, Muenster, Germany, [2]Sahlgrenska University Hospital, Göteborg, Sweden, [3]Mayo Clinic, Rochester, MN, [4]Fundacio Puigvert, Barcelona, Spain, [5]Mayo Clinic, Rochester, USA

Two pedigrees with negative family histories had in utero onset massive polycystic kidney disease (PKD) and at birth oligohydramnios and pulmonary hypoplasia. Although the presentation was indicative of autosomal recessive PKD (ARPKD), they were unlinked to the known gene, PKHD1. Mutation analysis of the autosomal dominant PKD (ADPKD) genes showed both families inherited, in trans, two incompletely penetrant PKD1 alleles. These cases illustrate that PKD1 mutations can superficially manifest as an ARPKD-like phenotype and the perils of performing linkage-based diagnostics in presumed ARPKD without positive mutation data. The phenotypic overlap between ADPKD and ARPKD further indicates likely related pathogenesis of these diseases.

P-MoleG-207

Identification and characterization of a genomic deletion in 2q32.1-q32.2 comprising the COL3A1 gene and the COL5A2 gene in a patient with a severe EDS phenotype including multiple aneurysms

Mayer K.[1], Datter S.[1], Heinrich U.[1], Gabert M.[1], Kotschote S.[2], Kaminsky E.[3], Rost I.[1]
[1]Center for Human Genetics and Laboratory Medicine, Martinsried, Germany, [2]IMGM Laboratories GmbH, Martinsried, Germany, [3]Praxis für Humangenetik, Hamburg, Germany

The vascular type of Ehlers-Danlos syndrome (EDS type IV) is an autosomal dominantly inherited disorder of connective tissue characterized by severe arterial and intestinal complications which are rarely observed in the other forms of EDS. Arterial ruptures account for the majority of deaths, whilst intestinal perforations, occurring mainly on the sigmoid colon, are less often fatal. EDS type IV is caused by mutations in the COL3A1 gene coding for type III procollagen.
We describe the molecular genetic analysis in a 49 year old male patient presenting EDS type IV typical clinical features including thin translucent skin, haematomas, early onset of varicose veins, multiple arterial ruptures, an aneurysm of the arteria hepatica propria, as well as intracranial complications like apoplexia and arteriovenous carotid-cavernous sinus fistulae as a consequence of dissections of the vertebral arteries and the carotids. In addition he exhibited a tall slender stature and hyperelastic skin not characteristic for EDS type IV.
Direct sequencing of all coding exons of the COL3A1 gene in genomic DNA revealed no mutation. Subsequent MLPA analysis of 10 of the 52 exons of the COL3A1 gene identified a heterozygous deletion of all investigated exons including exon 1 and 52. To verify the result of a complete gene deletion we first applied oligo array CGH using a medium density CytoChip 2x105k microarray (BlueGnome, Cambridge, UK). Subsequently, the minimal and maximal deletion size was esti-

mated to be 638 kb and 720 kb, respectively. The deletion comprises not only the COL3A1 gene but also the distally adjacent COL5A2 gene elucidating clinical features of the classical type of EDS. For fine mapping of the deletion boundaries we designed a 4x44k custom microarray with a high coverage of 20.555 oligonucleotides in the pre-estimated deleted region and the flanking regions on chromosome 2q32.1-q32.2 and about 22.000 oligonucleotides spread all over the human genome (4x44k format, Agilent Technologies). So far, less than 5% of all published COL3A1 mutations represent genomic deletions, most of these are intragenic. To our knowledge this is the second report of a large hemizygous genomic deletion simultaneously affecting the COL3A1 and the COL5A2 gene and causing a severe clinical EDS phenotype with arterial ruptures. This finding emphasizes the enclosure of an expanded deletion screening of the COL3A1 and the COL5A2 gene at least in complex EDS phenotypes.

P-MoleG-208

From promoters to protein half-life: An insight into the regulatory machinery of the Fanconi Anemia core complex

Meier D.[1], Hoehn H.[1], Schindler D.[1]
[1]Department of Human genetics, Wuerzburg, Germany

Fanconi anemia (FA) is a rare genome-instability disorder with the frequent presence of congenital malformations, bone marrow failure and FA typical malignancies and cellular hypersensivity to DNA-interstrand crosslinking agents such as mitomycin C. At least 13 complementation groups represent defects in any one of the corresponding genes. Eight of the FA- (FANCA, -B, -C, -E, -F, -G, -L and -M) and FA-associated proteins assemble in a nuclear complex, the FA "core complex".
Little is known about elements of common regulation of the FA core complex proteins. Our work included studies at the genomic, transcript and protein levels. We identified the promoters of the core complex genes as TATA-less, monodirectional promoters comparable to the SV40 type in their strength. The promoters are marked by a distinct transcriptional start site (TSS), surrounded by several alternatives TSSs. 5' of each promoter we identified regions that, when isolated, negatively regulate promoter activity. The middle region of the promoters showed a similar distribution of several transcription factor binding sites like STAT, SMAD, E2F an YY1. With an electro mobility shift assay (EMSA) we showed that the binding sites, predicted in silico are used in vitro.
Codon usage analysis of the transcripts showed no significant difference to random control genes. The mean difference of the FA core complex genes compared to the homo sapiens codon usage table is 23,5%. GLI3, HSP90, V-Raf, ß-Catenin IGF-1 and APC showed a mean difference of 27%. However, parallels in codon usage, especially for Isoleucin, suggested coevolution of the core complex genes. Our results identified FANCM as the evolutionary oldest member of the core complex and FANCA as the most recent gene. The core complex proteins are characterized by a short half-life with a mean of approximately 3,6h.

P-MoleG-209

Rates of splicing noise in the NF1 gene do not correlate to age in cultured human fibroblasts and peripheral blood cells

Mellert K.[1], Kaufmann D.[1]
[1]Institute of Human Genetics University Ulm, Ulm, Germany

In low frequencies errors occur in splicing of human hnRNA generating dysfunctional splice products. This noise in hnRNA splicing increases in vitro e.g. by cold shock or inhibiting of NMD by puromycin treatment. Here we investigated if splicing noise is increased in aged cultured cells and peripheral blood cells of human donors at different age. Splicing noise was measured by RT-qPCR of the expression of two skipped in frame and two out of frame NF1 exons in relation to the expression of the regular transcript. An increase in splicing noise could be detected in cold shock or puromycin treated young fibroblasts in the investigated NF1 exons. In the cultured fibroblasts of four controls differences in splicing noise rates were detected in the investigated exons but no correlation was found to age of the donors or the in vitro passages. In peripheral blood cells of 11 controls of different age (15y - 85y) again no correlation to age was found in the splicing noise rates. However, the splicing noise rate increased with the length of the upstream intron. Our studies show, that splicing is an highly controlled process with a high fidelity during ageing in the investigated cells indicating the significance of correct splicing in proliferating cells.

P-MoleG-210

Homozygous microdeletions 15q13.3 resulting in nullisomy for the small recurrent 680-kb segment containing CHRNA7 cause severe encephalopathy and refractory seizures

Moog U.[1], Endris V.[1], Neuhann T.[2], Tinschert S.[2], Hackmann K.[2], Haug U.[3], Grasshoff U.[4], Bonin M.[4], Schröck E.[2], Rappold G.[1]
[1]Institute of Human Genetics, Heidelberg, Germany, [2]Institute of Clinical Genetics, Dresden, Germany, [3]Center for Child Neurology and Social Pediatrics Maulbronn, Maulbronn, Germany, [4]Institute of Human Genetics, Tübingen, Germany

Background: A recurrent 1.4 Mb deletion 15q13.3 was recognised in 2008 as causative for a wide range of neurodevelopmental disorders including mental retardation, seizures, autism spectrum disorders and psychiatric disease. The 15q13.3 microdeletion syndrome is characterized both by highly variable expressivity and by incomplete penetrance. Very recently, Shinawi et al. (2009) reported on a recurrent smaller heterozygous 680-kb deletion within the same region which encompasses the entire CHRNA7 gene and one exon of OTUD7A, and which gives rise to the same spectrum of developmental abnormalities.
Methods and case reports: We report on two unrelated patients with compound heterozygous deletions 15q13.3, most severe encephalopathy, and therapy resistant seizures with onset in the first year of life. Patient 1 died at 13 years of age, patient 2 is currently 2 years old. As shown by high resolution array and FISH-analyses, both were heterozygous for the recurrent 1.4-Mb deletion (BP4-BP5) in 15q13.3. Additionally, patient 1 had the smaller 680-kb deletion, and patient 2 a larger 3.4-Mb deletion (BP3-BP5). The parents of both patients were healthy carriers of heterozygous deletions and had a normal intelligence. On MRI brain, patient 2 showed cerebral atrophy with demyelination, hypoplasia of corpus callosum and both optic nerves, and a macrocerebellum with retrocerebellar arachnoid cysts. So far, cerebellar malformations are unreported in patients with deletions 15q13.3 extending from BP3 to BP4 or BP5. In patient 1, the smallest overlapping region of both deletions contains CHRNA7 which encodes the alfa-subunit of a neuronal nicotinic acetylcholine receptor and one exon of OTUD7A. Expression studies of OTUD7A in fibroblasts, including fibroblasts of patient 1, and human brain showed a normal expression of OTUD7A in all cases.
Conclusion: For the first time these two cases show that a homozygous deletions for at least the 680kb-segment within 15q13.3 can be compatible with life but leads to a very severe neurodevelopmental disorder. Our data support the hypothesis reported previously that CHRNA7 (and not OTUD7A) conveys the phenotype of heterozygous and homozygous 15q13.3 deletions.

P-MoleG-211

Warburg MICRO Syndrome in patients from different ethnic backgrounds: new mutations in the RAB3GAP1 gene and a possible founder effect in the Danish population

Morris-Rosendahl DJ.[1], Segel RA.[2], Born AP.[3], Conrad C.[4], Bart Loeys B.[5], Sklower Brooks S.[6], Müller L.[1], Zeschnigk C.[1], Uyanik G.[7], Degen I.[4], Fraes F.[8]
[1]Institute for Human Genetics; University Clinic, Freiburg, Germany, [2]Medical Genetics Institute; Shaare Zedek Medical Center, Jerusalem, Israel, [3]Department of Paediatrics; Rigshospitalet, Copenhagen, Denmark, [4]Dept. of Child Neurology; Paediatric section; St. Elisabeth Hospital, Neuwied, Germany, [5]Center for Medical Genetics; Ghent University Hospital, Ghent, Belgium, [6]Department of Pediatrics; Division Genetics; Robert Wood Johnson Medical School, New Brunswick, USA, [7]Institut for Human Genetics; University Medical Center, Hamburg-Eppendorf, Germany, [8]Paediatric Neurology; University of Ghent, Ghent, Belgium

Warburg MICRO Syndrome (WARBM, OMIM: 600118) is a rare, autosomal recessive syndrome characterized by microcephaly, microphthalmia, microcornia, congenital cataracts, optic atrophy, cortical dysplasia, in particular corpus callosum hypoplasia, severe mental retardation, spastic diplegia, and hypogonadism. To date nine different mutations in the RAB3GAP1 gene have been found in patients from 14 families worldwide. We have found seven new mutations in RAB3GAP1 in eight patients with suspected MICRO Syndrome, from families with Turkish, Palestinian, Danish, French and Guatemalan backgrounds. A thorough clinical investigation of the patients has allowed the delineation of symptoms which are consistently present in the patients and may aid the differential diagnosis of MICRO Syndrome for patients in the future. All patients had postnatal microcephaly, micropthalmia, microcornia, bilateral congenital cataracts, short palpebral fissures, optic atrophy, severe mental retardation, and congenital hypotonia with subsequent spasticity. Only one patient had microcephaly at birth, highlighting the fact that congenital microcephaly is not a consistent feature of MICRO syndrome. Analysis of the brain MRIs revealed a consistent pattern of abnormal gyration in the frontal and post-frontal lobes, wide sylvian fissures, a thin hypoplastic corpus callosum and increased subdural spaces. All patients were homozygous for the mutations detected and all mutations were predicted to result in a truncated RAB3GAP1 protein. The analysis of nine polymorphic markers flanking the RAB3GAP1 gene showed that the mutation c.1410C>A (p.Tyr470X), for which a Danish patient was homozygous, occurred on a haplotype that is shared by the unrelated heterozygous parents of the patient. This suggests a possible founder effect for this mutation in the Danish population.

P-MoleG-212

Genetic modifiers of retinal degeneration caused by a PRPH2 mutation

Neidhardt J.[1], Bach M.[2], Lagreze W.A.[2], Glaus E.[1], Lemke J.R.[3], Berger W.[1], Poloschek C.M.[2]
[1]Division of Medical Molecular Genetics and Gene Diagnostics, University of Zurich, Switzerland, [2]Department of Ophthalmology, University of Freiburg, Germany, [3]Institute of Medical Genetics, University of Tübingen, Germany

We identified a five-generation family in which autosomal dominant macular dystrophy, cone dystrophy and cone-rod dystrophy occurred. We aimed to identify the causative mutation and possible genetic modifiers that explain the observed high intrafamilial phenotypic variation. Following chip-based mutation screening (Asper Ophthalmics), patients' genomic DNA was analyzed by sequencing analysis of PRPH2, ABCA4 and ROM1. Fifteen family members underwent detailed ophthalmic and electrophysiologic phenotyping.
We identified heterozygous mutations in three genes and found five different genotype combinations within the studied family. All clearly affected family members carried the heterozygous PRPH2 mutation p.R172W. Patients with heterozygous sequence alterations only in ROM1 (p.R229H) or ABCA4 (p.V2050L) showed a mild ocular phenotype and were otherwise asymptomatic. The phenotypic severity of patients carrying the PRPH2 mutation increased with additional mutations in ABCA4 or ROM1. Patients carrying all three mutations were the most severely affected.
Our study shows that features of a PRPH2-associated phenotype can be modulated by additional mutations in other genes (in this family ABCA4 and/or ROM1), accounting for intrafamilial variability and resulting in a cumulative effect, worsening the phenotype. We suggest that families showing a variable macular dystrophy phenotype caused by mutations in PRPH2 should be analyzed for additional mutations in ABCA4 and ROM1 as they might alter the progression of the PRPH2 phenotype implying different needs for genetic testing and counseling.

P-MoleG-213

Genetic predisposition to severe course of IBD

Nelke-Lozynska A.[1], Kubinska I.[2], Czkwanianc E.[2], Krokowicz P.[1], Podralska M.[3], Meissner W.[1], Drews M.[1], Lipinski D.[3], Roszak D.[1], Cichy W.[1], Plawski A.[3]
[1]Karol Marcinkowski University of Medical Sciences, Poznan, Poland, [2]Department of Pediatrics and Gastroenterology Institute of Polish Mother's Memorial Hospital, Lódz Poland, [3]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland

Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation in gastrointestinal tract. This autoimmune disease is dived into two subtypes Crohn disease (CD) and ulcerative colitis (UC). If it is not possible to differentiate these IBDs the patients are diagnosed as indeterminate colitis (IC). The genetic bases of predispositions have been still studied. In our 160 severe IBD patients with average age of diagnosis 26 years, the youngest patient was diagnosed when was 3 years old and the oldest one was diagnose at the age of 69. In this group we investigated frequency of alleles in NOD2/CARD15 gene and 15-PGHD gene. The 15-PGDH gene codes dehydrogenase which is a prostaglandin-degrading enzyme and acts as an antagonist to enzyme called cyclooxygenase 2. We also studied frequency of haplotype in q31 region on 5th chromosome. We estimated frequency of alleles SLC22A4 1672T and SLC22A5 T207C. In studied group we observed increased frequency of INV4+39C>T 15-PGDH homozygotes in group of patient under 18 years old with UC (12%) in comparison to adult patients where the INV4+39C>T 15-PGDH homozygotes were not been observed. The frequency of A at position 168 in PGDH gene were higher in patient under 18 years old (49%) than in adult patients (34%). In NOD2 gene we observed statistically significant differences of frequency of P268S, R702W and 2030insC in group of patient with severe course of IBD in comparison to unselected IBD patients and control group
The study was supported by the Polish Ministry of Science and Higher Education projects no. 2P05A06929

P-MoleG-214

A repeat mediated homozygous deletion in the ADAMTSL4 gene leads to isolated ectopia lentis

Neuhann TM.[1], Artelt J.[1], Tinschert S.[1], Rump A.[1]
[1]Institut für Klinische Genetik, Dresden, Germany

Background: Ahram et al. (2009) reported a homozygous nonsense mutation in the ADAMTSL4 gene in one family with isolated ectopia lentis. ADAMTSL4 encodes a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) protein family. We report two further families with isolated ectopia lentis with a homozygous mutation in ADAMTSL4.
Methods and Results: We have performed a mutational screening of the ADAMTSL4 gene in two unrelated families with isolated ectopia lentis. In each of the three affected sons (two from family I, one from

family II) 20 bp of coding sequence are homozygously deleted within exon 6 of the ADAMTSL4 gene (NM_019032.4:c.759_778del20). This leads probably either to a truncated ADAMTSL4 protein (NP_061905.2: p.Q256PfsX38) or to nonsense-mediated decay of the aberrant mRNA. The deletion is flanked by a perfectly matching 8 bp direct repeat as well as two perfect DNA polymerase alpha frameshift hotspots. The unaffected sister in family I as well as the non-consanguineous parents in both families are healthy heterozygote carriers of this deletion.

Conclusion: Our results strongly support the association of ADAMTSL4 null-mutations to isolated ectopia lentis. Since the parents are non-consanguineous in both families, probably the heterozygous deletion has not been inherited from a common relative but has been generated in unrelated ancestors by independent events, most likely due to the properties of the local DNA sequence environment. We suggest that many cases with so-called sporadic ectopia lentis might actually be autosomal recessive and caused by mutations in ADAMTSL4.

Reference:

Ahram D, Sato TS, Kohilan A, et al. A homozygous mutation in ADAMTSL4 causes autosomal-recessive isolated ectopia lentis. Am J Hum Genet 2009;84(2):274-8

P-MoleG-215

Update of the PKHD1 mutation database for autosomal recessive polycystic kidney disease (ARPKD)

Ortiz Brüchle N.[1], Venghaus A.[1], Rottländer C.[1], Bergmann C.[2], Zerres K.[1]
[1]Institute of Human Genetics, Aachen, Germany, [2]Bioscientia Center for Human Genetics & Institute of Human Genetics, Aachen, Germany

Mutations in the PKHD1 (Polycystic Kidney and Hepatic Disease 1) gene on 6p12 are associated with autosomal recessive polycystic kidney disease (ARPKD). The disease frequency is about 1 in 20,000 live births and therefore ARPKD is the most common childhood-onset ciliopathy. ARPKD is characterized by massively enlarged bilateral polycystic kidneys and congenital hepatic fibrosis. Almost one half of the ARPKD patients present with oligohydramnios and pulmonary hypoplasia. The PKHD1 gene (86 exons, longest ORF 66 exons) is highly complex due to its genomic size (470 kb) and its transcript structure with multiple alternatively spliced transcripts. Its gene product polyductin/fibrocystin contains 4074 aa. In our patients with typical clinical signs the mutation detection rate is up to 90% with at least one detected mutation. The mutations are scattered throughout the 66 coding exons of the largest transcript. Common mutations account for only 10–20% of all PKHD1 mutations and the remaining mutations are largely unique to individual families (private mutations). Genotype-phenotype analyses reveal that patients with two truncating mutations usually do not survive the neonatal period; survival requires the presence of at least one missense mutation. Our PKHD1 mutation database (http://www.humgen.rwth-aachen.de) has been established to catalogue all changes detected in the PKHD1 gene in a disease specific database e. g. for use in clinical practice. More than 300 PKHD1 variants have been listed in the database so far, mainly detected by our group or extracted from the literature. Approximately 60% of the PKHD1 mutations are truncating. The assessment of the pathogenicity of the remaining 40% of mutations (mostly missense) is difficult. To strengthen the conclusions regarding the pathogenicity of variants obtained from segregation analyses or healthy control-comparison studies bioinformatic validation with web-applications as SIFT, PolyPhen and others has become a useful tool. We are currently reevaluating the listed variants to provide the users of the database with this additional information. Furthermore we are introducing a lot of new variants.

P-MoleG-216

Improved protocol and characterization of neuronal differentiation of skin derived precursor cells

Pamminger T.[1], Liebmann L.[1], Hübner A.K.[1], Beetz C.[2], Thorwarth M.[3], Deufel T.[1], Hübner C.A.[1]
[1]Institute of Clinical Chemistry, Jena, Germany, [2]Institute of Clincal Chemistry, Jena, Germany, [3]Clinic for Plastic Surgery, Jena, Germany

To study the cellular mechanisms underlying hereditary neurodegenerative disorders of individual patients we tried to improve the culture of skin derived precursor cells and the protocol for neuronal differentiation. Skin derived precursor cells were isolated from small skin biopsies of control persons and patients with proven SPG4 and maintained in a 3:1 mixture of DMEM and F12 medium. Skin derived precursor cells growed as non adherent cell clusters that proliferated and self renewed as floating spheres in the presence of epidermal growth factor, fibroblast growth factor 2 and additional components including B-27 and N-2 supplement and chicken embryo extract. The spheres were passaged every 7 days and cultured for more than 8-10 weeks in total. Then differentiation into cells with a neuronal morphology was induced by removal of fibroblast growth factor 2 and epidermal growth factor and addition of the neurotrophic factors BDNF, beta-NGF, neurotrophin-3 and retinoic acid and laminine. 3 days after initiation of differentiation conditions more than 50% of cells co-expressed neuronal markers including nestin, beta III tubuline and MAP2c. 2 weeks later cells also co-expressed neurofilament-L, NeuN and synaptophysin. Electrophysiological analysis confirmed the expression of Na+ and K+ channels and the appearance of action potentials. Functional assays in comparison between control and patient derived neuronal like cells are currently under way to address basic cell features in neurodegenerative disorders.

P-MoleG-217

Identification of three novel protein S gene mutations in patients with protein S-deficiency and thrombosis

Pfanner G.[1], Schmitt U.[2], Steidle M.[1], Arlt M.[2], Burchert-Graeve M.[1]
[1]Laborärzte Sindelfingen, Sindelfingen, Germany, [2]LaboMed GmbH, Stuttgart, Germany

Protein S (PS) is a vitamin K-dependent plasma glycoprotein that plays a central role in the regulation of blood coagulation by acting as a co-factor of activated protein C (APC) in the proteolytic degradation of activated blood coagulation factors V and VIII and additionally by possessing APC-independent anticoagulant activities.

Hereditary PS deficiency is an autosomal dominant disorder that is associated with an increased risk of venous thrombosis and is caused by mutations within the gene encoding protein S (PROS1) located on chromosome 3 at position 3q11.2. To date, more than 200 different PROS1 mutations have been reported.

Herein, we describe three mutations within the PS gene that, to our knowledge, have not been reported previously. By means of direct sequencing of all coding regions (exons 1 to 15) and adjacent exon/intron boundaries of the PROS1 gene in a patient with low protein S activity the novel mutation c.25_34dup (p.Leu12Argfs*30) in exon 1 was detected in heterozygous form. Sequence analysis of the PROS1 gene from a protein S-deficient patient who experienced the first thrombotic event by the age of 26 and her asymptomatic protein S-deficient 16 and 18 years old sons revealed the presence of a heterozygous 2 bp-deletion mutation (c. 152_153delAG; p.Gln51Argfs*2) in exon 2. Furthermore, in a patient with low protein S activity and thrombosis mutational analysis identified heterozygous c.995_996delAT (p.Tyr332*) in exon 10.

All detected mutations are predicted to cause protein S-deficiency through the introduction of a premature translation termination codon elicting nonsense-mediated mRNA decay.

In conclusion, we detected three novel mutations in patients with protein S-deficiency and thrombosis, thereby expanding the spectrum of PROS1 gene mutations considered to be disease-causing.

P-MoleG-218

Germline mutations in the STK11 gene in Polish Peutz- Jeghers syndrome patients

Podralska M.[1], Czkwanianc E.[2], Nowakowska D.[3], Kozak-Klonowska B.[4], Teisseyre M.[5], Slomski R.[1], Pieczarkowski S.[6], Siwinska-Mrozek Z.[7], Cichy W.[7], Plawski A.[1]

[1]Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland, [2]Department of Pediatrics and Gastroenterology Institute of Polish Mother's Memorial Hospital, Lodz, Poland, [3]Genetic Counseling Unit Cancer Center and Institute of Oncology, Warsaw, Poland, [4]Regional Oncology Center, Kielce, Poland, [5]Department of Gastroenterology Hepatology and Immunology The Children's Memorial Health Institute, Warsaw, Poland, [6]Institute of Pediatrics Polish-American Children's Hospital, Krakow, Poland, [7]Karol Marcinkowski University of Medical Sciences, Poznan, Poland

Peutz-Jeghers syndrome (PJS) is rare, genetically conditioned disease. PJS is heredited in autosomal dominant manner and is characterized by occurrence of hamartomatous polys. The hamartomatous polyps are manifested during second or third decade of life. The polyps can be located throughout digestive tract. Occurrence hamartomatous polys in PJS may cause of many gastrointestinal discomforts. Although in PJS patients the risk of malignant transformation is lower then others hereditary neoplastic disease, an increased risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed. The second characteristic manifestations of JPS are brown, dark or blue spots. PJS is caused by mutations in the LKB1 (STK11) on chromosome 19. LKB1 gene encodes a serine/threonine protein kinase participating in very important cell signaling pathways. Here we present the study considering 20 patients diagnosed with PJS. PJS diagnosis was based on presence of two or more polyps, or one polyp and typical pigmented lesions, or one polyp and a family history of PJS. Mutations screening analysis encompassing SSCP, HA and direct sequencing of the LKB1gene are reveled seven mutations and one polymorphism. These mutations are located in different position in gene. With the Multiplex Ligation-dependent Probe Amplification (MLPA) – assay we detected additional genomic mutations. For our screening we used the SALSA P101 STK11 kit which contains MLPA probes for most STK11 exons. In seven patients we identified exonic deletions or duplications range from one to five exons.

The study was financed by the Polish Ministry of Science and Higher Education, project no. N402 481537

P-MoleG-219

Analysis of the endothelial nitric oxide synthase protein levels in different areas of thoracic aortic aneurysms

Radtke A.[1], Belge G.[2], Nimzyk R.[2], Sievers H. H.[1], Bullerdiek J.[2], Mohamed S. A.[1]

[1]Department of Cardiac Surgery, Luebeck, Germany, [2]Center for Humangenetics, Bremen, Germany

Introduction: Deficiency of the enzyme endothelial nitric oxide synthase (eNOS) is associated with formation of a bicuspid aortic valve (BAV) in mice. Its expression is dependent of physiological shear stress in the aorta.

Methods and Results: During surgery aortic aneurysmal specimen of 21 patients (15 BAV, mean age 52.3±13.9 years and 6 normal aortic valve (TAV), mean age 54.7±14 years) were collected. eNOS protein levels were detected and quantified in 4 different areas of the same patients (the aorta outer curve, (convexity), the opposite site (concavity), the distal- and the proximal aneurysm) by western-blotting semiquantitative analyses.

eNOS concentration in TAV patients did not differ between aneurysmal areas (distal-, proximal aneurysm and convexity) compared to the opposite areas (concavity), whereas eNOS levels were significantly lower in BAV convex than concave aortic sites (P = 0.01). Cross-comparison between aortic areas of BAV and TAV patients showed a significantly lower expression of eNOS in BAV proximal aneurysm in contrast to TAV proximal aneurysm (P = 0.04).

Discussion: Aneurysmal areas of the convex aortic site as well as distal- and proximal aneurysm in BAV patients showed lower eNOS protein levels compared to the concave aortic site which leads to the assumption that a loss of eNOS produced NO might be responsible for the formation of aneurysm in these areas. eNOS concentration in the proximal aneurysm of BAV patients is lower than in the same area in TAV patients. This might indicate a shear stress induced downregulation in BAV proximal aneurysm or at least a minor upregulation when compared to TAV.

P-MoleG-220

Analysis of splice-site mutations in the human hairless gene in a cell-based system

Refke M.[1], Pasternack SM.[1], Fiebig B.[2], Ishorst N.[1], Seyger MM.[3], Nöthen MM.[4], Hamel BC.[5], Betz RC.[1]

[1]Institute of Human Genetics, Bonn, Germany, [2]Center for Human Genetics, Regensburg, Germany, [3]Department of Dermatology, Nijmegen, the Netherlands, [4]Institute of Human Genetics and Life & Brain Center, Bonn, Germany, [5]Department of Human Genetics, Nijmegen, the Netherlands

Congenital atrichia (AUC) is a form of isolated alopecia with an autosomal recessive mode of inheritance. Patients are born with normal hair but this is shed almost completely during the first weeks or months of life and never regrows. In many families the development of papular lesions is noted as an additional phenotypic feature, which defines a related phenotype designated as atrichia with papular lesions (APL). Using positional cloning strategies and the molecular findings in hairless recessive (hr/hr) mice, an animal model for AUC, mutations in the human hairless gene (HR) have been identified as a cause of AUC and APL. To date, nearly 50 different mutations of the HR gene have been reported in AUC and APL including different mutation types scattered over the entire HR gene length.

In this study, we identified two novel mutations in the HR gene: a T-insertion in exon 2 which leads to a frame shift and premature stop codon and a splice-site mutation in exon 14 abolishing normal splicing. So far six different splice-site mutations in HR have been published as being causal for hair loss. None of these mutations has been tested if they affect splicing, mostly due to the absence of mRNA samples.

To look for potential consequences of the splice-site mutations, we used the neural network (NN) Splice server (http://www.fruitfly.org/seq_tools/splice.html) and the web application termed CRYP-SKIP (http://www.dbass.org.uk/cryp-skip/), which shows the probability of exon skipping (EXSK) and cryptic site activation (CR-E). In order to analyse the newly identified homozygous splice-site mutation and the six so far known splice-site mutations, all mutants and an equivalent wild type were cloned into the Exontrap Cloning Vector (MoBiTec) using site-directed mutagenesis and primer combined with the restriction sites.

In summary, we found four times skipping of the proceeding exon, two times one base-pair deletions with premature stop codons and one time an activation of a cryptic splice site with an in-frame insertion of 12 amino acids. The results of two third of the splicing mutations had been expected due to the predicted computer splice programs whereas two splice mutations led to completely different situations. This study underscores the importance of splicing experiments in a cell-based system.

P-MoleG-221

SNP analysis in the DNA damage mediator checkpoint 1 (MDC1) gene in 386 British patients with systemic sclerosis

Rittner G.[1], Fonseca C.[2], Abraham D.[2], Zechner U.[1], Bartsch O.[1], Haaf T.[3]
[1]Institute of Human Genetics, Mainz, Germany, [2]Centre for Rheumatology, London, United Kingdom, [3]Institute of Human Genetics, Würzburg, Germany

Systemic sclerosis (SSc) is an autoimmune disorder characterized by excessive fibrosis, vascular abnormalities and immune system dysfunction that can affect several organs or tissues (mainly skin, lungs and kidneys). The disease is the end result of a complex interaction of genetic factors and unknown environmental influences. A linkage of increased chromosomal breakage rates in cultured lymphocytes of SSc patients and some of their family members with the Major Histocompatibility Complex (MHC) on the short arm of chromosome 6 has been described. This observation is suggestive for a DNA repair defect. One candidate gene within the MHC region is the DNA damage mediator checkpoint 1 (MDC1) gene. This gene has an amino-terminal forkhead-associated (FHA) domain, a tandem repeat of breast cancer susceptibility gene 1 carboxyl terminal (BRCT) motif and a central domain with 13 repetitions of an approximately 41-amino acid sequence. After DNA damage resulting in chromosomal double strand breaks, MDC1 binds to NBS1 (component of the MRN complex) mediating the formation of gamma-H2AX foci as a first step of DNA repair.

In a pilot study, all 15 exons of the MDC1 gene were sequenced in 28 German scleroderma patients. We identified very rare missense variants in exon 5 (SNP rs2517560) in one patient and in exon 7 (novel variant not registered as a SNP in the NCBI SNP database) in two patients. In addition, higher frequencies of the non-synonymous SNPs rs9262152 (exon 5) and rs28994875 (exon 11) were observed in the patients compared to the NCBI SNP general population. Further analysis of the SNP rs2517560 in the DNA of 386 British patients with systemic sclerosis (200 patients with limited scleroderma and 186 with diffuse scleroderma) and 208 German control DNAs detected the minor allele once in every subgroup of the British patients, but not in the German control DNAs. For the second SNP in the NBS1 binding region (rs9262152), no frequency differences between British patients and German controls were found. However, the frequency of the minor allele (A) of SNP rs28994875 located in between the BRCT motif was increased in the group of British diffuse scleroderma patients (0.04) compared to the group of British limited scleroderma patients (0.02). In addition, we investigated the frequency of the novel variant in exon 7 in the British patients and 500 German controls. The variant was detected in the German controls with an allele frequency for the minor allele of 0.003, but not in the British patients. Due to the low frequencies observed these results are not significant and so far provide no evidence for an association between MDC1 and systemic sclerosis.

P-MoleG-222

Characterization of the mechanisms associated with mitotic NAHR: type-2 NF1 deletions as a model

Roehl A.C.[1], Vogt J.[1], Zickler A.[1], Wimmer K.[2], Kluwe L.[3], Mautner V.M.[3], Kehrer-Sawatzki H.[1]
[1]Institute of Human Genetics, Ulm, Germany, [2]Department of Medical Genetics, Innsbruck, Austria, [3]Department of Maxillofacial Surgery, Hamburg, Germany

Recombination between highly homologous duplicated sequences (non-allelic homologous recombination, NAHR) gives rise to rearrangements such as deletions and duplications that are responsible for genomic disorders, a diverse group of heritable genetic diseases characterized by copy number changes of dosage-sensitive genes. Although NAHR occurring during meiosis in certain hotspot regions has been investigated thoroughly, the mechanism associated with mitotic NAHR is less well understood. In this study, we have used type-2 NF1 microdeletions as a model system in order to investigate the molecular mechanism and positional preference of somatic deletions in the NF1 gene region at 17q11.2. Type-2 NF1 deletions encompass 1.2Mb and have breakpoints located in the SUZ12 gene sequences flanking the NF1 gene region. These deletions are unique among known rearrangements associated with genomic disorders since they are generally caused by postzygotic mitotic recombination between the duplicated SUZ12 sequences, leading to somatic mosaicism in the affected NF1 patients. The reason why these deletions occur predominantly during mitotic cell divisions is still unknown. By contrast, many other genomic disorders, including the recurrent 1.4Mb spanning type-1 NF1 deletions with breakpoints located in the NF1-REPs, tend to be caused by aberrant recombination during parental meioses and are observed as germline deletions in the affected offspring.

In this study, we have investigated the mechanism underlying 18 examples of the type-2 NF1 deletion by breakpoint sequencing, SNP-array and microsatellite marker analysis of the patients, their relatives and somatic cell hybrids containing single chromosomes 17 of the respective patients. We deduce from these analyses that the type-2 NF1 deletions associated with somatic mosaicism in the affected patients are mediated by intrachromosomal non-allelic homologous recombination. We further infer that the deletions must have occurred during early embryonic development affecting cells derived from all three germinal layers, since high frequencies of cells harbouring the deletion were observed in different parental tissues. Finally, a mild but generalized form of NF1 was noted in all patients exhibiting somatic mosaicism for the type-2 NF1 deletion. The striking predominance of intrachromosomal NAHR and the mitotic origin of the deletions suggest that, in actively dividing cells with short cell cycles, the phases of interchromosomal pairing might be too short for interchromosomal NAHR to occur at high frequency. An alternative explanation for the observed association between intrachromosomal NAHR and the mitotic origin of most type-2 deletions would be that the genomic architecture of the 17q11.2 region in chromatin serves to alleviate illegitimate non-allelic pairing of the SUZ12 sequences and non-allelic homologous recombination either within one chromatid or between chromosomes.

P-MoleG-223

Inhibition of prostate cancer cell growth and the cellular invasiveness of prostate cancer cells by the new natural androgen receptor antagonist atraric acid

Roell D.[1], Papaioannou M.[1], Schleich S.[2], Prade I.[1], Degen S.[1], Schuber U.[1], Tanner T.[3], Claessens F.[3], Matusch R.[2], Baniahmad A.[1]
[1]Institute of Human Genetics and Anthropology, Jena, Germany, [2]Institute of Pharmaceutical Chemistry, Marburg, Germany, [3]Department of Molecular Cell Biology, Leuven, Belgium

Extracts from Pygeum africanum are used in the treatment of prostatitis, benign prostatic hyperplasia and prostate cancer (PCa), major health problems of men in Western countries. The ligand-activated human androgen receptor (AR) supports the growth of the prostate gland. Inhibition of human AR by androgen ablation therapy and by applying synthetic antiandrogens is therefore the primary goal in treatment of patients. A central issue in androgen ablation therapy is cancer resistance that occurs after 1 to 2 years of treatment with the current commonly used antiandrogens. The resistance often arises by reason of AR mutation. One often detected AR point mutation in PCa is the AR T877A mutant. This change of one amino acid renders some therapeuitcal applied antiandrogens into AR agonists and promote PCa progression. Therefore it is important to identify new antiandrogens, that inhibit besides the wild type (wt) AR also the AR T877A mutant.
Here, we show that atraric acid (AA) isolated from bark material of Pygeum africanum has antiandrogenic activity, inhibiting the transactivation mediated by both, the ligand-activated human wt AR and the

T877A mutant. This androgen antagonistic activity is receptor specific and does not inhibit the closely related glucocorticoid or progesterone receptors. Mechanistically, AA inhibits nuclear transport of AR. Importantly, AA is able to efficiently repress the growth of both the human androgen-dependent LNCaP cells, that contain the AR T877A mutant and also the androgen-independent C4-2 prostate cancer cells but not that of PC3 or CV1 cells lacking AR. In line with this, AA inhibits the expression of the endogenous prostate specific antigen (PSA) gene in both LNCaP und C4-2 cells. Analyses of cell invasion revealed that AA inhibits the invasiveness of LNCaP cells through extracellular matrix. Thus, this study provides a molecular insight for AA as a natural antiandrogenic compound inhibiting wt and mutant AR and may serve as a basis for AA derivatives as a new chemical lead structure for novel therapeutic compounds as AR antagonists, that can be used for prophylaxis or treatment of prostatic diseases.

P-MoleG-224

Detection of duplication breakpoints in the factor VIII gene

Rost S.[1], Zimmermann M.[1], Löffler S.[1], Emmert G.[1], Oldenburg J.[2], Müller C. R.[1]

[1]Department of Human Genetics, University of Würzburg, Germany, [2]Institute of Exp. Haematology and Transfusion Med., University Bonn, Germany

Introduction: Haemophilia A is caused by a variety of mutations in the factor VIII (F8) gene: missense and nonsense mutations, small and large deletions and insertions, as well as large inversions. In a recent study, we have used MLPA for the detection of large duplications comprising whole exons in haemophilia A patients. We have characterised some of the duplication breakpoints in order to understand the molecular mechanisms.

Methods and Results: MLPA analysis (multiplex ligation-dependent probe amplification, MRC-Holland) of 80 haemophilia A patients which showed no mutations by sequencing or inversion PCRs revealed exon-spanning duplications in a total of nine patients. The duplications affected single exons in two cases (exon 13 and 14, respectively) or more than one exon (exons 1-5, exons 5-25, exons 23-25, exons 2-25, exons 14-21 and exons 7-11, respectively) in seven cases. Assuming that these duplications are arranged in tandem, we performed long range PCRs with the corresponding DNA samples using primers located in the exons or introns presumably flanking the duplication breakpoints. Sequencing of the obtained fragments revealed the breakpoints of seven duplications. Some of the breakpoints could be mapped to short or long interspersed nuclear elements (SINEs or LINEs): two duplications were mediated by recombination events of Alu-repetitive sequences, three duplications were presumably induced by LINEs.

Conclusion: We characterized the breakpoints of seven exon-spanning duplications in the F8 gene of haemophilia A patients. We could show that these duplications are arranged in tandem and are therefore disrupting the F8 gene. Recombination events between repetitive sequences such as SINEs and LINEs in intronic sequences presumably contribute to the molecular mechanisms for the formation of duplications in the F8 gene.

P-MoleG-225

Expression levels of a truncated protein and clinical phenotype in Nijmegen Breakage Syndrome

Salewsky B.[1], Lins S.[1], Radszewski J.[1], Krüger L.[1], Rothe S.[1], Seemanova E.[2], Chrzanowska K.H.[3], Sperling K.[1], Digweed M.[1]

[1]Institut für Humangenetik, Charité - Universitätsmedizin Berlin, Germany, [2]Medical School of Charles University, Prague, Czech Republic, [3]Children's Memorial Health Institute, Warsaw, Poland

Patients affected by the autosomal recessive Nijmegen Breakage Syndrome (NBS [MIM 251260]) have possibly the highest risk for developing a malignancy of all the chromosomal instability syndromes. This reflects the profound disturbance to genomic integrity and cellular homeostasis that is caused by the mutation of the essential mammalian gene, NBN. Indeed, null-mutation of NBN is lethal in the mouse and NBS patients survive only due to the fact that the common human founder mutation, found in over 90% of patients, is in fact hypomorphic and leads, by alternative translation, to a partially functional carboxyterminal protein fragment. This 555 amino acid protein migrates on SDS-polyacrylamide gels as 70kDa and is termed here, p70-nibrin. p70-nibrin is clearly able to sustain vital cellular functions of the full-length protein.

Examining a panel of patient cell lines has indicated that there is considerable interindividual variation in the expression level of p70-nibrin. Cellular levels of p70-nibrin in cell lines correlate with the incidence of malignancy in patients. Family studies were carried out and suggest that p70-nibrin levels is subject to polygenic regulation. Using real-time PCR we have now found that the variation in p70-nibrin expression is not due differences in mRNA quantity. Thus, transcription rates and nonsense mediated mRNA decay (NMD) are not likely to be responsible for the observed variation. In agreement with this, inhibition of NMD did not lead to increases in NBN mRNA levels.

In contrast, we found a considerable increase in p70-nibrin levels when degradation by the proteasome was specifically inhibited. Thus protein turnover rates may be more relevant for determining p70-nibrin steady state levels. The results of analysing p70-nibrin degradation rates in patient cells will be presented and discussed with respect to clinical variability in NBS.

P-MoleG-226

The TAT gene in tyrosinemia type II: A mutational update

Scherer G.[1], Adler S.[1], Bausch E.[1], Fernández-Burriel M.[2], Fumic K.[3], Giguère Y.[4], Huppke P.[5], Lee N.C.[6], Mitchell G.A.[7], Mönch E.[8], Trimble E.R.[9], Trump D.[10], Vianey-Saban C.[11]

[1]Institute of Human Genetics, Freiburg, Germany, [2]Hospital Materno-Infantil, Las Palmas de Gran Canaria, Spain, [3]Zagreb University School of Medicine, Zagreb, Kroatia, [4]Service Génétique de Laboratoire, St-Foy, Canada, [5]Kinderklinik und Poliklinik, Göttingen, Germany, [6]Taipei Veterans General Hospital, Taipei, Taiwan, [7]Service de Génétique Médicale, Montréal, Canada, [8]Charité University Medical Center, Berlin, Germany, [9]Royal Victoria Hospital, Belfast, UK, [10]Addenbrooke´s Hospital, Cambridge, UK, [11]Hopital Debrousse, Lyon, France

Tyrosinemia type II (Richner-Hanhart syndrome, RHS; MIM 276600) is a rare autosomal-recessive disorder characterized by keratitis with photophobia, palmoplantar hyperkeratosis, variable mental impairment, and elevated blood tyrosine levels. The disorder results from deficiency in the liver-specific enzyme tyrosine aminotransferase (TAT). The 11 kb TAT gene at 16q22 encompasses 12 exons encoding a 454 amino acid protein. Starting with our initial publication (Natt et al., PNAS 89:9297, 1992), TAT mutations have up to now been identified in 18 RHS families totalling 18 different mutations: 1 deletion, 3 frameshift, 3 splice, 3 nonsense, and 8 missense mutations. We now identified 11 missense and 2 identical R297X nonsense mutations in 12 RHS families from 9 different countries. Of the 11 missense mutations, 2 recurred twice each, and 1 (R119W) has been described previously, so 8 missense mutations are novel: L76Q, A147V, T209I, A237P, N303I, D389N, P406L, R417Q. Notably, 4 of the 10 different point mutations are CpG to TpG or CpA transitions, confirming the CpG dinucleotide as a mutational hotspot in RHS (Hühn et al., Hum. Genet. 102:305, 1998). The relevance of the amino acid substitutions identified will be interpreted with respect to evolutionary conservation of TAT sequences and to 3D structures of related aminotransferases.

P-MoleG-227

CCM3 regulates endothelial cell proliferation, migration, and tube formation

Schleider E.[1], Stahl S.[1], Wüstehube J.[2], Walter U.[3], Fischer A.[2], Felbor U.[4]
[1]Department of Human Genetics and Institute of Clinical Biochemistry and Pathobiochemistry, Würzburg, Germany, [2]Joint Research Division Vascular Biology of the Medical Faculty Mannheim, Mannheim, Germany, [3]Institute of Clinical Biochemistry and Pathobiochemistry, Würzburg, Germany, [4]Institute of Human Genetics, Greifswald, Germany

Mutations in CCM1, CCM2 or CCM3 lead to cerebral cavernous malformation, one of the most common hereditary vascular diseases of the brain. Endothelial cells within these lesions are the main disease compartment. As of this moment, not much is known about cellular functions of CCM3. To gain better understanding of those, angiogenesis in vitro assays using HUVECs were conducted. While overexpression of CCM3 leads to dramatic negative effects on cell proliferation, migration, and tube formation, downregulation of CCM3 resulted in increased tube formation and unaltered proliferation and migration rates. Moreover, phospho-tyrosine-kinase arrays revealed a potential role for CCM3 modulating the PDK-1/Akt pathway. Together, we could demonstrate that CCM3 plays an important role in angiogenesis in vitro and that kinase profiling shows great promise for novel target identification for CCM treatment.

P-MoleG-228

The protein phosphatase Calcineurin: A modifying factor in Spinocerebellar ataxia type 3?

Schmidt T.[1], Elfert S.C.[1], Boy J.[1], Zimmermann M.[1], Lauber K.[2], Scoles D.R.[3], Lasbleiz C.[4], Wesselborg S.[2], Pulst S.-M.[3], Tricoire H.[4], Riess O.[1]
[1]Medical Genetics, Tuebingen, Germany, [2]Department of Internal Medicine I, Tuebingen, Germany, [3]Department of Neurology, Salt Lake City, USA, [4]Laboratory for genetics of stress and aging, Paris, France

Calcineurin (Protein phosphatase 3, PPP3) is a Calcium/Calmodulin dependent protein phosphatase consisting of the catalytical subunit Calcineurin A (60 kDa) and the regulatory subunit Calcineurin B (19 kDa). Calcineurin is highly abundant in the brain and has been implicated in several neurodegenerative disorders including Down syndrome, Alzheimer's disease as well as Huntington's disease. For Alzheimer's disease it has been shown that calcineurin inhibition can prevent neuronal dysmorphalogy and improve memory function. Calcineurin inhibition prevented in a tissue culture model of Huntington's disease the polyglutamine-mediated cell death. In a different study, however, Calcineurin inhibition intensified the phenotype in a transgenic mouse model.

Here, we analyzed the possible role of Calcineurin in Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD). SCA3/MJD is a neurodegenerative disorder which is caused by the expansion of a CAG repeat in the MJD1 gene resulting in a polyglutamine expansion in the encoded ataxin-3 protein. Therefore, SCA3/MJD, like Huntington's disease, belongs to the group of polyglutamine diseases.

In brain samples of SCA3/MJD patients, we detected Calcineurin within neuronal intranuclear inclusion bodies, one hallmark of SCA3/MJD. We also observed a co-localization between ataxin-3 and Calcineurin but could not confirm a direct interaction between both proteins. In addition, we measured a reduced Calcineurin activity in cells transfected with expanded ataxin-3 constructs.

In order to clarify these results, we analyzed the role of Calcineurin for polyglutamine-induced pathogenesis in vivo using a transgenic fly model. The SCA3 fly model was analyzed for degeneration of neurons as well as differences in the lifespan of the flies as consequences of both increased and decreased expression of Calcineurin. To analyze the effect of an increased amount of Calcineurin, we crossed the SCA3 flies with flies containing a duplication of the Calcineurin locus. We also analyzed whether partial deletion of Calcineurin modifies the phenotype and fully deleted the Calcineurin gene in the fly. Since homozygous flies are lethal we then analyzed whether heterozygous deletion influences the lifespan of an SCA3 fly model. However, neither the overexpression nor the reduction of Calcineurin influenced the phenotype of flies expressing expanded ataxin-3. We therefore concluded that, although in vitro data might implicate a role of the protein, Calcineurin does not influence the phenotype in SCA3.

P-MoleG-229

A multi-method molecular approach to identify novel pathogenic alleles in hearing loss patients with monoallelic GJB2 mutations

Schmitt B.[1], Schneider E.[2], Wetzig C.[1], Kohlschmidt N.[1], Keilmann A.[3], Bartsch O.[1], Haaf T.[2], Zechner U.[1]
[1]Institute of Human Genetics, Mainz, Germany, [2]Institute of Human Genetics, Würzburg, Germany, [3]Department of Communication Disorders, Mainz, Germany

Most cases of congenital non-syndromic sensorineural hearing loss are caused by biallelic (compound heterozygous or homozygous) mutations in the GJB2 gene encoding connexin 26. In a significant portion of patients (10-30%), only monoallelic (heterozygous) GJB2 mutations are identified. Some of these cases can be explained by the presence, in trans, of a 309-kb deletion, del(GJB6-D13S1830), or a 232-kb deletion, del(GJB6-D13S1854), truncating the GJB6 gene (encoding connexin 30) upstream of GJB2 and also preventing the expression of the GJB2 allele in cis. Here, we present a multi-method molecular approach to identify novel possibly pathogenic alleles in patients with monoallelic GJB2 mutations or variants for whom the presence of del(GJB6-D13S1830) and del(GJB6-D13S1854) had already been excluded. Mutation analysis of the coding regions of GJB6, GJB4 (encoding connexin 30.3) and GJA1 (encoding connexin 43) in 38 patients identified three patients with already previously reported monoallelic mutations in the GJB4 gene (p.C169W and c.154_157del, respectively) and one patient with a novel monoallelic variant in the GJA1 gene (p.G321V). In addition, we performed methylation analysis by bisulphite pyrosequencing of the GJB2 promoter in blood DNA of 32 patients, analysis of GJB2 replication timing in lymphocytes of 25 patients and allele-specific GJB2 expression analysis of RNA from lymphoblastoid cell lines of 7 patients which all provided no evidence for silencing of the normal GJB2 allele. To search for smaller deletions or duplications affecting possible GJB2 regulatory elements, the blood DNA of 30 patients was further analyzed by genomic quantitative PCR with five different amplicons equally distributed over the 135-kb genomic region upstream of the GJB6 gene. We found two patients with a duplication of the amplicon located 38 kb upstream of the GJB6 transcriptional start site, one patient with a deletion of the amplicon located 88 kb upstream of the GJB6 transcriptional start site and six patients with a duplication of the amplicon located 135 kb upstream of the GJB6 transcriptional start site. Allele-specific GJB2 expression analysis of buccal and saliva RNA of these patients will be carried out to verify if these copy number changes are associated with a silencing of the normal GJB2 allele. Our studies may lead to the discovery of further deafness-associated GJB2 regulatory mutations and/or digenic inheritance of deafness caused by monoallelic mutations in GJB2 and other unlinked genes encoding connexin proteins.

P-MoleG-230

Hypo- and hypermorphic nuclear changes in cells with up to three functional copies of the lamin B receptor gene - A model for studying gene-dosage effects and autosomal dosage compensation in humans

Schnipper N.[1], Gravemann S.[1], Meyer H.[1], Vaya A.[2], Nowaczyk MJM.[3], Rajab A.[4], Hofmann WK.[5], Salewsky B.[6], Tönnies H.[6], Neitzel H.[6], Stassen HH.[7], Sperling K.[6], Hoffmann K.[1]

[1]Institute of Medical Genetics - Charité University Medicine, Berlin, Germany, [2]Department of Clinical Pathology - La Fe University Hospital, Valencia, Spain, [3]Departments of Pediatrics and of Pathology and Molecular Medicine - McMaster University, Hamilton - Ontario, Canada, [4]Genetic Unit - Ministry of Health, Muscat, Sultanate of Oman, [5]Department of Hematology and Oncology and Transfusion Medicine - Charité University Medicine, Berlin, Germany, [6]Institute of Human Genetics - Charité University Medicine, Berlin, Germany, [7]Research Group‚Psychiatric Genetics and Genetic Epidemiology' - Psychiatric University Hospital, Zurich, Switzerland

Background: The lamin B receptor (LBR) is a component of the inner nuclear membrane with dual function. The nucleoplasmic part has structural impact on nuclear shape and chromatin organization; the transmembrane part belongs to the sterol reductase family. We have previously shown that LBR mutations result in hyposegmentation of neutrophil nuclei (Pelger anomaly). The typical nuclear structure of human neutrophils is an evolutionarily evolved and conserved characteristic: Ovoid nuclei are found in most non-vertebrates, followed by rodlike or bilobed nuclei in fish and birds, and multi-segmented nuclei in neutrophils of most mammals. This is reminiscent of the stages in human granulopoiesis since CD34+ progenitor cells in the bone marrow do also present with ovoid nuclei progressing to rod-like or bilobed forms and ending in a multi-segmented nuclear shape in mature neutrophils.

Aims: The aim of the project was to exactly quantify the gene-dosage effect of LBR on nuclear structure in vivo and in vitro.

Material and methods: We quantified the number of nuclear segments in neutrophils in blood smears, analyzed the effect of different mutation types and studied the LBR expression during regular granulopoiesis. We analyzed blood smears from 2 homozygous and 9 heterozygous Pelger individuals, 12 controls, and 3 individuals with triplication of the LBR gene. Three copies occur naturally in patients with cytogenetic duplications that include the LBR gene on chromosome 1q42. This extends the previously described three conditions to 4 possible LBR doses. To study LBR expression granulopoiesis, we used an in vitro granulocyte differentiation model.

Results: We found that the number of functional copies of the LBR gene (0-3) is proportional to the amount of LBR protein and significantly associated with the segmentation grade of neutrophil nuclei ($P<0.0001$). However, we also observed that the increase in the lobulation index from 2 to three LBR copies as compared to 0?1 and 1?2 is less expressed, pointing to a dosage compensation effect.

Interestingly, we found that heterozygous persons with the splice mutation IVS12-5-10del had more nuclear segments than those of the four heterozygous persons with null mutations ($P<0.0001$), demonstrating significant allelic-dosage effect of LBR.

We further show that LBR expression continually increases during granulopoiesis in vitro from human precursor cells with ovoid nuclei to multi-segmented neutrophil nuclei 11 days later indicating relevance for regular human granulopoiesis.

Conclusions: Our data demonstrate that there is a highly significant gene-dosage effect between the amount of LBR and the degree of nuclear segmentation. As to our knowledge, this is one of the few or the only human gene where 4 doses can be studied by a simple routine clinical setting such as blood smears. Further, we showed that this effect is also relevant for regular human granulopoiesis; the differentiation process of neutrophil nuclei is correlated with an increase in the expression of the LBR gene.

The results indicate that the lamin B receptor is an ideal model to study gene-dosage effects systematically and even to analyze the consequences of perturbations by exogenous factors. The affected cellular phenotype (nuclear hyposegmentation of neutrophils) is relevant for frequent clinical conditions such as inflammation, myelodysplasia, acute myelogenous leukemia, toxic drug reaction, and vitamin B12 or folate deficiency.

P-MoleG-231

A new transgenic mouse model for TSPY, the TSPY transgenic mice line Tg(TSPY)15Jshm

Scholz M.[1], Gonzalez D.[1], Adham I.[2], Meinhardt A.[3], Schmidtke J.[1], Schubert S.[1]

[1]Hannover Medical School, Institute of Human Genetics, Germany, [2]University of Göttingen, Institute of Human Genetics, Germany, [3]Justus-Liebig-University of Giessen, Department of Anatomy and Cell Biology, Germany

TSPY encodes the testis-specific protein, Y encoded. In man, expression of TSPY is restricted to germ cells of the testis, and epithelial cells of the prostate. There is circumstantial evidence that TSPY is involved in spermatogonial proliferation and gonadal and prostate tumorigenesis. Because the laboratory mouse carries the Tspy gene in a naturally silenced state, we previously restored TSPY activity in mice and generated a TSPY transgenic mouse line, Tg(TSPY)9Jshm, in which the organization and expression of the human TSPY transgene follow the human pattern. We here describe a second TSPY transgenic mice line, Tg(TSPY)15Jshm, harbouring a complete structural TSPY gene and 2.110 bp instead of 2.923 bp of the putative human TSPY promoter region. In this line, a single copy of the human transgene was inserted in 3'-5' orientation at a single integration site on the murine chromosome 8, in contrast to our previuos transgenic attempt with Y-chromosomal integration (Tg(TSPY)9Jshm). The transgene is abundantly transcribed in the testis in male transgenic mice, ectopically transcribed at basal levels in all analysed male and female organs, and spliced according to the human pattern. The testicular cell-specific expression pattern of the human TSPY transgene is different in comparison to that observed in the TSPY transgenic mouse line Tg(TSPY)9Jshm and in humans. TSPY is expressed in primary spermatocytes of the leptotene, zygotene and pachytene stage and in round spermatids, but not in spermatogonia, in which TSPY is mainly expressed within human testes. Our findings indicate that 2.110 bp of human TSPY promoter is not capable of directing the expression of TSPY in spermatogonia of transgenic mice. Histological examinations of testes of 12 to 16 months old homozygous TSPY transgenic males from the mouse line Tg(TSPY)15Jshm indicated a significant vacuolation of the seminiferous germinal epithelium in comparison to age-matched controls. The human TSPY transgene is also expressed in the luminal glandular epithelial cells of the prostate. The TSPY transgenic line Tg(TSPY)15Jshm will serve as an animal model for further investigations of TSPY function in the human prostate and its putative role in the tumorigenesis of prostate cancer.

P-MoleG-232

Characterization of selected aberrant splice sites in the human F7 gene

Schröder W.[1], Astermark J.[2], Auerswald G.[3], Felbor U.[1], Herrmann F.H.[1], Wulff K.[1]

[1]Institute for Human Genetics, Greifswald, Germany, [2]Dept. Coagulation Disorders, Malmö, Sweden, [3]Hämophilie Zentrum, Bremen, Germany

In the „Greifswald Registry FVII Deficiency", molecular defects of more than 1000 FVII deficient (FVIID) patients were described. 14% of mutations affect acceptor or donor splice sites. Selected splice sites in the F7 gene were characterized by in vitro studies using the exon trap vector pET01. To further confirm the causality of these mutations total RNA from patients was isolated from peripheral leucocytes and analysed by one step RT-PCR and sequencing.

Most mutations in donor and acceptor splice sites cause exon skipping while intron retention was observed for short intron 3 in about 50% of cases. Atypical was a novel variation in intron 5 (c.571+78G>A) detect-

ed in 7 FVIID patients. This intronic nucleotide substitution creates an aberrant donor splice site 79 bp downstream of exon 5 which was exclusively used in the mRNA of the affected allele. Furthermore, it could be demonstrated in vitro that the consensus value is the most important factor for the selection of the donor splice site of F7 exon 5. Another intronic mutation, c.805+1G>A, results in activation of a cryptic splice site located within the first repeat of a minisatellite characterized by 4 to 8 repeated 37 bp elements at the 5'end of intron 7.
Most splice site mutations result in a frameshift which predicts premature termination.
By transcript analysis, the causality of all splice site variations investigated could be confirmed. Furthermore, it was demonstrated that mutations deep in the intron can be causative for FVIID.

P-MoleG-233
Screening of the CCDC66 gene in patients with Retinitis Pigmentosa and Leber Congenital Amaurosis
Schulte-Middelmann T.[1], Kropatsch R.[1], Petrasch-Parwez E.[2], Gerding W. M.[1], Epplen J. T.[1], Gal A.[3], Dekomien G.[1]
[1]Human Genetics, Bochum, Germany, [2]Department of Neuroanatomy and Molecular Brain Research, Bochum, Germany, [3]Department of Human Genetics, Hamburg, Germany

Background: Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies in which the loss of photoreceptor cells via apoptosis leads to blindness. The homologous disease in dogs, generalised progressive retinal atrophy (gPRA), is also a heterogeneous group of inherited retinopathies. The dog represents a well established animal model for human RP because canine mutations are paralleled by disease-causing mutations in homologous human RP genes. Recently, two separate mutations were found in the newly identified canine gene coiled-coil domain containing 66 (CCDC66) which cause autosomal recessive transmitted gPRA in dogs. Although the function of the CCDC66 gene is not known, CCDC66 appears to be preferentially expressed in inner segments of photoreceptor cells. Hence, CCDC66 is a candidate gene for RP in human.
Methods: We screened 80 RP patients and 20 cases of Leber Congenital Amaurosis (LCA) for CCDC66 mutations by SSCP and DNA sequence analyses of conspicuous banding patterns.
Results: Until now, we identified 20 sequence variations in the human CCDC66 alleles, three variations had not been published yet. A known three base pair insertion was observed in CCDC66 exon 15. For comparison, 200 healthy controls were screened for the aforementioned variation. A causal role of this variation in retinal diseases is not evident as of today.
Conclusion: Although no pathogenic mutation has been found in the CCDC66 gene in RP and LCA patients so far, this gene represents still a promising candidate for retinal diseases. We continue to screen larger cohorts of respective patients.

P-MoleG-234
Fanconi anemia patients of type I depend on hypomorphic mutations in FANCI
Schuster B.[1], Neveling K.[1], Hoehn H.[1], Smogorzewska A.[2], Hanenberg H.[3], Schindler D.[1]
[1]Department of Human Genetics, University of Wuerzburg, Germany, [2]Laboratory of Genome Maintenance, Rockefeller University New York, USA, [3]Department of Pediatric Oncology Hematology and Clinical Immunology, Heinrich Heine University Duesseldorf, Germany

Fanconi Anemia (FA) is a rare autosomal or X-chromosomal recessive disease with great genetic and phenotypic heterogeneity. FA is characterized by bone marrow failure, high cancer risk and various, non-obligatory, yet typical developmental anomalies. Hypersensitivity of FA cells to DNA crosslinking agents such as mitomycin C (MMC) results in chromosomal breakage and G2-phase arrest in the cell cycle. To date, 13 FA genes FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M and -N have been reported. Their products and additional proteins interact in the FA/BRCA DNA damage response network. The most recently detected FA gene - FANCI - was first reported in 2007. To date, altogether nine FA-I patients and their FANCI mutations have been published. Four additional unpublished cases are listed in the Fanconi Anemia Mutation Database. Since molecular and clinical information is still limited, it appears worthy to study more FA-I patients in detail in order to characterize the clinical and mutational spectrum. We used retroviral complementation strategies to exclude patients` cells from belonging to the upstream complementation groups (FA-A, -B, -C, -E, -F, -G, -L). Subtypes FA-M, -D2, -J, -D1 and -N were ruled out by immunoblotting. Assignment of cells to group FA-I was also by Western Blotting. DNA sequencing with mutation detection confirmed positive classifications. We present three additional patients of complementation group FA-I. All of them carry private mutations that have not been reported previously. Among them are three splice site mutations, two nonsense mutations and one small deletion. This deletion is in frame and removes just one amino acid. The other mutation in patient 1 is a splice site mutation that retains low levels of correctly spliced transcript. Patient 2 carries two splice site mutations. At least one of them gives rise to small amounts of correctly spliced transcript. Patient 3 carries two nonsense mutations in exon 36 and 37 and shows truncated protein. Low levels of residual protein were detected in cells from all of these patients, consistent with the hypomorphic nature of the underlying mutations. The cellular FA-I phenotype included hypersensitivity to DNA crosslinking agents upon chromosome breakage and cell cycle analysis, and cultured fibroblasts also revealed hypersensitivity to ambient oxygen concentration. Clinical features of these patients showed an average FA spectrum of severity. To sum up, FANCI mutations appear to be private. Most or all FA-I patients seem to carry hypomorphic combinations of mutations and show residual protein levels. These findings are consistent with the report of Kalb et al. (2007) for FANCD2 and the hypothesis of Sims et al. (2007) for FANCI that biallelic null mutations may be lethal in humans. Clinical and cellular features of group FA-I are indistinguishable from those of the major FA subtypes. Definitive data on FA-I patients and FANCI mutations may have prognostic value and may influence therapeutical strategies.

P-MoleG-235
Shank3 mutations found in autism patients increase binding of Shank3 to Sharpin by releasing an intramolecular blockade.
Sieber S.[1], Kollmorgen G.[1], Mameza M.G.[2], Güler T.[1], Hönck H.-H.[1], Kreienkamp H.-J.[1]
[1]Institut für Humangenetik, Hamburg, Germany, [2]Institut für Zellbiochemie und klinische Neurobiologie, Hamburg, Germany

Recent investigations into the genetic causes for autism spectrum disorders have identified mutations in several genes coding for synaptic proteins in affected individuals. In case of the gene coding for the postsynaptic scaffolding protein Shank3, several deletions and nonsense mutations were found which are likely to lead to a loss of Shank3 function. In addition, several missense mutations were identified in the region coding for the N-terminal portion of Shank3, which contains an ankyrin repeat domain. The relevance of these mutations for Shank3 function remained unclear. By expression of wild type and mutant Shank3 in a human cell line we show here that mutations L68P, R300C and Q321R affect binding of Shank3 to its interaction partner Sharpin in a positive manner, such that more Sharpin can be coimmunoprecipitated with mutant Shank3 than with wild type Shank3. Sharpin binds to the ankyrin repeat region of Shank3, which includes residues R300 and Q321, but not L68. A yeast two hybrid screen shows that the ankyrin repeat region is also involved in an intramolecular interaction with the N-terminal region (residues 1-82) of Shank3; binding of Sharpin to the ankyrin repeat region of Shank3 is blocked by this intramolecular interaction. The L68P mutation (within the N-terminal segment) renders the N-terminal part unable to bind to

the ankyrin repeat region, allowing for unrestricted binding of Sharpin to mutant Shank. Thus the Shank3 mutations observed in autism patients present a gain of function phenotype with respect to Sharpin binding, which may provide an explanation why mutations may be pathogenic when found in heterozygous patients.

P-MoleG-236

A clear effect of chromosomal constitution on autosomal and X-linked LINE-1 methylation: a correlation tendency of higher autosomal L1 methylation in smaller genomes

Singer H.[1], Meesters C.[2], Nüsgen N.[1], Schreiner F.[3], Woelfle J.[3], Kress W.[4], Kalscheuer V.[5], Schwaab R.[1], Wienker T.[2], Oldenburg J.[1], Becker T.[2], El-Maarri O.[1]
[1]Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany, [2]Institute of Medical Biometry, Informatics and Epidemiology, Bonn, Germany, [3]Pediatric Endocrinology Division Children's Hospital, Bonn, Germany, [4]Institute of Human Genetics, Würzburg, Germany, [5]Max Planck Institute for Molecular Genetics, Berlin, Germany

LINE-1 repeats account for about 17% of the human genome, however little is known about their individual methylation levels and patterns. This is mainly because their repetitive identical sequence makes them difficult to be specifically targeted at a given locus. In this study, we used bisulfite conversion followed by semi-nested PCR approach to study individual LINE-1 repeats; the methylation at different CpG sites was than quantified by SIRPH or pyrosequencing. Using this approach we studied the effect of the chromosomal constitution on the patterns and levels of methylation at individual LINE-1 repeats; this included 6 X-linked loci subject to inactivation, 6 X-linked loci that escape inactivation and 5 autosomal loci in 45 Xo, 46 XY, 46 XX and 47 XXY individuals. Our analysis revealed a clear difference in levels of methylation at X-linked regions that are subject to inactivation. XX females and XXY males (Klinefelter Syndrom) showed about 30-50% less methylation than XY males and Xo females (Turner Syndrom), which shows that these repeats, like the body of X-linked genes, contribute to the hypomethylation of the inactive X. Moreover, LINE-1 X-linked loci at the telomere or centromere that escape the inactivation process, have methylation levels similar to what is seen on autosomes. The fact that the regions escaping inactivation show LINE-1 methylation levels, in all 4 genotypes, similar to autosomal LINE-1 loci suggests a potential role of these repeats in maintaining or spreading the inactivation process. Most interestingly, methylation levels at autosomal LINE-1 showed a correlation tendency between the level of methylation and the size of the genome, with the highest methylation observed in Xo and the lowest in XXY individuals. This novel observation point to a limiting factor enforced by the number of methylatable CpG sites or the genome size; such factors could be the availability of the methyl donor group or the enzymes involved in the methylation process.

P-MoleG-237

Craniosynostotic syndrome with mild phenotypic expression caused by a novel splice-site mutation in the FGFR2-gene

Stelzer C.[1], Trübenbach J.[1], Scheele R.[2], Wildhardt G.[1], Steinberger D.[1], Steinberger D.[3]
[1]bio.logis Center for Human Genetics, Frankfurt am Main, Germany, [2]Kaiserswerther Diakonie Florence-Nightingale-Krankenhaus, Düsseldorf, Gemany, [3]Institute of Human Genetics Justus Liebig University, Gießen, Germany

Craniosynostosis syndromes comprise a spectrum of phenotypic expressions that were before the era of molecular classification designated as Crouzon, Pfeiffer, Apert and Jackson-Weiss syndrome. These syndromes are caused by mutations in the genes coding for Fibroblast Growth Factor Receptors 1, 2 and 3 (FGFR1, FGFR2, FGFR3). A portion of FGFR-associated craniosynostotis are caused by genetic alterations affecting the IgIII-loop of the FGFR2-gene.

The IgIII domain of the FGFR2 protein is encoded by exons that undergo an alternative splicing of a common 5´exon, IgIIIa, onto either an IgIIIb or an IgIIIc variant exon. The significiance of this alternative splicing in the IgIII domain is that it indicates ligand specificity.
Here, we report the results of the molecular genetic analysis of a two and a half year old girl with complete synostosis of the sagittal and lambdoid sutures. The girl carries the nucleotide change c.1084+2T>A in intron 9 of the FGFR2-gene in a heterozygous state. Computer assisted analyses indicate that splicing of the IgIIIc domain will be affected by this mutation. Clinical features seen in the affected patient is discussed in the context of our molecular findings.

P-MoleG-238

Mutational analysis of FHL1 in patients suffering from Emery-Dreifuss muscular dystrophy

Sukalo M.[1], Schröder W.[1], Felbor U.[1], Wehnert M.[1]
[1]Institute of Human Genetics, Greifswald, Germany

Emery-Dreifuss muscular dystrophy (EDMD) is a rare neuromuscular disease characterized by early contractures, slowly progressive muscular weakness and life-threatening cardiac arrhythmias. Clinically and genetically, a wide intra- and interfamilial variability can be observed. Genetically, five EDMD types can be distinguished caused by mutations in LMNA, EMD, SYNE1 and SYNE2. Our findings revealed that mutations of these genes only cover 43% of unrelated EDMD patients. Consequently, EDMD is supposed to show further genetic heterogeneity. Recently, mutations in FHL1 (four and a half LIM domains 1) have been associated to XMPMA (X-linked myopathy with postural muscle atrophy) which has a phenotype similar to EDMD. Hence, FHL1 was considered in the present study as a candidate gene to be associated to EDMD. The coding regions of 151 patients that clinically expressed EDMD and similar diseases but were negative for mutations in LMNA, EMD, SYNE1 and SYNE2 have been analysed by direct Sanger sequencing. A novel putative amino acid substitution, p.C209R, was found affecting a highly conserved cysteine residue in the third LIM domain of the FHL1 peptide. Loss of the cystein residue is supposed to have deleterious effects on the tertiary structure of FHL1 which might lead to disturbances in the regulation of cytoskeletal dynamics. Co-segregation with the rather variable clinical manifestation in a large German family confirmed the pathogenic effect of the mutation. In four unrelated German families, the previously published amino acid exchange p.C244W was identified most likely indicating a mutational hot spot or an old mutation in the population. A further previously described putative amino acid exchange, p.D275N, affected four unrelated patients. It modifies a less conserved residue at the C-terminus of the FHL1 peptide and was found with a frequency of 3.1% in a normal population (n=225 alleles tested). Moreover, the lack of co-segregation with the disease phenotype qualifies this variation most likely as a non-pathogenic SNP.
In the present study, 3.5% of the patients from the LMNA, EMD, SYNE1 and SYNE2 negative EDMD-cohort showed FHL1 mutations. Consequently, EDMD patients that were excluded to carry mutations in LMNA and EMD should also be screened for FHL1 mutations. However, further genetic heterogeneity in EDMD has to be expected.

P-MoleG-239

Studies on the proteolysis of huntingtin in the transgenic rat model of Huntington's Disease

Thiele K.[1], Wree A.[2], Pahnke J.[3], Nguyen H. P.[4], Riess O.[4], Holzmann C.[1]
[1]Department of Medical Genetics, Rostock, Germany, [2]Institute for Anatomy, Rostock, Germany, [3]Department of Neurology, Rostock, Germany, [4]Department of Medical Genetics, Tuebingen, Germany

Huntington's disease (HD) is a polyglutamine disorder based on the expansion of a CAG triplet repeat in the huntingtin gene (htt) leading to cerebral and striatal neurodegeneration. The clinical triad of motor

dysfunction, cognitive decline, and psychiatric manifestations characterizes HD.
We have recently generated a transgenic rat model of HD expressing truncated huntingtin protein (exons 1- 14) with 51 CAG repeats. This rat model closely resembles the human HD phenotype exhibiting emotional disturbance, motor deficits and cognitive decline.
Proteolysis of mutant huntingtin is crucial to the development of Huntington disease. The group of Michael Hayden generated YAC mice expressing caspase-3- and caspase-6-resistant mutant htt (Graham et al. (2006), Cell 125:1179). Mice expressing mutant caspase-6-resistant htt maintain normal neuronal function and do not develop striatal neurodegeneration. In contrast, preventing caspase-3 cleavage of mhtt provides no protection from striatal atrophy in vivo.
To investigate the cleavage of htt in the transgenic rat model we performed western blot of brain lysates using several N-terminal anti-htt and anti-polyglutamine antibodies. As expected, endogenous and transgenic (mutant) rat htt is cleaved by both recombinant caspase-3 and caspase-6. We found a significant decrease of endogenoeus htt and mutant htt in the striatum of transgenic rats older than 12 month. In contrast, we identified no significant changes in the cortex of the same animals. These results will be verified by immunohistochemistry and expression analysis.
Currently we investigate the toxicity of mutant rat htt resistant to cleavage by caspase-3 and caspase-6, the subcellular distribution of caspase-3 and -6 cleaved rat htt and the age-dependent expression of apoptosis-related genes in brains of tgHD rats.
Supported by the FORUN-program of the Medical Faculty of the University of Rostock.

P-MoleG-240

Validating an in-silico score to determine the nature of putative splice site variants on the basis of PKHD1 alterations

Venghaus A.[1], Ortiz Brüchle N.[1], Zerres K.[1], Bergmann C.[2]
[1]Insitute of Human genetics, Aachen, Germany, [2]Bioscientia Center for Human Genetics & Institute of Human Genetics, Aachen, Germany

Mutations in the PKHD1 gene on chromosome 6p12 cause autosomal recessive polycystic kidney disease (ARPKD) characterized by massively enlarged bilateral polycystic kidneys and congenital liver fibrosis. The longest open reading frame of the PKHD1 gene contains 66 exons and encodes a membrane protein called polyductin. In about 90% of patients with typical clinical features at least one PKHD1 mutation can be detected. Most of the variants alter the amino acid sequence but approximately 5% are splice site mutations. Potential effects in alterations others than canonical splice-site defects are generally difficult to assess in a routine-diagnostic setting. In addition to potential splice site alterations the correct formation of the spliceosome is influenced by intronic splicing enhancers and silencers (ISE and ISS) and their exonic counterparts (ESE and ESS). In case of PKHD1, the validation is even further complicated by the gene's large size and lack of expression in lymphocytes. Many web applications exist for splice site analyses and splice enhancer/silencer binding site prediction but they differ in the used algorithm and in their accuracy. We are currently evaluating criteria how to use these different web tools to estimate whether a single nucleotide alteration affects splicing or not. Using these criteria we will develop a score to assess the results of the different tools. We are focusing on two different groups of possible splice sites: a) single nucleotide alterations within the first twenty basepairs up- and downstream of the intron/exon boundary. b) silent and neutral changes in the coding sequence which might lead to exon skipping. We are validating the score by comparing our data with those from known unambiguous splicing mutations from our PKHD1 database and further data from the literature. This score will be helpful for a more accurate determination of the pathogenic nature of 60 further putative PKHD1 splicing relevant variants in our database and other variants of unknown significance.

P-MoleG-241

Distinct AZFb gene deletions cause different testicular pathologies

Vogt PH.[1], Raabe-Meyer G.[2], Bender U.[1], Schulze B.[2]
[1]Molecular Genetics and Infertility Unit; Department of Gynecological Endocrinology and Reproductive Medicine, Heidelberg, Germany, [2]Praxis für Humangenetik, Hannover, Germany

Deletion of the AZFb region in the long arm of the human Y chromosome encompasses 11 Y genes which encode proteins expressed in testicular tissue (last review: Vogt, 2008; in: Int. J. Androl. 31: 383-394). Its classic extension was first characterized by the presence or absence of a series of genomic Y probes and STS markers in the long arm of the human Y chromosome (Yq11). After having established the corresponding genomic Y sequence (Skaletsky et al. 2003; in: Nature 423: 825-837) it was found that the molecular mechanism of its origin was based on some intrachromosomal non-allelic homologous recombinations (NAHRs) of long repetitive sequence blocks also called "amplicons" located in different palindromes of the AZFb and AZFc region in Yq11.
It has been repeatedly reported that the occurrence of these "classic" AZFb deletions in men with idiopathic infertility is always associated with the same testicular pathology, namely, a meiotic arrest of the patients´ spermatogenesis process. That means whereas all types of premeiotic spermatogonia and spermatocytes are present, no postmeiotic spermatids and mature sperms could be observed in their testis tissue sections. In contrast of this, smaller AZFb deletions were found to be associated with different testicular pathologies including the complete absence of germ cells called Sertoli-Cell-Only (SCO) syndrome.
Because we wanted to know whether these distinct testicular pathologies were probably associated with the deletion of different Y genes located in AZFb, we have set up some PCR-multiplex assays to distinguish the deletion of each AZFb gene, separately. Additionally, we developed some novel PCR assays to study the molecular location of the proximal and distal AZFb breakpoint regions in the distinct amplicons in more detail.
When we applied this AZF gene deletion assays on some infertile male patients with the indication of a classical AZFb deletion but with variable testicular pathologies not including meiotic arrest we recently found a partial AZFb deletion in a man with severe Oligo-Astheno-Terato (OAT)-zoospermia. The patient displayed a normal 46,XY karyotype and PCR STS analyses for the AZF microdeletions pointed to the presence of a classical AZFb deletion. Both genomic AZFb routine STS markers were absent. However, extension of this AZFb deletion assay by detailed analyses of the proposed proximal and distal AZFb breakpoints and a screen for deletion of all AZFb genes revealed a novel distal AZFb breakpoint region between the repetitive RBMY and PRY gene families. We like to discuss the possibility that this pattern of AZFb gene deletions most likely have caused the patient´s sperm pathology

P-MoleG-242

Dnmt1 Overexpressing Mice as a Model for Epigenetic Diseases

Wagner N.[1], Vaßen L.[2], Horsthemke B.[1]
[1]Institut für Humangenetik, Universitätsklinikum Essen, Germany, [2]Institut für Zellbiologie, Universitätsklinikum Essen, Germany

One of the epigenetic mechanisms by which gene expression is regulated is the methylation of DNA. Erroneous DNA methylation can cause a vast spectrum of diseases. A mouse model was created in this study to investigate whether the overexpression of the somatic form of Dnmt1, Dnmt1s, can predispose to erroneous methylation and disease.
A CAG promoter-driven transgene containing the eGFP marker protein and the Dnmt1s DNA methyltransferase was constructed. Transgene functionality was tested in 3T3 murine fibroblasts in cell culture. Because the ubiquitous overexpression of Dnmt1 has been reported to be embryonic lethal, a conditional transgene was constructed using

the Cre-LoxP system. This technique allows the initial expression of the eGFP marker protein only. Upon cross-ins with Cre-recombinant mouse lines, the eGFP sequence is cut out and degraded. This leads to the expression of the Dnmt1s under control of the CAG promoter. To easily distinguish between endogenous and transgenic methyltransferase, a RGS-His-tag was added to the N-terminal end of the Dnmt1s transgene.
Pronucleus injections with the Dnmt1s transgene resulted in six founder lines verified by PCR, Southern blot and eGFP fluorescence. Cross-ins of these founders with CMV-Cre mouse lines yielded viable offspring. The ubiquitous overexpression of Dnmt1s was investigated in cross-ins that carry the recombined version of the Dnmt1s transgene and express RGS-His-tagged Dnmt1 as verified by RT-PCR. TaqMan analyses of total Dnmt1 expression of the line with the strongest overexpression showed a ~ 3-fold (3.17 ± 0.65) overexpression in liver, a ~ 3.5-fold (3.47 ± 0.15) overexpression in testis, a ~ 5.5-fold (5.44 ± 0.39) overexpression in brain and a ~ 6-fold (6.24 ± 1.48) overexpression in kidney, but no overexpression in the spleen of CMV-Cre recombined transgenic mice in comparison to sibling wild type controls. Dnmt1 overexpression was confirmed on the protein level by quantitative western blots, which detected ~ 3-fold Dnmt1 levels in brain and ~ 6-fold Dnmt1 levels in testis of transgenic mice in comparison to sibling wild type controls. Upcoming analyses will test the in vivo functionality of the transgenic protein and the phenotypic consequences of Dnmt1s overexpression.

P-MoleG-243
Array-based resequencing data underpin the role of exhaustive mutation scanning in familial hypertrophic
Waldmüller S.[1], Lawrenz T.[2], Borisov K.[3], Haremza J.[4], Müller M.[4], Rackebrandt K.[4], Davies J.[4], Binner P.[4], Scheffold T.[1]
[1]University of Witten/Herdecke, Dortmund, Germany, [2]Städtische Kliniken Bielefeld-Mitte, Bielefeld, Germany, [3]Federal State Institution „Rehabilitation Center", Moscow, Russia, [4]CorTAG Gesellschaft für molekulare Diagnostik mbH, Dortmund, Germany

Objective. Genetic testing can help confirm the diagnosis of sporadic and familial forms of hypertrophic cardiomyopathy. Furthermore, it provides a basis for the timely identification of presymptomatic individuals at risk of sudden cardiac death. Nowadays, due to the high costs of conventional sequencing approaches, routine genetic testing in HCM nowadays encompasses only the three "major disease genes". The objective of the present sudy was to elucidate how often further screening of the less frequently affected "minor disease genes" reveals additional mutations worth considering in genetic counseling.
Methods. Patients were enrolled in the CMP (cardiomyopathy) study which was approved by the local advisory board. DNA samples of 10 Patients presenting with suspected familial HCM were each subjected to array-based resequencing using two different custom design Affymetrix arrays referred to herein as CardArray_1 (interrogating the major HCM genes MYH7, MYBPC3 and TNNT2) and CardArray_2 (covering the minor HCM genes TCAP, TNNI3, MYH6, TPM1, MYL2, MYL3, CRYAB, CSRP3, ACTC1, TNNC1, CAV3, a kinase-domain encoding part of the TTN gene, as well as two genes, GLA and PRKAG2 associated with storage diseases mimicking HCM). Arrays were hybridized with a mixture of either 9 (CardArray_1) or 26 (CardArray_2) short- or long-range PCR amplicons (amplicon size: 0.3-12 kilo bases). Mutations were confirmed by conventional Sanger sequencing.
Results. Two of ten index patients tested had no conclusive mutation at all in any of the 16 genes resequencened with both arrays. Five patients carried a single probably pathogenic mutation in one of the major HCM genes addressed by the CardArray_1. Two of these mutations were "novel" (i.e. not previously described: MYH7 c.4159G>A/p.E1387K and MYBPC3 c.236_237insA/p.Y79fsX1). One patient carried one mutation in each of two different major HCM genes, one of which was novel (MYBPC3 c.2125G>A/p.D709N). Another patient had two mutations, one in each of two minor HCM genes: TNNI3 c.146T>A/p.L49Q was novel and CAV3 c.233C>T/p.T78M has previously been linked to the Long QT syndrome and Hyper-CKemia. Mutations in both the major and the minor HCM genes were found in one single patient who carried two allelic MYBPC3 mutations known to cause HCM and in addition a novel PRKAG2 mutation, c.247C>T/p.P83S, suspected of causing pathological glycogen storage. In summary, the screening of the minor in addition to the major disease genes uncovered three probably disease-causing or modifying mutations in two out of ten patients suspected of having familial HCM.
Conclusion. Screening only the major HCM genes misses mutations that may cause or modify the disease and should therefore be considered upon counseling and predictive testing. Resequencing microarrays and other high-throughput tools may help to bring exhaustive mutation scanning in HCM to routine practice.

P-MoleG-244
Analysis of F8 mRNA in haemophilia A patients with silent mutations or presumptive splice site mutations
Zimmermann M.[1], Gehrig A.[1], Rost S.[1], Müller E.[1], Oldenburg J.[2], Müller C. R.[1]
[1]Department of Human Genetics, University of Würzburg, Germany, [2]Institute of Exp. Haematology and Transfusion Med., University Bonn, Germany

Introduction: Mutation screenings in haemophilia A patients identify a great variety of mutations in the factor VIII (F8) gene: about 40% missense mutations, 25-30% intron 22 inversions and less than 10% nonsense mutations, small or large deletions, insertions and splice site mutations, respectively.

Mutations which do not result in amino acid substitutions (silent mutations) and intronic variants which are located outside the splice site consensus sequences cannot be easily classified as causative for haemophilia A. Further insight can be gained by mRNA analysis.
Methods and Results: F8 mRNA was prepared for four haemophilia A patients with silent mutations and five patients with presumptive splice site mutations. Amplification of cDNA fragments was done using exonic primers located adjacent to the mutation-containing exons or introns. The obtained fragments were sequenced on an ABI automatic sequencer and compared to F8 wildtype cDNA. In this way, three of the nine mutations examined could be shown to have an effect on F8 mRNA splicing.
The silent mutation Tyr2017Tyr (c.6108C>T) resulted in the partial loss of exon 19 in the F8 mRNA. In addition, two presumptive splice site mutations have a similar effect. The point-mutation c.1752+5G>A caused a loss of exon 11. A small deletion in intron 13 (c.2114-8_-25del18) resulted in the loss of the whole exon 14 in the F8 mRNA.
Conclusion: Analysis of F8 mRNA from nine haemophilia A patients with silent mutations or presumptive splice site mutations has revealed that three of the examined mutations cause an aberrant F8 mRNA due to loss of an existing or activation of a cryptic splice site.
This study also shows that examination of splice sites should not only rely on special prediction software but rather must be proved in mRNA analysis experiments.

P-MoleG-245
HNF1ß mutations as a common cause of cystic kidney disease - Mutation update
von Bothmer J.[1], Eggermann K.[1], Jia Y.[1], Zerres K.[1], Bergmann C.[2], Ortiz Brüchle N.[1]
[1]Institute of Human Genetics, Aachen, Germany, [2]Bioscientia Center for Human Genetics & Institute of Human Genetics, Aachen, Germany

Mutations in the HNF1ß gene (17q12) are responsible for MODY (Maturity Onset Diabetes of the Young) type V usually occurring before the age of 25. In addition, they can cause a wide spectrum of kidney

malformations. The most common renal manifestations are renal agenesis and cystic dysplasia (type POTTER II). Besides cystic kidneys resembling ARPKD (Autosomal Recessive Polycystic Kidney Disease) and early-onset ADPKD (Autosomal Dominant Polycystic Kidney Disease), glomerulocystic hypoplastic kidneys can be observed. Hyperuricemia, abnormal liver function and urogenital malformation can be part of the spectrum which is also denoted as RCAD- (Renal Cysts and Diabetes) syndrome following an autosomal dominant mode of inheritance with incomplete penetrance and variable expression.
The clinical spectrum in family members carrying identical mutations is often extremely variable. The reason for this intrafamilial variability is not yet understood. In some of our pedigrees, phenotypes range from prenatal Potter sequence to very mild manifestations in elderly affected family members.
We analysed more than 260 patients with cystic kidneys by direct sequencing and MLPA (Multiplex Ligation dependent Probe Amplification) analysis and identified several new mutations. Mutations in the HNF1ß gene may be responsible for a broad spectrum of renal malformations. Finally one should keep in mind that HNF1ß mutations might mimic clinical pictures similar to ARPKD or early-onset ADPKD.

P-NEUROGENETICS

P-Neuro-246
Linkage analysis of 14 consanguineous families with non-syndromic autosomal recessive mental retardation

Abou Jamra R.[1], Ekici A.[2], Alblas M.[1], Kluck N.[1], Giesebrecht S.[1], Abboud A.[3], Al-Hallak B.[4], Al-Jabban L.[5], Atik S.[6], Issa S.[7], Ibrahim S.[8], Muhammad S.[9], Sabbagh A.[10], Schumacher J.[1], Reis A.[2], Nöthen MM.[1]
[1]Institute of Human Genetics, Bonn, Germany, [2]Institute of Human Genetics, Erlangen, Germany, [3]Private Clinic, Jdaidet Artouz, Syria, [4]Private Clinic, Kaferenbel, Syria, [5]Private Clinic, Damascus, Syria, [6]Private Clinic, Jesser El-Sheghour, Syria, [7]Private Clinic, Kafesejneh, Syria, [8]Private Clinic, Kameshli, Syria, [9]Private Clinic, Lattakia, Syria, [10]Private Clinic, Hama, Syria

Mental retardation (MR, IQ<70) affects approximately 1-3% of the general population and can be divided into syndromic and non-syndromic forms. In contrast to syndromic MR, only a few causative genes have been reported for non-syndromic MR (NSMR). It has been postulated that almost a quarter of undiagnosed NSMR cases have an autosomal recessive etiology. Studies of consanguineous families with affected children represent the best available strategy for the identification of further genes.
We recruited 22 consanguineous families from Syria with 1 – 4 affected individuals with NSMR each. The inbreeding coefficient between the parents of the affected children varied between 0.004 and 0.135. All affected individuals presented with severe mental retardation, in some cases with additional neurological symptoms such as epilepsy and muscular tonus abnormalities.
We first excluded fragile X syndrome when suspected. Afterwards, we genotyped families with Illumina 610K-chips (3 families) and the CytoSNP-chip (all others), and excluded deletions and duplications. Based on the family history we assumed an autosomal recessive etiology and performed genome wide linkage analyses. Preliminary analyses show in 8 families one linked candidate region, each. These regions are located on chromosome 1p13-q23 (~42Mb), 1q42-q43 (~6Mb), 4q26-4q28 (~14Mb), 5q31-q32 (~10Mb), 6q11-q21 (~20Mb), 6q16-q22 (~22Mb), 1212q 22-q24 (~13Mb), and 22q13-tel (~3Mb). In further 4 families we identified two candidate regions, while in the remainder more than two regions were observed.
Our study defines circumscribed risk regions and thus represents an essential step in the identification of autosomal recessive genes for NSMR. Our ongoing work aims at identifying the causal mutations by sequencing of positional candidate genes. Our goal is to facilitate the development of diagnostic strategies for NSMR and to understand the complex neuronal network which underlies normal and abnormal brain functioning.

P-Neuro-247
SCA15 is the most common non-repeat SCA

Bauer P.[1], Beetz C.[2], Bauer C.[1], Bonin M.[1], Schmitz-Hübsch T.[3], Wüllner U.[3], Nägele T.[4], Riess O.[1], Schöls L.[5], Synofzik M.[5]
[1]Medcial Genetics, Tübingen, Germany, [2]Institute of Clinical Chemistry, Jena, Germany, [3]Dept. of Neurology, Bonn, Germany, [4]Dept. of Neuroradiology, Tübingen, Germany, [5]Dept. of Neurodegenerative Diseases, Tübingen, Germany

Genetic diagnosis in autosomal-dominant spinocerebellar ataxias (SCAs) is a major challenge when common CAG repeat expansions have been excluded. To guide time- and cost-intensive analyses of the increasing number of SCA genes, more information about phenotypic characteristics and frequency distribution in different populations is highly warranted. SCA15 has recently been shown to be caused by multi-exon deletions of the ITPR1-gene. To assess the prevalence and phenotypic spectrum of SCA15 we here screened German SCA families for multi-exon deletions in the ITPR1-gene. Moreover, to determine the most efficient diagnostic method for deletion detection two molecular-genetic techniques were used in parallel, namely multiplex ligation-dependent probe amplification (MLPA) and real-time PCR combined with copy number analysis by SNP array. SCA15 accounted for 1.8% (5/274) of all SCA families and for 7.2% (5/69) of SCA families negative for common SCA mutations, thus yielding SCA15 the most common non-trinucleotide SCA. MLPA demonstrated less false positives and less false negatives and is more cost-effective than qPCR combined with SNP array. All SCA15 patients (n=10) presented with slowly progressive cerebellar ataxia, whereas marked postural, action and intention tremor often developed later in the disease. Other extra-cerebellar signs like e.g. pyramidal tract affection were infrequent. Cerebellar atrophy was uniformly most pronounced in the superior vermis and was present before patients regarded themselves as affected. In conclusion, MLPA-screening for ITPR1 deletions should be considered in patients with slowly progressive SCA, vermal cerebellar atrophy and prominent tremor after common SCA repeat expansions have been excluded.

P-Neuro-248
Screening for copy number variations in recessive ataxias by MLPA

Bernard V.[1], Edener U.[1], Gehlken U.[1], Gillessen-Kaesbach G.[1], Zühlke C.[1]
[1]Institut für Humangenetik, Lübeck, Germany

The autosomal recessive cerebellar ataxias (ARCAs) are a heterogeneous group of rare neurological disorders, characterized by degeneration or abnormal development of the cerebellum and spinal cord and juvenile age of onset. To date, at least 20 different ARCAs are known. Friedreich ataxia (FRDA) is the most frequent form in Europe followed by ataxia with oculomotor apraxia type 2 (AOA2) and type 1 (AOA1). In about 96% of the patients, FRDA is caused by a homozygous GAA repeat expansion in the first intron of the FXN gene. Furthermore at least 24 point mutations and one intragenic deletion are described in the FXN gene. AOA2 is genetically defined by mutations in the SETX gene while AOA1 is associated with mutations in the APTX gene. AOA2 and AOA1 are predominantly caused by missense and nonsense mutations. A few intragenic deletions or duplications are reported for the SETX gene, whereas only one deletion of the entire APTX gene is known.
By sequencing analyses of all coding exons and flanking intronic sequences in about 100 ataxia patients, we identified 16 with a heterozygous mutation in the SETX gene. In another patient exon 11 to 15 of the SETX gene could not be amplified by PCR. Additionally, fragment analyses revealed a heterozygous GAA repeat expansion in intron 1 of the FXN gene in one affected person. These 18 patients were screened

for gene and exon deletions or duplications using MLPA, covering the respective genes for FRDA, AOA2 and AOA1.
In 4 of 18 patients we identified large exon deletions in the SETX gene and the patient heterozygous for the repeat expansion in the FXN gene carried a deletion of exon 2 and exon 3. In the other 13 patients no abnormal copy numbers were detected, so that the diagnosis of AOA2 is not approved definitely. It is possible that the heterozygous mutations identified by sequencing analysis are rare polymorphisms or that the second mutation still escapes MLPA and sequencing methods. For example large insertions, as known for the SETX gene, could be overlooked by these methods. However, altogether these results indicate that gross mutations are a possible cause of AOA2 and FRDA and, moreover, reveal the importance of additional MLPA analysis for routine diagnostic.

P-Neuro-249
High-density SNP array linkage and CNV analyses in familial forms of common migraine
Borck G.[1], Todt U.[1], Nürnberg G.[2], Heinze A.[3], Lessel D.[1], Göbel I.[1], Netzer C.[1], Nürnberg P.[2], Göbel H.[3], Kubisch C.[1]
[1]Institute of Human Genetics, University of Cologne, Germany, [2]Cologne Center for Genomics, University of Cologne, Germany, [3]Pain Clinic, Kiel, Germany

Migraine is a prevalent primary headache disorder with a high heritability but a poorly understood genetic basis. With the long-term goal of identifying genes underlying common forms of migraine, we have collected a large number of multiplex migraine families, parent-child trios and single patients of German origin, enriched for migraine with aura (MA), and started genetic mapping studies. The initial family sample contains 380 individuals in 41 families, with 3-12 affected individuals available for genotyping in each family. We used Affymetrix 6.0 arrays for genomewide high-density genotyping which allows us not only to perform linkage analysis with SNP markers but also to detect copy number variations (CNVs) associated with migraine. In the large multiplex families, we did not detect linkage to any of the three known genes involved in familial hemiplegic migraine (FHM), confirming that FHM genes are not responsible for common migraine in most families. Our preliminary results have identified a single MA locus in one family which overlaps a previously reported MA locus, and next-generation sequencing of this genomic region is underway to uncover the causative MA mutation. Moreover, we identified candidate linkage regions in most of the other families, some of which are < 3 Mb and would have been missed by microsatellite-based linkage scans. Our ongoing sequencing efforts concentrate on functional candidate genes mapping to overlapping regions putatively linked in two or more families and on strong functional candidate genes like ion-channel genes or genes involved in neurotransmitter metabolism and cortical excitability. In addition, we perform CNV analyses and family-based association studies, which are complemented by genome-wide association studies within an international consortium. Our results may ultimately help to better understand the complex genetic architecture of common forms of migraine.

P-Neuro-250
Using a conditional mouse model to analyse proteasomal impairment in the pathogenesis of SCA3 in vivo
Boy J.[1], Schmidt T.[1], Unser S.[1], Hilke F.[1], Schumann U.[1], Schmitt I.[2], Zimmermann F.[3], Rieß O.[1]
[1]Department of Medical Genetics, Tuebingen, Germany, [2]Clinic for Neurology, Bonn, Germany, [3]Center for Molecular Biology, Heidelberg, Germany

Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene. The formation of intracellular inclusion bodies is a hallmark shared by SCA3 as well as by several other neurodegenerative diseases. Several indications point to a role of proteasomal dysfunction in the pathogenesis of SCA3, but up to now no in vivo results exist supporting this thesis.

We chose a conditional mouse model of SCA3 generated using the "Tet-Off-System" and two different indicator mouse lines to elucidate this question. In our inducible mice, the Ca2+/Calmoduline-dependent protein kinase II (CamKII) promoter leads to strong expression of expanded ataxin-3 in the forebrain. In double transgenic mice, the formation of ataxin-3 positive accumulation is detectable by Western blotting and immunohistochemistry. Mice show motor impairment as measured by Rotarod as well as Beam walking tests. As indicator mice we used two different mouse lines for which the suitability to study proteasomal impairment in vivo has previously been demonstrated. Both lines (GFPdgn and UbG76V-GFP) are based on alterations in the Green Fluorescence Protein (GFP) which lead to the rapid degradation of this protein if the proteasomal function is intact. Only if the proteasomal function is impaired, GFP fluorescence can be detected. By crossbreeding these indicator mice with mice of the conditional SCA3 model (CamKII/MJD77) we generated triple transgenic mice which were used for the analyses. We performed immunofluorescence analyses of brains sections of mice to examine whether GFP fluorescence and, thus, proteasomal impairment is detectable.

P-Neuro-251
Novel mutation in the AFG3L2 gene causing SCA28
Edener U.[1], Wöllner J.[1], Hehr U.[2], Kohl Z.[3], Schilling S.[4], Kreuz F.[5], Bauer P.[6], Bernard V.[1], Gillessen-Kaesbach G.[1], Zühlke C.[1]
[1]Institut für Humangenetik, Lübeck, Germany, [2]Zentrum und Institut für Humangenetik, Regensburg, Germany, [3]Abteilung für Molekulare Neurologie, Erlangen-Nürnberg, Germany, [4]Klinik und Poliklinik für Neurologie,Regensburg, Germany, [5]Gemeinschaftspraxis für Humangenetik, Dresden, Germany, [6]Institut für Humangenetik, Tübingen, Germany

The autosomal dominantly inherited spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders primarily affecting the cerebellum. Typical signs are progressive incoordination, dysarthria and impairment of eye movements. Genetically, 26 different loci have been identified so far whereas the corresponding gene has not yet been determined for 10 of them. Recently, mutations in the ATPase family gene 3-like 2 gene were presented to cause spinocerebellar ataxia type 28 (SCA28). This type of SCA is characterised by early onset as well as slowly progressive gait and limb ataxia accompanied by dysarthria, hyperreflexia at lower limbs, nystagmus and ophthalmoparesis.
In order to define the frequency of SCA28 mutations in the German population, we performed molecular genetic analyses in 140 unrelated familial cases with ataxia. Amongst other variations, we found a novel missense mutation at an evolutionary conserved amino acid position using a single strand conformation polymorphism approach followed by DNA sequencing. This amino acid exchange p.E700K was detected in five ataxia patients of a four-generation German family and was not observed in the DNA sample of an unaffected family member as well as in a survey of 400 chromosomes from healthy control individuals.
Altogether, these data show that SCA28 is not a frequent cause for dominantly inherited ataxias in the German population.

P-Neuro-252

Hereditary motor and sensory neuropathy (HMSN) type X1 in an Argentinean family reveals independent GJB1/Cx32 mutations at identical nucleotide position

Gerding W.M.[1], Kötting J.[1], Rey L.[2], Bibas Bonet H.[2], Epplen J.T.[1], Carrero-Valenzuela R.D.[3]
[1]Humangenetik, Ruhr-Universität Bochum, Germany, [2]Collaborator of the Biomedical Department Genetic Orientation UNT Faculty of Medicine, San Miguel de Tucuman, Argentina, [3]Biomedical Department Genetic Orientation UNT Faculty of Medicine, San Miguel de Tucuman, Argentina

X-linked Charcot-Marie-Tooth (CMT) disease (HMSN Type X1, OMIM #302800) represents a frequent cause of hereditary peripheral motor and sensory neuropathies and is associated with mutations in GJB1/Cx32 encoding gap junction protein beta 1. Studying an Argentinean family with 7 affected males in five generations exhibiting clinical signs of CMT, 8 obligate female carriers could be identified genealogically. Direct sequencing of exon 2 and adjacent regions of GJB1/Cx32 in two symptomatic males whose respective maternal grandfathers, both affected, were brothers, revealed mutations in GJB1/Cx32. Surprisingly, each of the two affected patients had a different mutation in hemizygous state in the same nucleotide position: c.383C>T (p.S128L) and c.383C>A (p.S128X). In both cases, the identified mutation was present in heterozygous state in the corresponding maternal genomic DNA. Furthermore, X-chromosomal microsatellite analysis showed identical marker alleles in both patients. Together with the genealogical information, these molecular data highly suggest that a primarily mutant allele mutated for a second time. In conclusion, two different mutations at the same nucleotide position in this Argentinean family represent an uncommon finding with a very low probability of occurrence.

P-Neuro-253

The regulatory role of DSC3 in X-linked dystonia- parkinsonism

Herzfeld T.[1], Grznarova M.[1], Nolte D.[1], Gaarz A.[2], Staratschek-Jox A.[2], Schultze J.-L.[2], Müller U.[1]
[1]Institut für Humangenetik, Giessen, Deutschland, [2]LIMES-Institut, Bonn, Deutschland

X-linked dystonia- parkinsonism (XDP) is one of 17 currently recognized monogenic primary dystonias. Based on the frequent association of dystonia with parkinsonism, XDP is classified as a "dystonia plus" syndrome [1]. The disease is caused by one or several of a total of 7 disease-specific sequence changes ("DSCs") within the disease gene. This gene is referred to as "TAF1/DYT3 multiple transcript system" and is composed of TAF1 (coding for TATA box binding protein associated factor) plus five downstream ("d") exons. [2-4]. In order to elucidate the molecular basis of XDP we have started to investigate the effect of DSCs on normal function of TAF1/DYT3 in vitro. We focused on DSC3, the only disease specific sequence change located in an exon, i.e. down-stream exon d4. Exon d4 forms part of a transcript composed of exons d2, d3, d4 which is regulated by a specific promoter. Alternatively, d4 together with d3 can be spliced to the 3´ UTR of TAF exons. We speculated that d4 plays a role in gene regulation and that this function is disturbed by DSC3. In order to test this hypothesis we overexpressed the transcript composed of exons d2, d3 and d4 either with or without DSC3 in different cell lines. We then extracted RNA and analyzed the pattern of gene expression in these cells interrogating Illumina microarrays (HumanWG-6 v3). We found significant alterations in the expression of several hundred genes (depending on cell line) in cells overexpressing the wild type transcript. This effect was almost completely abolished by overexpressing DSC3- containing transcript. We found the same effect even in a more artificial environment of cells overexpressing exons d3 and d4 alone (WT or DSC3). Genes dysregulated by the DSC3 containing transcript included some involved in synaptosomal function and in Ca-homeostasis. Significantly, these genes are expressed in the striatum, the area of the brain most severely affected in XDP.

[1] Müller U. (2009) Brain. 132(Pt 8), 2005-25
[2] Nolte D, Niemann S, Müller U (2003) Proc Natl Acad Sci. 100(18), 10347-52
[3] Makino S, Kaji R, Ando S, Tomizawa M, Yasuno K, Goto S, Matsumoto S, Tabuena MD, Maranon E, Dantes M, Lee LV, Ogasawara K, Tooyama I, Akatsu H, Nishimura M, Tamiya G (2007) Am J Hum Genet. 80(3), 393-406.
[4] Herzfeld T, Nolte D, Müller U (2007) Mamm Genome. 18(11), 787-95.

P-Neuro-254

Allelic variants in CARD15 and post surgical opioid requirements in Crohn's disease

Huehne K.[1], Maas S.[1], Lascorz J.[2], Muenster T.[3], Winter S.[3], Foertsch T.[4], Croner R.[4], Rautenstrauss B.[5], Winterpacht A.[1]
[1]Institute of Human Genetics, Erlangen, Germany, [2]German Cancer Research Center, Heidelberg, Germany, [3]Department of Anaesthesiology, Erlangen, Germany, [4]Department of Surgery, Erlangen, Germany, [5]Medical Genetics Center, Munich, Germany

Crohn's disease (CD) is a painful inflammatory bowl disease with complex polygenic inheritance. CD patients require significantly higher post operative opioid doses than patients undergoing comparable severe abdominal surgery. Crohn's disease, therefore, may be a suitable model for the identification of novel pain susceptibility genes. We could recently demonstrate that higher postsurgical opioid requirement in CD patients is not due to a general change in pain sensitivity caused by the most common variants in components of opioid metabolism. Therefore, we now investigated whether polymorphisms in CD susceptibility genes might be associated with altered opioid needs. We focused on the caspase-activating recruitment domain 15 (CARD15) gene located in a region with the strongest evidence for linkage with susceptibility to CD. In this gene three common variants have been associated with disease suscepticility: R702W, G908R and a frameshift mutation l1007fsinsC. We screened 181 CD patients and 356 healthy controls. In a first step, we were able to replicate the association of CD with these three variants: allelic variants were present in 45% of the CD patients. Of the CD patients, 30% were heterozygous and 18 % homozygous or compound heterozygous for at least one of the CARD15 exchanges. In a next step, a subset of patients who had undergone major abdominal surgery and for whom post surgical opiod requirements were determined by patient controlled analgesia (PCA) were classified according to their post surgical morphine consumption as high, average and low morphine consumers. The 13 low consumers showed the typical CD distribution of the three CARD15 polymorphisms: allelic variants were present in 69% of low consumers. 23 % (3) were homozygous or compound heterozygous for CARD15 mutations. However, only 23 % of the 11 high morphine consumers revealed a CARD15 variant. The other eight high consumers revealed wt alleles. Although the number of CD patients is to low to draw final conclusions, the present data at least suggest that the presence/absence of the three CARD15 variants may be associated with altered post surgical opiod requirement in Crohn's disease.

P-Neuro-255
Systematic mutation search in families with XLMR by next-generation sequencing
Kalscheuer VM.[1], Chen W.[1,2,6], Haas S.[1], Hu C.[1], Emde AK.[1], Menzel C.[1], Bienek M.[1], Zemojtel T.[1], O'Keeffe S.[1], Vingron M.[1], Ullmann R.[1], Wrogemann K.[17], Tzschach A.[1], Strobl-Wildemann G.[3], Wieacker P.[4], EURO MRX Consortium.[5], Ropers HH.[1]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany, [2]Max-Delbrück Center Berlin Germany, [3]Praxis, Passau, Germany, [4]Institute for Human Genetics, University Hospital Münster, Germany, [5]Berlin Germany, Nijmegen The Netherlands, Tours France, Paris France, Leuven Belgium, [6]Berlin Institute for Medical Systems Biology, Berlin, Germany, [7]University of Manitoba, Winnipeg, Canada

X-linked mental retardation (XLMR) affects 1-2/1,000 males and accounts for approximately 10% of all mental retardation (MR). To date, almost 90 different XLMR genes have been identified. Mutations in these genes are seen in approximately 50% of the families. Thus, many more XLMR genes remain to be identified, especially for the non-syndromic forms of XLMR. We have set out to find the causative gene defect in a total of 200 families with this condition. To this end, we systematically enrich X-chromosomal exons employing several gene partitioning techniques followed by next generation sequencing, and depending on the outcome, by sequencing flow-sorted X-chromosomes. In contrast to previous studies (Tarpey et al., Nat. Genet. 2009) this strategy should enable us to detect mutations in the entire non-repetitive portion of the X-chromosome. We will report our results on the first 60 families, several of which carry mutations in known XLMR genes, and including a family with Wieacker-Wolff syndrome (MIM 31458). Patients with this disorder suffer from with congenital contractures of the feet at birth, a slowly progressive predominantly distal muscle atrophy, dyspraxia of the eyes, face and tongue muscles, and mild mental retardation. In this disorder, we have identified a missense mutation in a zinc finger gene located on the proximal long arm of the X-chromosome.
This work is supported by NGFNplus (BMBF).

P-Neuro-256
TOWARDS A GENETIC SCREENING TEST FOR DYSLEXIA ALLOWING FUNCTIONAL REGENERATION: A Strategy for Identification and Analysis of Genetic Risk Factors
Kirsten H.[1], Wilcke A.[1], Ahnert P.[2], Boltze J.[3]
[1]TRM Universität Leipzig und IZI Fraunhofer, Leipzig, Germany, [2]IMISE Universität Leipzig, Leipzig, Germany, [3]IZI Fraunhofer, Leipzig, Germany

Our aim is to develop a genetic screening test for dyslexia, a severe disorder of reading and frequently of writing, affecting approx. 4 % of all schoolchildren. A significant problem is late diagnosis resulting in a decreased chance of functional regeneration. Our solution is an early genetic test that will initiate existing early training programs. Genetic dyslexia markers necessary for this test are identified in a micro array based fine screen supplemented by polymorphisms of highly relevant candidate genes. Validation of these markers is done by A) genotyping an independent cohort; B) by characterising markers in functional magnet resonance imaging (fMRI) and C) by characterising markers in allele specific mRNA-expression analysis or allele-specific chromatin immunoprecipitation (ChIP). The final test will neither include fMRI/EEG nor expression analysis. It translates genetic findings into a clinical assay. This test would allow early identification of children at risk, enabling early support resulting in functional regeneration.

P-Neuro-257
Proximal myotonic myopathy (PROMM) unmasked by statin treatment
Knoblauch H.[1], Schöwel V.[1], Kress W.[2], Rosada A.[1], Kassner U.[1], Steinhagen-Thiessen E.[1], Spuler S.[1]
[1]Charité, Berlin, Germany, [2]Institute of Human Genetics,Würzburg, Germany

Introduction: Statins are ingested by millions of patients to reduce their cardiovascular risk. Nonetheless, about 5% develop skeletal muscle symptoms and elevations in creatine kinase (CK). A genetic susceptibility has been identified, namely a genetic variant in SLCO1B1, that increases the susceptibility for this side effect of statin treatment. SLCO1B1 variation alone does not explain the aetiology and variability of statin-induced myopathy. We therefore reasoned that other genetic causes could occur in patients that were referred to us because of CK elevations and muscle complaints.
Clinical summary: Fifty men were referred to our neuromuscular outpatient clinic because of myalgia and/or elevated CK after initiation of statin treatment for hypercholesterloemia. In three men (age 56 – 70 yrs.; CK 250 – 1700 U/l) neurological examination revealed mild paresis paresis of proximal muscles and head flexion. All men were physically active before initiation of statin treatment. They had no history of skeletal muscle disorder. One patient had catarcts. Family history was unremarkable.
Muscle biopsy: We performed a muscle biopsy in two of the patients and observed multiple central nuclei in the myocytes, variable fiber size, and increased connective tissue, consistent with myotonic dystrophy type-2 (DM2).
Molecular genetic testing: After due approval and written informed consent, we next sequenced the ZNF9 gene, focusing on the known (CCTG)n expansion in intron 1 in all three patients. Each patient harboured the typical molecular defect underlying DM2.
Discussion: Nothing indicated that our three patients had an underlying chronic muscle disease until they were begun on statin therapy. Clues to test for DM2 were the progressive course of the complaints years into statin medication plus the lack of relief after statins were discontinued. Our report argues for some precautions before describing statins, like measuring CK levels, and subsequent consultation with a neuromuscular specialist.

P-Neuro-258
Non-classical FSHD phenotypes and the importance to establish a D4Z4 haplotype at the 4q subtelomeric region
Kress w.[1], Meng G.[1], Halliger-Keller B.[1], Kottlors M.[2]
[1]Institute of Human Genetics, Würzburg, Germany, [2]Seidel-Klinik, Bad Bellingen, Germany

The Facioscapulohumeral muscular dystrophy (FSHD) is the second most common autosomal dominant myopathy of adult onset presenting as a highly variable disorder with weakness starting from infancy to late adulthood. The beginning of the disease in typical cases is characterized by (sometimes asymmetric) muscle weakness of the shoulder girdle, (sometimes discreet) involvement of facial muscles and then a slowly progressive involvement of pelvic and leg muscles. Several atypical phenotypes have been reported. There was always some doubt about the subclassification of these phenotypes as variants of autosomal dominant FSHD.
A diagnosis of FSHD is normally established by a deletion of D4Z4 repeat elements (>10 in healthy probands) on chromosome 4q detected by shortened Eco RI and EcoRI/BlnI fragments on a Southern-Blot. The double digestion differentiates between 4q and 10q repeat elements, which are highly homologous and do cross- hybridization. In the last few years, new elements at the D4Z4 locus were found which are necessary to render the deletion pathogenic. The most important element is the presence (haplotype A) or absence (haplotype B) of a beta-satellite sequence at the telomeric end of D4Z4 repeat. Only haplotype A is pathogenic.

It is shown in two families that the haplotype determination, which can be done also by Southern hybridization, could be a decisive diagnostic criterion in questionable FSHD phenotypes.
In one family the index patient (male, 65 years old) has bent spine syndrome whereas his daughter presents with a classical FSHD phenotype. Bent spine syndrome is characterized by a stooping posture on standing position, exaggerated while walking or with exercise, disappearing in a supine position that is different from simple fixed dorsal kyphosis. It is combined with a marked atrophy of the paraspinal muscles. A shortened D4Z4 repeat number of nine elements on an A haplotype segregates in the family causing the highly variable phenotypes. So bent spine syndrome can be a rare presentation of FSHD.
In the second family a girl suffers from an unknown form of LGMD with rather high CK values (>1200U/l). A shortened D4Z4 fragment on chromosome 4q was detected in her DNA and in the DNA of her obviously healthy father. But in this case the shortened D4Z4 repeat was shown to be on a B haplotype. So FSHD could be rejected as a differential diagnosis, which indeed was questionable on account of clinical reasons.

P-Neuro-259

ARSACS – first analysis of SACS gene in Germany and identification of novel mutations

Kreuz F. R.[1], Timmann D.[2], Gerwig M.[2], Synofzik M.[3], Schöls L.[3], Schwarz J.[4], Della Marina A.[5], Sarpong A.[6], Hoffjan S.[7], Minnerop M.[8], Bier A.[1], Reif S.[1], Plaschke J.[1], Krüger S.[1]
[1]Gemeinschaftspraxis für Humangenetik, Dresden, Germany, [2]Neurologische Universitätsklinik, Essen, Germany, [3]Neurologische Universitätsklinik, Tübingen, Germany, [4]Neurologische Universitätsklinik, Leipzig, Germany, [5]Universitäts-Kinderklinik, Essen, Germany, [6]Department of Neuropaediatrics Charité University Hospital, Berlin, Germany, [7]Abteilung für Humangenetik der Ruhr-Universität, Bochum, Germany, [8]Institut für Neurowissenschaften und Medizin/Neurologische Universitätsklinik, Jülich/Bonn, Germany

In 1978 Autosomal Recessive Spastic Ataxia Charlevoix-Saguenay (ARSACS) was first described in the French-Canadian population of the Charlevoix-Saguenay-Lac-Saint-Jean region of north eastern Quebec in Canada and was initially thought to occur only in this region. The original phenotypic description included in addition to ataxia: spasticity, dysarthria, distal muscle wasting, foot deformities, truncal ataxia, absence of sensory nerve conducting potentials in the lower limbs, retinal striation, and often mitral valve prolapse. Other common features are nystagmus, finger deformities, and skoliosis. Disease onset is usually in early childhood. Typical findings in cerebral CT or MRI imaging are atrophy of the vermis followed by progressive atrophy of the cerebellar hemispheres. Recently, the underlying gene, SACS (chromosome 13q12.12, encoding Sacsin), was identified. Mutations have been reported not only from Quebec but also from Japan, Italy, Tunisia, Spain, Turkey, France, Belgian, and The Netherlands. Whereas the phenotype of the Quebec patients appeared relatively uniform according to the original description, some non-Quebec patients showed atypical features such as later disease onset, absence of hypermyelinated retinal fibers, intellectual impairment, and lack of spasticity. Initially, the SACS gene was thought to comprise a single large exon with an ORF of 11.5 kb. Meanwhile, eight additional exons upstream of the original exon were identified, generating an ORF of 13.7 kb. Most of the currently reported 53 different mutations are either truncating point or missense mutations.
Here we report first results of SACS gene analysis in Germany. Fourteen non-related index patients were analysed by sequencing of all nine coding exons of SACS including the exon-intron-boundaries in altogether 45 single fragments. All 14 patients had an age of onset before 25 years and showed typical symptoms of ARSACS, such as ataxia, spasticity, and vermis atrophy.
Two patients were found to be homozygous for the SACS mutations c.9305_9306insT (p.Leu3102PhefsX8) and c.4076T>G (p.Met1359Arg), respectively. Two further patients carried two heterozygous SACS mutations (c.1373C>T, p.Thr458Ile plus c.2983G>T, p.Val995Phe and c.2080G>A, p.Ala694Thr plus c.12875A>G, p.His4292Arg, respectively). Only one of these mutations had been described before. Interestingly, one mutation (c.1373C>T) did not occur in the large exon 9 but in exon 7, stressing the need for analysing the whole gene. We applied four splice site prediction programs as well as three computer models for the functional characterisation of missense mutations. The c.4076T>G mutation was predicted to create an alternative splice acceptor site. The mutations p.Thr458Ile and p.Val995Phe were predicted to impair protein function. We consider these three mutations as pathogenic. The two remaining sequence changes (p.Ala694Thr and p.His4292Arg) were not predicted to affect splicing or protein function. Thus, we suggest that these variations are likely unclassified variants and not disease causing. All carriers of pathogenic mutations had moderate to severe gait ataxia, spasticity, and atrophy of cerebellar vermis with an age of onset before 20 years.
In conclusion, here we describe the first SACS mutations identified in Germany. In 21.4% (3 out of 14) of patients with early onset spastic ataxia the diagnosis of ARSACS was made. One additional patient carried 2 heterozygous unclassified variants. Our findings underscore that ARSACS based on SACS mutations occurs worldwide and should be considered in the diagnostic work up of ataxia patients.

P-Neuro-260

Molecular characterization of translocation breakpoints in two cases with either a familial or a de novo constitutional translocation co-segregating with phenotypic anomalies as an attempt to directly identify genes involved.

Kroisel P.M.[1], Obenauf A.C.[1], Eichwalder B.[2], Gruber-Sedlmayr U.[3], Wagner K.[1], Schwarzbraun T.[1], Speicher M.R.[1], Auer M.[1], Hoffmann E.M.[1], Rötzer K.M.[1], Geigl J.B.[1]
[1]Institute of Human Genetics/Medical University of Graz, Graz, Austria, [2]Department of Pediatrics/LKH Villach, Villach, Austria, [3]Department of Pediatrics/Medical University of Graz, Graz, Austria

Identification of genes affected by constitutional chromosomal rearrangements like balanced translocations can be achieved by different strategies. A more conventional approach is based first on fluorescence in situ hybridization (FISH) using various genomic clones to isolate subsequently breakpoint spanning clones followed by further characterization and sequencing of junction fragments. This however represents a very laborious, time consuming and tedious method. Recently a new approach called array painting was described and demonstrated to be successful to achieve this goal and potentially allows to speed up the entire process. A sophisticated and highly efficient amplification technique, that allows generation of complex probes from single chromosomes suited for high resolution array CGH was developed at our Institute (Geigl et. al).
This technique is now combined with noncontact laser microdissection or microdissection using glass needles to obtain array painting probes in two particular cases. In a familial case, a 1/6 translocation was found to segregate with microcephaly, brachycephaly, moderate mental retardation and several minor dysmorphic features through at least three generations. The mother showing the karyotype of 46,XX,t(1;6)(p36.3;p21.1) as well as the maternal grandmother and one of her two sons are affected. In a non-familial case, a currently twelve year old male patient with a severe mental retardation and specific mainly facial dysmorphic features was found to carry a de novo 11/19 translocation resembling the karyotype of 46,XY,t(11;19)(q23.3;q13.3). These cytogenetic findings were also confirmed by using whole chromosome painting probes to rule out involvement of further chromosomal segments. Array painting probes obtained from single copies of the rearranged chromosomes were used to more precisely identify and map the genomic regions located at all translocation breakpoints. In both cases a high resolution array CGH analysis using a 1.000 k or 44 k oligonucleotide array was

applied first to rule out a significant chromosomal imbalance related to specific phenotype anomalies like microcephaly, mental retardation and distinct facial dysmorphism.

P-Neuro-261

Small Deletions in Monogenic Primary Dystonias

Köhler A.[1], Heinrich S.[1], Schramm A.[2], Hoffjan S.[3], Müller U.[1]
[1]Institut für Humangenetik, Gießen, Germany, [2]Neurologische Universitätsklinik, Erlangen, Germany, [3]Abteilung für Humangenetik, Bochum, Germany

Monogenic primary dystonias can be divided into three groups: the "pure" forms with dystonia as the sole or main manifestation, "dystonia plus syndromes" with dystonia as a main sign accompanied by additional neurological manifestations such as myoclonus and parkinsonism, and the "paroxysmal dystonias" with episodes of dystonic involvement. The disease gene has been identified in nine, and disease loci have been assigned in 7 forms (Müller, 2009). In an attempt to identify the genetic basis in additional primary dystonias, we performed SNP array analysis (Illumina, Human660W-Quad beadchips) in dystonias in which no mutation was detected in any of the known disease genes. We found a 1.3 Mb deletion in the short arm of chromosome 18 (18p11.21) in a patient with focal dystonia that resulted in the deletion of 4 genes. His similarly affected father is currently being tested. Previously, a disease locus has been assigned to the short arm of chromosome 18 in a family with focal dystonia (dystonia 7) by linkage analysis (Leube et al., 1996). An additional locus has been assigned to the short arm of chromosome 18 (18p11) in a dystonia plus syndrome (myoclonus dystonia; dystonia 15) (Grimes et al., 2002). In another case with generalized dystonia we found a small deletion (415 kb) in the short arm of chromosome 10 (p12.33). Testing of additional affected and unaffected family members will show whether the deletion is the cause of dystonia in this family. Presently no disease locus has been assigned to this region of chromosome 10.

Ref.:

Müller U: The monogenic primary dystonias. Brain 132: 2005–2025, 2009
Grimes DA, Han F, Lang AE, St George-Hyssop P, Racacho L, Bulman DE: A novel locus for inherited myoclonus-dystonia on 18p11. Neurology 2002; 59: 1183–1186.
Leube B, Rudnicki D, Ratzlaff T, Kessler KR, Benecke R, Auburger G: Idiopathic torsion dystonia: assignment of a gene to chromosome 18p in a German family with adult onset, autosomal dominant inheritance and purely focal distribution. Hum Mol Genet 1996; 5: 1673–7.

P-Neuro-262

Genome-wide association mapping of susceptibility alleles predisposing to idiopathic generalised epilepsy

Leu C.[1], Steffens M.[2], de Kovel C.G.F.[3], Koeleman B.P.C.[3], Lindhout D.[3], Weber Y.G.[4], Lerche H.[4], Kleefuß-Lie A.A.[5], Hallmann K.[5], Elger C.E.[5], Muhle H.[6], Stephani U.[6], Rückert I.M.[7], Wichmann H.E.[7], Franke A.[8], Schreiber S.[8], Trucks H.[1], Toliat M.[1], Nürnberg P.[1], Sander T.[1], EPICURE Consortium.[9]
[1]Cologne Center for Genomics, University of Cologne, Germany, [2]Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany, [3]Section Complex Genetics, University Medical Center Utrecht, The Netherlands, [4]Neurological Clinic, University of Ulm, Germany, [5]Department of Epileptology, University of Bonn, Germany, [6]Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Germany, [7]Institute of Epidemiology, Helmholtz Zentrum München, Germany, [8]Institute for Clinical Molecular Biology, University Medical Center Schleswig-Holstein, Germany, [9]EPICURE Consortium, EUR, http://www.epicureproject.eu

Idiopathic generalised epilepsies (IGEs) are genetically determined (heritability: 80%) and presumably display a polygenic predisposition in the majority of affected individuals. Up to date, the genetic variants predisposing to common IGE syndromes remain elusive. To search for common genetic risk factors, we have set up a two-staged genome-wide association (GWA) study on common IGE syndromes, using the Affymetrix Genome-Wide Human SNP Array 6.0. The Stage-1 discovery sample included 1426 IGE patients of Western-European ancestry and 2883 German population controls (PopGen & KORA cohorts). Association analysis was conducted for the entire IGE sample and two subsamples, comprising 668 IGE probands affected by idiopathic absence epilepsy (IAE) and 539 probands affected by juvenile myoclonic epilepsy (JME). The GWA results revealed a promising number of genome-wide significant associations. The allele frequency difference test (AFD) showed 35 association hints achieving a significance threshold of AFDp<5x10E-7 in the three IGE samples (IGE: n=10; IAE: n=19; JME: n=6). Two regions at 2p16.1 and 17q21.31 displayed multiple consistent association signals achieving genome-wide significance in the IGE sample. A significant association was observed at 2p16.1 in the IAE subsample whereas the strongest association lead was found at 17q21.31 in the JME subsample, suggesting that different gene configurations contribute to the phenoptypic variance of either absence or myoclonic seizures. We are currently evaluating 132 Stage-1 association hints (AFDp<10E-5) in a Stage-2 confirmation sample including 1040 European IGE patients and 904 ethnically matched controls. The comprehensive survey of the most relevant genomic risk factors will enhance prospects to specify individual risk profiles and to differentiate their leading epileptogenic pathways.

P-Neuro-263

Recurrent expansion of a 700kb deletion on chromosome 14 triggered by an 13kb duplication at one break point

Lohmann K.[1], Tönnies H.[2], Saunders-Pullman R.[3], Rakovic A.[1], Martin-Subero I.[2], Wiegers K.[1], Hinrichs F.[4], Hellenbroich Y.[4], Raymond D.[3], Ozelius L.J.[3], Schwinger E.[4], Bressman S.B.[3], Siebert R.[2], Klein C.[1]
[1]Section of Clinical and Molecular Neurogenetics, Lübeck, Germany, [2]Institute of Human Genetics, Kiel, Germany, [3]Beth Israel Medical Center, New York, USA, [4]Institute of Human Genetics, Lübeck, Germany

Dopa-responsive dystonia (DRD) is a childhood-onset movement disorder characterized by sustained muscle contractions causing twisting and repetitive movements or abnormal postures with an excellent response to levodopa. Most DRD is autosomal dominantly inherited with reduced penetrance and associated with mutations in the GTP cyclohydrolase 1 (GCH1) gene on chromosome 14q22. We have previously identified a three-generation family with four DRD patients and six unaffected individuals. All four affected and one unaffected individual carried a heterozygous deletion of all six exons of GCH1. All five mutation carriers also presented with ptosis. Surprisingly, one obligate carrier (II.3) of the deletion, whose sister and son were both affected, did not carry this deletion, despite confirmed paternity.
To elucidate the cause of this apparent inconsistency, we performed haplotype analysis with microsatellite markers on chromosome 14q, quantitative PCR, fluorescence in situ hybridization (FISH) with three different probes, and array comparative genomic hybridization (aCGH). aCGH was carried out with the Human Genome Microarray 244A platform (Agilent) and with a customized high resolution chip for the region of interest on chromosome 14 (Nimblegen).
Haplotype analysis of the GCH1 region revealed several incompatibilities that could be explained by a smaller deletion localized downstream of GCH1 in II.3. His mother carried the same deletion of about 700kb. These deletions were confirmed by quantitative PCR and by aCGH. One of the three FISH probes also verified the small deletion. Interestingly, this deletion expanded twice independently in this family to a deletion of ~3600kb involving about 20 genes including GCH1. This was also demonstrated by quantitative PCR, the deletion of all three FISH probes, and by aCGH. Further, high-resolution aCGH revealed a duplication of about 13 kb at the break point whereto the smaller deletion expands, indicating the duplication functions as a trigger for expansion.

In conclusion, co-occurrence of apparently unrelated disorders (DRD and ptosis) may be considered a clinical red flag pointing to a deletion involving different genes. Elucidating the genetic mechanism leading to recurrent expansion of a deletion twice in this family will contribute to a better understanding of the origin of large deletions, an important type of mutation.

P-Neuro-264

Linkage and Mutation Analysis in Families with Primary Microcephaly (MCPH)

Mueller K.[1], Liu S.[1], Uyanik G.[2], Morlot S.[3], Albrecht B.[4], Spranger S.[5], Zeschnigk C.[1], Hehr U.[6], Wolff G.[1], Morris-Rosendahl D.J.[1]
[1]Institut für Humangenetik, Universitätsklinikum Freiburg, Germany, [2]Institut für Humangenetik, Universitätsklinikum Hamburg-Eppendorf, Germany, [3]Praxis für Humangenetik, MVZ Wagnerstibbe Hannover, Germany, [4]Institut für Humangenetik, Universitätsklinikum Essen, Germany, [5]Praxis für Humangenetik, Bremen, Germany, [6]Zentrum für Humangenetik, Universitätsklinikum Regensburg, Germany

Brain size is determined by the number of neural cells, which is reduced in primary microcephaly (MCPH). MCPH is a recessive neurodevelopmental disorder resulting in a marked reduction in brain size (-3SD below the norm) without other gross structural brain malformations or severe neurological deficits. It is genetically heterogenous with seven known loci and five genes: Microcephalin (MCPH1), MCPH2, CDK5RAP2 (MCPH3), MCPH4, ASPM (MCPH5), CENPJ (MCPH6) and STIL (MCPH7). All of these genes code for centrosomal proteins, which have an important function in cell division early in neurogenesis.
We have performed linkage analysis in six families with one or more individuals affected with microcephaly, using at least five polymorphic markers within or around the seven MCPH loci. In four families the parents are related. Haplotype analysis revealed linkage to ASPM and CENPJ in an Afghan and Turkish family, respectively. Sequencing of the ASPM gene in the Afghan family is in progress. Based on this relatively small sample of families, it appears that approximately one third of cases of familial autosomal recessive primary microcephaly may be attributable to mutations in a gene at one of the seven loci. The initial screening for linkage to these loci is proving to be an effective method for elucidating the genetic cause of the MCPH in such families.

P-Neuro-265

The XLMR protein PQBP1 plays a role in alternative splicing

Musante L.[1], Fischer U.[1], Grimmer A.[1], Kalscheuer VM.[1]
[1]Max-Planck-Institute for Molecular Genetics, Berlin, Germany

The polyglutamine binding protein 1 (PQBP1) gene plays an important role in X-linked mental retardation (XLMR). Nine of the thirteen PQBP1 mutations known to date affect the AG hexamer in exon 4 and cause frameshifts introducing premature termination codons (PTCs). Using RT-PCR we have shown previously that in patients with a PQBP1 mutation the PQBP1 mRNA is degraded in a mutation-dependent manner via nonsense mediated mRNA decay. Furthermore, we have shown that patients with insertion/deletion mutations in the AG hexamer express significantly increased levels of a PQBP1 isoform which is very likely encoded by the transcripts without exon 4 (Musante et al., 2009) indicating a mechanism compensatory for the loss of PQBP1 function in patients that is based on the control of PQBP1 alternative splicing.
Now we have searched for novel PQBP1 binding partners and have found a splicing factor (PQBP1IP1) which belongs to the family of the SR proteins and is highly expressed in brain. PQBP1IP1 plays a role in the regulation of alternative splicing events. By influencing alternative splicing of its own mRNA this protein is able to autoregulate its protein level in the cell. In RNA-protein immunoprecipitation experiments we could further show that, in addition to the PQBP1IP1 protein, the PQBP1 protein complex also contains the PQBP1IP1 mRNA. Based on these physical interactions we hypothesized that PQBP1 is involved in the feedback mechanism regulating PQBP1IP1 protein expression. To test whether PQBP1 has an effect on alternative splicing of the PQBP1IP1 mRNA, we have knocked-down PQBP1 and have overexpressed a minigene containing an exon that can be alternatively spliced into the PQBP1IP1 mRNA. RT-PCR experiments and quantification of the proportion of the transcripts with and without the alternative exon indicated that knock-down of PQBP1 significantly reduced the inclusion of the alternative exon ($p<0.001$). Experiments assessing the impact of PQBP1 in the splicing regulation of further target genes involved in neuronal development are in progress.

P-Neuro-266

Expression pattern of WHSC3 and NAT8L, two genes from the Wolf-Hirschhorn syndrome critical region 1, suggests a basic role in CNS development

Nelkenbrecher C.[1], Bördlein A.[1], Endele S.[1], Schlickum S.[1], Winterpacht A.[1]
[1]Institute of Human Genetics, Erlangen, Germany

The Wolf-Hirschhorn syndrome (WHS; OMIM 194 190) is a variable, complex malformation syndrome caused by partial deletion of the distal short arm (4p16.3) of one chromosome 4 resulting in segmental aneuploidy with an unknown number of genes contributing to the different aspects of the phenotype. WHS is a complex disorder with severe neuromuscular involvement which includes mental retardation, muscular hypotonia and seizures. The neuromuscular abnormalities are thought to be caused by an additive effect resulting from the hemizygous loss of several genes. In order to elucidate the resulting pathomechanisms, it is indispensable to identify and functionally characterize all genes affected by the variable deletions in WHS patients, correlate their presence or absence with the clinical phenotype.
Here, we report detailed expression analysis of the genes, WHSC3 and NAT8L, from the Wolf-Hirschhorn syndrome critical region 1. We could show that WHSC3 (Wolf-Hirschhorn candidate 3) most probably represents a novel neuropeptide while NAT8L encodes an N-acetyltransferase. Recently it has been shown, that NAT8L is responsible for N-acetylaspartate (NAA) synthesis, which is the second most abundant metabolite in the brain. The functions of NAA in CNS development and neuron activity remains unclear. Consequently, a homozygous loss-of-function mutation in NAT8L has been described in one patient with NAA deficiency (hypoacetylaspartia). WHSC3 and NAT8L are located in a 165 Kb interval deleted in a patient with mild WHS features and mild mental retardation with attention deficit syndrome. Focusing on this neurological aspect we performed detailed expression studies during CNS development in mouse and chicken. Both genes are expressed during neocortical and cerebellar development in opposing cell layers, as well as during neural tube development. In addition, WHSC3 is also expressed in interneurons of the adult cerebral cortex and cerebellum. From the observed expression patterns and the putative protein functions we conclude that both genes are likely involved in neuronal migration during brain development. Thus, both genes represent strong candidates for an involvement in the variable neurological phenotype observed in WHS patients. Overexpression experiments for both genes in the chicken embryo are currently in progress.

P-Neuro-267

Molecular characterization of pure Hereditary Spastic Paraplegia in an extended Chilean pedigree.

Ramirez A.[1], Freimann K.[1], Wiegers K.[1], Goebel I.[2], Kubisch C.[2], Klein C.[3], Castiglioni C.[4]

[1]Section of Clinical and Molecular Neurogenetics at the Department of Neurology at the University Lübeck, Lübeck, Germany, [2]Institute of Human Genetics at the University of Cologne, Cologne, Germany, [3]Section of Clinical and Molecular Neurogenetics at the Department of Neurology, Lübeck, Germany, [4]Department of Neurology at Clinica Las Condes, Santiago, Chile

Hereditary Spastic Paraplegias (HSPs) are a heterogeneous group of inherited motor neuron disorders. HSPs are divided into pure (or uncomplicated) and complex (or complicated) forms. While pure HSPs are characterized by the presence of isolated pyramidal motor neuron dysfunction, in complex HSPs, the pyramidal dysfunction is accompanied by additional neurologic or extraneurologic features. The genetic heterogeneity of HSPs is illustrated by a growing list of genomic loci harboring genes responsible for HSP, referred to by "SPG" acronyms. Currently, 45 SPG loci and 20 causative genes have been identified. Among the known HSP genes, Spastin (SPG4/SPAST) accounts for 40-50% of all pure HSPs. Here, we report clinical and genetic findings of an extended Chilean pedigree affected by pure HSP. First, we sequenced all coding exons and intron/exon boundaries of SPG4 in the index patient and detected a novel mutation in SPG4 (c.1360G>A, p.E454K) at a highly conserved position of the protein. Mutagenesis experiments at the corresponding position in the Drosophila orthologue of Spastin showed a relevant functional role of this position in the protein (Roll-Mecak A, Vale RD.Nature. 2008. 451(7176)). Segregation analysis of this mutation revealed that only the HSP-affected mother of the index patient carried the mutation but not her unaffected parents despite confirmed paternity. Thus, these data demonstrate that c.1360G>A is a de-novo mutation. Five additional family members affected by HSP.did also not carry this mutation. Interestingly, the age of disease onset in the index patient and her mother was in the first year of life, whereas all other affected members showed a disease onset after the second decade of life, suggesting genetic heterogeneity of HSP in this family. In summary, we described here a novel de-novo mutation in SPG4 that explains only in part the HSP phenotype found in this pedigree. Therefore, we performed a genome-wide scan using a 10K GeneChip array from Affymetrix leading to the identification of a new HSP locus confirming the presence of a second disease-causing gene in this family.

P-Neuro-268

The prospective German Charcot-Mary-Tooth patient registry

Rautenstrauss B.[1], Sereda M. W.[2], Walter M. C.[3]

[1]Medizinisch Genetisches Zentrum, Munich, Germany, [2]Max-Planck-Institute of Experimental Medicine, Goettingen, Germany, [3]Friedrich-Baur-Institute, Munich, Germany

A prerequisite for clinical trials are standardized patients registries. A small number of treatments / drugs have yet been proposed as therapeutic options in CMT, but more will be available in the future. New treatments need to be tested to determine their effects: Clinical trials are research studies that evaluate these new treatments under controlled circumstances. However, inherited peripheral neuropathies are summarized in the group of rare disorders and thus some bottlenecks exist: lack of interest by industry, politics, professionals and wider public. As consequence multi-national collaboration is essential to pool resources. The bottlenecks have been identified by experts and patient organizations (AFM, DGM etc.) and include: fragmentation of data, lack of harmonization, lack of suitable infrastructures, lack of trial readiness. Trial readiness implies standardized patient registries with many benefits to registered patients, such as feedback on standards of care and new research developments, feeling a sense of "belonging" to a broader community, not being left behind as clinical trials develop, a link to the research community. For industry the benefits are easy access to the patient community, a clear concept of the target market, feasibility and planning of clinical trials, recruitment of patients into clinical trials. As a consequence, the CMT patient registry under construction is harmonized by using a minimal data set for international exchange and will be implemented in the global TREAT-NMD registry as well as the upcoming international CMT database supported by the CMTA STAR initiative. Disorders will include not only CMT but also related neuropathies as HMN and HSAN. The registration is planned as a two step process: patients self registration - questionnaire part 1; detailed clinical and genetic data: - questionnaire part 2.

A TREAT-NMD charter for the global database has been developed and revised with the help of partners, lawyers, ethicists and patient organizations and sets the framework for the cooperation between the global database, national registries and third parties. It defines best practice guidelines for the global database and national registries, has been adopted by national registries and TREAT-NMD, shall be part of contracts and agreements with third parties and is publicly available. With regard to all these international efforts the upcoming German CMT patient registry is part of a promising approach to improve research, treatment and information exchange among the interested groups.

P-Neuro-269

Periventricular nodular heterotopia (PNH) due to a novel Filamin A (FLNA) mutation in a 3 year old, moderately retarded, boy with total agenesis of the corpus callosum

Rittinger O.[1], Zeschnigk C.[2], Forstner R.[3], Morris-Rosendahl D.J.[2]

[1]UK für Kinder-und Jugendheilkunde der PMU Salzburg, Salzburg, Austria, [2]Institut für Humangenetik des Universitätsklinikums Freiburg, Freiburg, Germany, [3]UI für Radiologie der PMU Salzburg, Salzburg, Austria

Background: X-linked periventricular nodular heterotopia (PNH) is a neuronal migration disorder usually caused by loss-of-function mutations in the FLNA gene. PNH is mainly observed in females due to an X-chromosomal dominant mode of inheritance and presumably lethality in males. More recently, reports of less severely affected males indicate that the particular mutation type may be crucial to the clinical findings and outcome.

Case report. We report on a 3 -year old boy who was admitted for moderate psychomotor retardation, speech delay and microcephaly. Family history as well as perinatal period was inconspicuous. There were only subtle dysmorphic facial features, weight and height were in the normal range. Epileptic seizures had so far not been observed. Neuroimaging revealed complete agenesis of the corpus callosum (ACC) and bilateral linear enhanced signal intensity along the nodulated margin of the cella media.The patient's mother was apparently healthy and without symptoms of epilepsy.

Molecular findings: Mutation analysis in the FLNA gene revealed a previously undescribed mutation, c.62_64 dupTCG in exon 2, in the patient's lymphocytes and hair roots. The mutation results in the insertion of an additional Valine at position 21 in the amino acid sequence (p.V21dup). The patient's mother carries the mutation as a weak mosaic in DNA extracted from lymphocytes, thus confirming vertical transmission of the mutation.

Conclusion: FLNA gene mutations are known to cause quite different clinical conditions, ranging from skeletal disorders to heart valve disease, depending on the type and position of the mutation. The clinical severity of PNH seems to be more variable, particularly in males, than previously appreciated. In addition to the particular type of mutation concomitant malformations like the complete ACC observed in our patient may further modify the clinical outcome.

P-Neuro-270

Histone deacetylase inhibitors induce cellular senescence in neuroblastoma and prostate cancer

Roediger J.[1], Lornez V.[1], Kyrylenko S.[2], Baniahmad A.[1]
[1]Jena University Hospital, Institute of Human Genetics and Anthropology, Germany, [2]University of Kuopio, Department of Biosciences, Finland

Aims: Cellular senescence leads to an irreversible block of cellular division capacity both in cell culture and in vivo. The induction of an irreversible cell cycle arrest is very useful in treatment of cancer. Thus, this mechanism could potentially be a new approach for cancer therapy. Histone deacetylases (HDACs) are considered as therapeutic targets to treat cancer patients. Since they inhibit cancer growth and are used in various clinical trials similarly. Reactive oxygen species (ROS) induces an irreversible senescent-like cell cycle arrest similar to senescence. Here, we analyzed whether specific inhibitors of HDAC induce senescence in neuroblastoma and two different human prostate cancer cell lines, an androgen-dependent and an androgen-independent.
Methods: Cellular senescence was detected by analysis of senescence markers such as the SA-beta-galactosidase, Senescence Associated Heterochromatin Foci (SAHFs) and western blot analysis of cell cycle factors.
Results: We find that HDAC inhibitors induce cellular senescence in neuroblastoma and in both used human prostate cancer cell lines. We also show that the cell cycle arrest is not reversible. Furthermore, we find that HDAC class I and II inhibitors down-regulate the E2F1 protein in neuroblastoma cells. However, the cell cycle inhibitor p21 protein levels seemed to be unchanged. Interestingly, the HDAC class III inhibitor induces both cellular senescence and ROS levels.
Conclusion: These results indicate an epigenetic regulation and an association of ROS production with cellular senescence in neuroblastoma cells. The data underline that tumor cells undergo cellular senescence and irreversible cell cycle arrest, as a new possibility for tumor suppression.

P-Neuro-271

Recurrent 16p11.2-p12.2 microdeletion syndrome: narrowing the minimal critical deletion region

Roetzer KM.[1], Schwarzbraun T.[1], Obenauf AC.[1], Plecko-Startinig B.[2], Brunner-Krainz M.[2], Speicher MR.[1]
[1]Institute of Human Genetics, Medical University of Graz, Austria, [2]Department of Pediatrics, Medical University of Graz, Austria

Microdeletions in 16p11.2-p12.2 have been recently described in a number of patients exhibiting mental retardation, speech delay, developmental delay, hypotonia, dysmorphic features, and recurrent infections. However, linkage of the deleted genes to the phenotype was difficult due to the size of the deletions (~ 8Mb) and the number of genes contained.

Here we report on an 11-year-old girl with muscular hypotonia, speech delay, ptosis, recurrent infections and dysmorphic features reminiscent of patients with microdeletions in 16p11.2-p12.2. A muscle biopsy at the age of 1 year showed unspecific changes, but structural abnormalities of the mitochondria were observed. However, biochemical investigations for mitochondriopathies were inconclusive. Classical chromosome analysis revealed a normal female karyotype (46,XX) and fragile X-syndrome was excluded. Finally, array-CGH analysis revealed a 205 kb microdeletion in 16p11.2, overlapping the deleted regions in the 6 recently reported patients. Further investigations revealed that in this family, the microdeletion was inherited by the mother, and a thorough history and physical examination showed that she also exhibits mild muscular hypotonia, ptosis, and recurrent ear infections. The microdeletion contains only three disease causing genes: TUFM, ATP2A1 and CD19. TUFM is a mitochondrial translation elongation factor, and homozygous missense-mutations have been described in combined oxidative phosphorylation defects. ATP2A1 (also called SERCA1) is a calcium transporting ATPase of the sarcoplasmic reticulum, and homozygous mutations in this gene are associated with autosomal-recessive Brody myopathy. It was shown that a truncated form of ATP2A1 (S1T) transports calcium from the ER to the mitochondria and thus bridges ER stress to the intrinsic pathway of apoptosis. The third gene, CD19, is a cell surface protein mainly expressed in B lymphocytes, and homozygous mutations in CD19 have been reported in hypogammaglobulinemia and increased susceptibility to infections.
We hypothesize that the muscular hypotonia could be due to deletion of ATP2A1 and the recurrent infections might be associated with CD19 deficiency. This theory is further supported by the observation that only one patient reported by Ballif et al. did not exhibit muscular hypotonia, and this was also the only patient with a microdeletion not including ATP2A1. However, further investigations are needed to confirm the central role of ATP2A1 and CD19 in the phenotypic presentation of patients with 16p11.2-p12.2 microdeletions.

P-Neuro-272

PCDH8 and SLC44A1 are candidate genes for cognitive impairment

Rosenberger G.[1], Fritsch A.[1], Lübker B.[1], Caliebe A.[2], Schürmann M.[3], Kutsche K.[1]
[1]Institute of Human Genetics, Hamburg, Germany, [2]Institute of Human Genetics, Kiel, Germany, [3]Institute of Human Genetics, Lübeck, Germany

Mental retardation defined by a global deficiency of cognitive abilities is one of the most frequent causes of serious handicaps in children. We report on a male patient with developmental delay, muscular hypotonia, microcephaly, and postaxial hexadactyly carrying a de novo chromosome translocation [46,XY,t(9;13)(q31.1;q21.1)]. Array CGH analysis did not reveal any genetic imbalance. Mapping of the breakpoint regions by fluorescence in situ hybridization identified BAC clones overlapping the two breakpoints. In 9q31.1 the breakpoint is located ~50 kb upstream of the SLC44A1 gene (solute carrier family 44, member 1) encoding a choline transporter protein. Choline is an essential nutrient for membrane phospholipids and cell signalling molecules. Most importantly, neural cells depend on continuous choline supply for the synthesis of the neurotransmitter acetylcholine. Abundance of the SLC44A1 protein in various human CNS regions, where it is found in neuronal, glial and endothelial cells, suggests that it may have an important role in development and/or maintenance of the nervous system [1]. We mapped the breakpoint in 13q21.1 ~50 kb downstream of the PCDH8 gene (protocadherin 8) which belongs to the protocadherin gene family. PCDH8 encodes an integral membrane protein that is thought to function in cell adhesion and signalling. The murine ortholog Pcdh8 is strongly expressed in the nervous system and, interestingly, also in limb interdigital mesenchyme [2]. In addition, Pcdh8 was found to trigger an intracellular signalling cascade regulating the number of dendritic spines in an activity-dependent manner [3]. Together, we suggest that regulatory mutation of either SLC44A1 or PCDH8 might be of functional importance for the clinical manifestations in the translocation patient.

[1] Machova E. et al., J Neurochem 110, 1297-309 (2009)
[2] Makarenkova H. et al., Biochim Biophys Actat 1681, 150-6 (2005)
[3] Yasuda S. et al., Neuron 56, 456-71 (2007)

P-Neuro-273

Combined mRNA and miRNA expression profiling of the CNGA3-/- mouse – a mouse model of achromatopsia

Schaeferhoff K.[1], Michalakis S.[2], Biel M.[2], Riess O.[1], Bonin M.[1]
[1]Department of Human Genetics, Tuebingen, Germany, [2]Munich Center of Integrated Protein Science and Dept. of Pharmacy, Muenchen, Germany

The CNGA3-/- mouse is an animal model lacking the A subunit of the cone specific cyclic nucleotide gated channel. 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 CNGA3-/- 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 10 transcripts per time point were analyzed by qRT-PCR. miRNA expression profiling was conducted on an Illumina whole genome mouse miRNA array.
496 transcripts were differentially regulated in the retinas of the 4 week old mice and 204 in those of the 8 week old animals. Gene regulation networks revealed misregulations of genes associated with RNA post-transcriptional modification and cellular growth. 80 % of the transcripts chosen for real-time validation could be verified. In the miRNA array analysis of 4 week old mice we found 97 differently regulated miRNAs which have potential target genes included in the differential gene list of our previous transcriptional analysis. Three miRNAs were validated by qRT PCR including miRNAs linked to RNA post-transcriptional modification or ophthalmological diseases.
Expression analysis of the CNGA3-/- mouse highlighted a misregulation of the phototransduction cascade 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 course of degeneration.

P-Neuro-274

Frequency of progranulin mutations in a German cohort of 79 frontotemporal dementia patients

Schlachetzki J.C.[1], Schmidtke K.[1], Beckervordersandforth J.[2], Borozdin W.[3], Wilhelm C.[3], Hüll M.[1], Kohlhase J.[3]
[1]Center for Geriatrics and Gerontology, University Hospital Freiburg, Germany, [2]Department of Neuropathology, University Hospital Freiburg, Germany, [3]Center for Human Genetics, Freiburg, Germany

Mutations of the progranulin gene lead to progranulin haploinsufficiency and to frontotemporal lobar degeneration (FTD) with TDP-43 positive inclusions. It is assumed that unknown genetic, epigenetic and environmental factors are responsible for the observed marked degree of phenotypic variability among mutation carriers. This is the first published series of German FTD cases screened for progranulin mutations. Mean age at onset was 62 years, 19 patients (24%) had a positive family history of dementia, and 11 patients (14%) had a positive family history for probable FTD. Data on FTD subtypes are presented. Two mutations were identified (3%), one of which has been described previously. Clinically, both patients showed the frontal-behavioural variant type of FTD. Remarkably, a sibling of one case presented with progressive non-fluent aphasia, clinically distinct from the brother. We also performed quantitative PCR analyses to detect potential whole progranulin gene and exon deletions, but did not identify any deletion.

P-Neuro-275

PGC-1alpha and NRF1 as modifier of onset age in Huntington disease

Taherzadeh-Fard E.[1], Saft C.[2], Andrich J.[2], Arning L.[1]
[1]Department of Human Genetics, Ruhr-University Bochum, Germany, [2]Department of Neurology, Ruhr-University Bochum, Germany

Background: Although there is a strong correlation between CAG repeat length and age at onset (AO) of motor symptoms, individual Huntington disease (HD) patients may differ dramatically in onset age and disease manifestations despite similar CAG repeat lengths. Polymorphisms in the peroxisome proliferators-activated receptor gamma coactivator 1 alpha PPARGC1A) gene have recently been associated with AO of HD. Since PGC-1alpha induces mitochondrial biogenesis by coactivating specific transcription factors such as the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) and the nuclear receptor oestrogen receptor alpha (ERR alpha) we have extended association analysis by investigating additional genes regulating biogenesis and maintenance of the mitochondrial network.
Methods: A total of 30 single nucleotide polymorphisms (SNPs) in the PGC-1alpha, PGC-1beta, NRF1, NRF2, MFN2, PPAR-gamma, PPAR-alpha and ER-alpha genes were selected for genotyping in a German HD cohort of more than 400 patients recruited from the Huntington Center NRW in Bochum. Genotyping was performed by restriction fragment length polymorphism (RFLP) and Taqman assays. Variability in AO was assessed by linear regression using logarithmically transformed AO as dependent and respective genotypes as independent variables.
Results: Beside PGC-1alpha, one polymorphism in NRF1 was significantly associated with the AO of HD. Interestingly, NRF1 and PGC-1alpha together explain more variability in AO than each gene alone.
Conclusions: These findings suggest that the observed association between PGC1-alpha and AO may be mediated through functional interaction with other members of the mitochondrial network. There is evidence that genetic interaction among these loci may further elucidate mechanisms involved in HD pathology and disease progression.

P-Neuro-276

Targeted re-sequencing of an 8 Mb linkage interval in a family with an unknown neuro-degenerative disorder reveals a novel variant of Aicardi-Goutières syndrome

Thiele H.[1], du Moulin M.[2], Barczyk K.[3], George C.[2], Schwindt[4] W.[4], Roth J.[3], Rutsch F.[2], Nürnberg P.[1]
[1]Cologne Center for Genomics, Cologne, Germany, [2]University Children's Hospital, Münster, Germany, [3]Institute for Immunology, Münster, Germany, [4]Department of Clinical Radiology, Münster, Germany

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

P-Neuro-277

Breaking the silence: Functional differences in mature nicotinic alpha4-receptors caused by silent SNPs

Wanischeck M.[1], Hoda J.-C.[2], Bertrand D.[2], Steinlein O.K.[1]
[1]Institute of Human Genetics, Munich, Germany, [2]Department of Neuroscience, Geneva, Switzerland

Single nucleotide polymorphisms (SNPs) are discussed as susceptibility factors for endophenotypes of drug dependence and several neuropsychiatric disorders. Especially variants within the genes coding for neuronal nicotinic acetylcholine receptor (nAChR) subunits have been shown to be associated with nicotine abuse and schizophrenia. Exon 5 of CHRNA4, one of the major nAChR subunits contains about 75% of entire coding sequence and hence 19 of 21 annotated SNPs are located within this exon. Six of these SNPs could be experimentally verified, and had minor allele frequencies suitable for being functionally relevant for the mentioned endophenotypes. The corresponding haplotypes for these SNPs were calculated computationally and the complementary haplotypes (differing in their alleles in each of the six SNP positions) were introduced into expression vectors by step-by-step in vitro mutagenesis. The resulting cDNAs were coinjected into Xenopus oocytes with CHRNB2 cDNA in equal amounts and electrophysiological properties of the receptors were determined two to five days later by voltage clamp experiments. Although all of these SNPs are silent, i.e. do not change the "meaning" of the codon they are located in, a small but nonetheless significant difference was observed on the concentration-activation curve. The rarer haplotype 1 causes an increased sensitivity to ACh and thus a shift of the activation-curve to the left. This means that a substantial minority of the West European population carries a haplotype that causes a moderate gain-of-function effect compared to the more common haplotype 2. These differences in agonist sensitivity are likely to increase the interindividual functional diversity in nAChR-related brain processes within the population.

P-DEVELOPMENTAL GENETICS

P-Devel-278

Intronic GLI3 enhancers executing broad-range expression control in fish development govern expression specifically in tetrapod CNS and limb patterning

Abbasi AA.[1], Paparidis Z.[1], Malik S.[2], Schmidt A.[3], Koch S.[3], Grzeschik KH.[1], Oeffner F.[1]
[1]Zentrum für Humangenetik, Marburg, Germany, [2]Faculty of Biological Sciences, Islamabad, Pakistan, [3]Abteilung für Pathologie, Marburg, Germany

The transcription factor GLI3 together with its paralogue GLI2 is a major mediator of Sonic hedgehog (SHH) signaling. Many studies in mice and other model organisms proved the existence of a GLI code, the interplay of different GLI proteins and a quantitatively and temporally fine tuned expression of the GLI genes in adjacent domains. This basic morphogenetic tool is repeatedly used in early development including morphogenesis of the neural tube, prosencephalon and cerebellum, patterning of the anterior-posterior limb axis, chondrocyte differentiation, and skeletal muscle generation. The importance of hedgehog signal transduction governing the GLI code and the function of downstream target genes have been scrutinized in a multitude of studies, but the role of cis-acting sequences and associated regulatory factors involved in spatiotemporal control of Gli3/GLI3 expression remained largely elusive.
Previously, we had demonstrated that 11 human GLI3 intragenic conserved non-coding elements (CNEs) act in zebrafish as time-specific enhancers, showing considerable redundancy in expression control. Our current work proves that elements, which had driven transcription in cultured cells and zebrafish, can induce reporter gene activation matching endogenous Gli3 expression in chicken and mice, as well. We show that three sequence elements (CNE1/2/9) control reporter gene expression in developing neural murine tissues in a time- and position- specific complementary way. They are more specifically targeted at distinct areas of the CNS in mice than in fish. CNE6 and CNE11 displayed non-redundant regulatory activities in the developing mouse limb, and were also able to drive reporter gene expression in chicken limb buds, whereas CNE1/9/10 were not. The spatiotemporal regulatory role of CNE6 and 11 in zebrafish had been more redundant and not especially deployed for fin/limb patterning as now seen in chicken and mice. Our results suggest that, though fish, birds, and mammals share an ancient catalog of regulatory elements within GLI3, the function of individual enhancers have diverged considerably. Ancient regulatory elements exerting redundant expression control in fish have adopted higher specificity essential for patterning of evolutionary novel structures such as fingers and toes.

P-Devel-279

Strain specific methylation goes along with a trans allele effect at the mouse scapinin (Phactr3) locus

Bauer S.[1], Grohmann J.[1], Eckenstaler R.[1], Fink C.[1], Tauber M.[1], Theumer T.[1], Worch S.[2], Hansmann I.[1], Schlote D.[1]
[1]Institut für Humangenetik und Medizinische Biologie, Universität Halle/Wittenberg, Germany, [2]Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany

Paramutation-like phenomena 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 in a mouse model by reciprocally mating NMRI mice and Mus musculus castaneus. Using a single-nucleotide polymorphism in leader exon 1C representing a restriction fragment length polymorphism, reverse transcription PCR products of the F1 hybrids of both crosses were transcribed from the NMRI allele only. Even several backcrosses against Mus musculus castaneus exhibit only NMRI specific transcripts in the analysed offspring. Therefore, the Phactr3 exon 1C splice variant is potentially strain specific regulated, leading to the expression of only one allele of the reciprocal crosses. In our previous work, we showed that the Phactr3 splicevariant 1C was mainly transcribed in the brain. To investigate a possible epigenetic basis for this expression pattern, we analysed the tissue as well as the strain specific methylation pattern of a CpG island located in the region of exon 1C. Based on a bisulfid sequencing approach we compared the methylation pattern between NMRI, C57/BL6 and Mus musculus castaneus. There were no difference between NMRI and C57/BL6, but a significantly increased methylation in Mus musculus castaneus (_ = 0,01) which could be associated with the strain specific expression pattern. Furthermore we could also find a significant difference in the methylation pattern between normal tissues and brain in all three analysed mouse strains which is consistent with the tissue specific expression pattern of exon 1C containing splicevariants. These results potentially provide new insight into non-Mendelian inheritance in mammals and may serve also as a model for investigating the regulation of allele-specific expression.

P-Devel-280

Inactivation of insulin-like factor 6 disrupts the progression of spermatogenesis at late meiotic prophase.

Burnicka-Turek O.[1], Shirneshan K.[1], Mohamed B.[1], Paprotta I.[1], Grzmil P.[1], Meinhardt A.[2], Engel W.[1], Adham I.[1]
[1]Institute of Human Genetics, Göttingen, Germany, [2]Department of Anatomy and Cell Biology, Giessen,Germany

Insulin-like factor 6 (INSL6), a member of the insulin-like superfamily, is predominantly expressed in male germ cells. Expression of the Insl6 is first detected in mouse testis at postnatal d 15 when the first wave

of spermatogenesis progresses to pachytene spermatocytes. To elucidate the role of INSL6 in germ cell development, we generated Insl6-deficient mice. The majority of the Insl6-deficient males on a hybrid genetic background exhibited impaired fertility, whereas females were fertile. The number of mature sperm and sperm motility were drastically reduced in the epididymis. The reduced sperm count could be due to apoptotic death of a significant number of developing germ cells. Analysis of germ cell development during the juvenile life showed an arrest of the first wave of spermatogenesis in late meiotic prophase. RNA analysis revealed a significant decrease in expression of late meiotic- and postmeiotic-specific marker genes, whereas expression of early meiotic-specific genes remains unaffected in the Insl6-/- testes. These results demonstrate that INSL6 is required for the progression of spermatogenesis.

P-Devel-281

A Genetrap Mouse Model for Gerodermia Osteodysplastica

Egerer J.[1], Zhang H.[1], Chan H.W.L.[2], Kolanczyk M.[1], Künisch J.[3], Chan D.[2], Mundlos S.[4], Kornak U.[4]
[1]Max Planck Institute for Molecular Genetics, Berlin, Germany, [2]Department of Biochemistry, University of Hongkong, Hongkong, [3]Institute for Medical Genetics Charité Universitaetsmedizin, Berlin, Germany, [4]Institute for Medical Genetics Charite Universitaetsmedizin, Berlin, Germany

The rare autosomal recessive disorder gerodermia osteodysplastica (GO; OMIM 231070) is caused by loss-of-function mutations in the gene GORAB (SCYL1BP1) encoding a Rab6-interacting Golgi protein with unknown function. Patients suffer from lax and wrinkly skin, hypermobile joints, jaw hypoplasia and reduced bone mass leading to osteoporosis and increased fracture risk.
In order to study developmental, physiological and cell biological aspects of this disease, we generated a mouse model by insertion of a genetrap cassette between exon 1 and exon 2 of the murine Gorab gene. Gorab is expressed mainly in cartilage, osteoblasts, lung and skin. Homozygous mutant animals displayed a nearly complete absence of Gorab mRNA and protein expression, indicating an efficient gene inactivation. Mutant animals were born alive, but died within hours after birth due to respiratory problems caused by a delay of lung development. The mutant skin appeared swollen, but showed no significant histological abnormalities except for strikingly disorganized dermal collagen bundles in ultrastructural analyses. A mild craniofacial phenotype with mandibular hypoplasia was evident and long bones were slightly shorter and mildly bent compared to wildtype littermates. The bone phenotype was furthermore characterized by an ossification delay during embryonic development and a thickened and disorganized bone collar in newborns. A severe reduction of sclerostin (Sost) and increased dentin matrix protein 1 (Dmp1) expression implied altered osteocyte maturation. Differences in lectin staining patterns indicated alterations in glycan structures in mutant animals.
Due to the phenotypic similarities between Gorab knockout mice and gerodermia osteodysplastica patients, this genetrap mouse line is an excellent tool to uncover the pathogenesis of this rare disease as well as the physiological function of Gorab.

P-Devel-282

Selenoprotein M is expressed in developing bone and tendon

Grosch M.[1], Bock F.[1], Winterpacht A.[1], Tagariello A.[1]
[1]Institute of Human Genetics, Erlangen, Germany

Introduction: The essential trace element selenium has been shown to be of great importance for the growth and homeostasis of cartilage and bone. Consequently, selenium deficiency, occurring in regions of low selenium intake in Central Africa and some provinces in China, is associated with a special form of osteoarthritis, the Kashin-Beck disease (KBD). KBD is primarily characterized by degeneration and necrosis, mainly in the growth plate and articular cartilage. Thus, KBD represents a highly interesting model to study the pathomechanisms of inherited skeletal dysplasias, as well. The role of selenium is largely attributed to its presence in selenoproteins as the 21st amino acid, selenocystein (Sec). Only very little is known about the function of these proteins in cartilage/bone and their role in the pathogenesis of KBD.
Aim: Recently, we isolated selenoprotein M (SELM) in a screen for differentially expressed genes in human fetal growth plate cartilage/bone. The aim of the present study was to investigate expression pattern of SELM during bone development in order to learn more about the destructive and/or protective effect of selenium on cartilage/bone tissue during development.
Results and Discussion: The expression pattern of Selm was determined during chicken limb development. After cloning and sequencing the complete coding sequence of the chicken Selm gene, we investigated Selm expression in sections from developing limb at embryonic (Hamburger Hamilton) stage 29 to 40 by RNA-in situ-hybridization. At all stages investigated Selm showed a highly specific expression in bony structures. Counterstaining and detailed histological investigation revealed expression in osteoblasts. In addition, we detected expression in tendon. Based on functional data present in the literature it is assumed that Selm encodes a thiol-disulfide oxidoreductase probably participating in isomerization or reduction of disulfide bonds in the endoplasmic reticulum. Together with the observed expression pattern this points to a putative function of SELM in the processing of extracellular matrix proteins during skeletal development. Investigation of possible target proteins is in progress.

P-GENOMICS, TECHNOLOGY AND BIOINFORMATICS

P-Techno-283

Quality Control and Quality Management for High-Throughput Genotyping Projects

Bettecken Th.[1], Wienker T.F.[2], TMF–QC/QM Genotyping Consortium T.[3]
[1]Max Planck Institute of Psychiatry, München, Germany, [2]University of Bonn Institute of Medical Biometry, Informatics and Epidemiology, Bonn, Germany, [3]Speaker Michael Krawczak University of Kiel Institute of Medical Informatics and Statistics, Kiel, Germany

Highly integrated microarrays dedicated to SNP genotyping, process automation, and a concomitant drop in cost per genotype have made genome-wide genotyping of thousands and ten thousands of DNA samples a feasible research strategy to unravel the genetic determinants of complex traits and diseases. With the vast majority of genetic variants being biallelic SNPs, genotype calls are categorical and fourfold: homozygous (AA), heterozygous (AB), counter-homozygous (BB), and no call (0). Copy number (CNV), other structural (SV) and epigenomic variants are or will be other types of markers of potential relevance in genome-wide association studies (GWAS). Current microarray technologies are able to interrogate more than one million SNPs respectively loci per sample.
There are two main platforms for microarray based genome-wide SNP genotyping: Affymetrix and Illumina. While the Affymetrix chips are based on hybridisation of labelled DNA, with one SNP locus represented by several probes ("features"), the Illumina assays are based on hybridisation with a subsequent minisequencing reaction, introducing a different fluorochrome for each of the two alleles. In both methods, the microarrays are scanned for fluorescence intensities after laser excitation. There is an extraordinary demand on both accuracy and reproducibility for each single genotype call in high-throughput genotyping methodologies. Error rates exceeding a rather low threshold will render results of association analyses unreliable and erroneous. This is particularly the case in large projects with very big sample sizes required for the detection of weak genetic effects.

A stringent quality control and quality management is mandatory there prior to statistical analyses. On the technical side, data quality should be managed at three levels: (i) low level (e.g. feature data from hybridization probes or from beads, fluorescence signal intensities), (ii) intermediate level (normalized signal intensities, cluster parameters and cluster assignments, genotype calls), and (iii) high level (single SNP statistics, Hardy-Weinberg deviation, global heterozygosity values, identity-by-state values, etc., and multilocus LD-based stats, improbable haplotypes etc., population heterogeneity). Plausibility checks such as systematic tests for congruence of phenotypic with genotypic gender, inadvertently duplicated samples and cryptic genetic relationship are additional QM and QC high level measures to be employed before a genotype data package should be released for comprehensive statistical analysis.
In any case, since most high-throughput genotyping projects are large and involve many dislocated research groups over a longer period of time, standardized documentation of data generating processes and analytic procedures is mandatory.
A multistep QC process implementing a series of stringent and tuned error filtration procedures is under development and evaluation by the BMBF funded TMF-QC/QM consortium. This consortium has adopted the mission to provide the research community with reliable tools for QC/QM necessary in all types of high throughput and genome-wide genetic association studies.
With the advent of next generation DNA sequencing and genome-wide epigenomics, future projects will produce even more demanding data, both with respect to sheer volume and more complicated structure. In order to prevent 'personalized standards' different between independent groups, it is of importance to agree on minimal data quality, core information content, and minimal data format conversion options. Matching these requirements will greatly foster the success of meta analyses , and will dramatically reduce the expenses for data management, data storage and computing hardware.

P-Techno-284
DARVIN & CONAN: Copy Number Variation Prediction and Analysis Software Solutions for Genomewide CNV Association Studies
Brandstätter A.[1], Haider F.[1], Kluckner T.[1], Forer L.[1], Weißensteiner H.[1], Schönherr S.[2], Specht G.[2], Kronenberg F.[1]
[1]Division of Genetic Epidemiology, Innsbruck, Austria, [2]Department of Database and Information Systems, Innsbruck, Austria

Background: Genome-wide association studies (GWAS) based on single nucleotide polymorphisms (SNPs) revolutionized our perception of the genetic regulation of complex traits and diseases. Copy number variations (CNVs) are structural variations of DNA greater than 1 kilobase in size and account for a considerable amount of human genetic variation. CNVs have gained increasing recognition as key factors in disease. However, available software packages for the determination of CNVs from SNP chip data are difficult to handle and highly inefficient for the analysis of thousands of samples as encountered in genetic epidemiology. In addition, the downstream statistical inference of CNV-phenotype interactions is still subject to complicated R-programming, thus strongly limiting the performance of GWAS based on CNVs.
Methodology: We present two freely available software packages for the detection of CNVs from SNP-array data and for a downstream GWAS developed in an academic environment. DARVIN (Database supported Algorithm for Rapid genomic Variation INference and prediction) provides a robust, integrated implementation of algorithms for interrogation of CNVs using Affymetrix 500K SNP arrays. Our algorithm consists of three main steps: normalization, copy number inference, and CNV prediction applying Hidden Markov Models. DARVIN is a server-client software solution with an intuitive graphical user interface providing economy, efficiency, and flexibility in experimental design. CONAN (COpy Number variation ANalysis software) is also a server-client software solution for performing GWAS based on CNVs with an intuitive graphical user interface for categorizing, analyzing and associating CNVs with phenotypes. Moreover, CONAN assists the evaluation process by visualizing detected associations via Manhattan plots in order to enable a rapid identification of genome-wide significant CNV regions.
Conclusions: DARVIN and CONAN facilitate the conduction of GWAS based on CNVs and the visual analysis of calculated results.

P-Techno-285
Genome & Transcriptome Analysis: Maximising the Benefits of Next-Generation Sequencing by Combining Technologies
Dr. Hinz M. (GATC Biotech AG, Konstanz, Germany)

The large data quantity and high sequence quality provided by the Illumina GAII can be used in combination with traditional Sanger sequencing or other Next-Gen technology, e.g. GS FLX. The benefits of ultradeep sequencing increase the information gained from de novo genome sequencing or transcriptome sequencing.
Genome Sequencing: GAII sequencing data can be combined with GS FLX sequencing data to improve the accuracy of the assembly. Homopolymers are corrected using the high accuracy of the 36bp,76bp, or longer reads. Paired end and mate pair data which are mapped to the de novo contigs orient the contigs and create scaffolds. Proprietary software developed at GATC is used to close gaps in the assembly and correct repeats in the genomic sequence.
Transcriptome Sequencing: Organisms without a genome reference or extensive EST data can be qualitatively and quantitatively analysed by combining data from the GS FLX and the GAII. A reference EST data set can be generated from normalised cDNA on the GS FLX. The ultra-high coverage from non-normalized cDNA can be mapped to these sequences. Using a tagging protocol developed at GATC it is possible to mulitplex samples on the GAII to generate expression profiles from several samples in a cost effective way. For both project types SNPs in the genomic sequence or the cDNA can be detected. This analysis allows detection of heterozygous alleles or mutations in the cDNA sequence that may have a phenotypic or disease relevant influence.
Conclusion: By optimally combining several Next Gen technologies with state of the art bioinformatic analysis it is possible to generate high quality project results.

P-Techno-286
The Heidelberg-Tübinger-MRNET experience: Molecular karyotyping of 175 parent-patient-trios with unexplained mental retardation by Mapping 6.0 SNP arrays
Dufke A.[1], Moog U.[2], Grasshoff U.[1], Riess A.[1], Rossier E.[1], Lemke J.[1], Riess O.[1], Böhringer B.[2], Steinlicht S.[2], Kapeller J.[2], Karch S.[2], Bonin M.[1], Rappold G.[2]
[1]Department of Medical Genetics, Tübingen, Germany, [2]Institute of Human Genetics, Heidelberg, Germany

Only few autosomal mental retardation (MR) genes have been identified to date and none seems to be a common cause of MR. The primary goal of the MRNET-project is to identify such genes using different technological approaches. Recent developments in genomic microarray technology (array-CGH / molecular karyotyping techniques) allow genome wide detection of submicroscopic chromosomal alterations. Among clinically unselected MR-patients with a normal karyotype, 10-15% carry submicroscopic de novo deletions and duplications that are not seen in healthy controls and are likely to be for the cause of their MR. In contrast, the majority of benign copy number variations (CNVs) are inherited. The goal of the MRNET study is to find and define small submicroscopic deletions/duplications in clinically well defined patients with MR for which other causes of their disorder have been excluded. Using this approach we expect to find novel genes which are candidates to be tested in a large cohort of MR patients.
In this study we took advantage of a so-called trio-analysis approach (father, mother, affected child with unexplained MR) to drastically

reduce elaborative, costly and time consuming experiments of yet undescribed CNVs using Mapping 6.0 SNP arrays. In a cohort of 175 patients a total of 5292 CNVs (mean: 30,24 CNVs/patient) had been found. Out of these, 346 CNVs (mean: 1,97 CNV/patient) have not been reported previously as common benign variants or where only partially overlapping with known CNVs. However, the number of de novo and thus potentially pathogenic CNVs was reduced to 29 by synchronous trio analysis. In conclusion, analysis of parental DNA was shown to be inevitable for the validation of CNVs in the majority of cases justifying a priori trio-analysis. Compared to array-CGH, trio analysis in SNP-arrays has furthermore the potential to unmask the parental origin of CNVs as well as the detection of Uniparental Disomies in a single experiment.

P-Techno-287

Massively parallel Sequencing as a promising diagnostic tool in heterogeneous monogenic disorders: the example of Hypertrophic Cardiomyopathy

Fokstuen S.[1], Iseli C.[2], Robyr D.[3], Munoz A.[3], Antonarakis SE.[4], Blouin JL.[1]
[1]Genetic Medicine, University Hospitals of Geneva, Switzerland, [2]Ludwig Institute for Cancer Research; Lausanne; Swiss Institute of Bioinformatics, Lausanne, Switzerland, [3]Genetic Medicine and Development, University of Geneva School of Medicine, Switzerland, [4]Genetic Medicine; University Hospitals of Geneva; Genetic Medicine and Development, University of Geneva School of Medicine, Switzerland

Hypertrophic Cardiomyopathy (HCM) is the most common inherited cardiac disorder (prevalence : 1/500) with a remarkable genetic and allelic heterogeneity (> 550 mutations in at least 22 genes). Molecular testing for HCM has a growing impact on the medical management of patients and their families. To overcome the extensive genetic heterogeneity we have developed a 30 Kbp microarray to resequence all exons (n=160), splice-sites and 5'-UTR of 12 HCM genes (HCM-custom-DNA-resequencing-array, HCM-RA, Fokstuen et al, 2008). This method is now used as a diagnostic tool in our clinical practice. It is rapid and very cost-effective but does not detect small indels (~14% of all known HCM mutations). Moreover, HCM-RA lacks flexibility as the addition of new genes requires a new design.
In order to solve these shortcomings we analysed the 12 HCM-RA genes (targeted by multi-exonic amplicons) using short read massively parallel sequencing (MPS) and a newly developed downstream data analysis pipeline. We initially reanalysed the DNA of 19 patients previously hybridized by the HCM-RA (11 without known mutations, 8 positive-controls as a composite-pool) in one lane of an Illumina Genome-Analyzer flow cell. All 8 known pathogenic mutations and SNPs previously identified were also found by MPS. Furthermore we identified novel variants: two indels and a nonsense mutation in MYBPC3 gene that likely cause the disease. In another experiment, we sequenced a single patient with no known mutation in one lane. No pathogenic mutation but many non-coding dbSNPs were identified.
Although improvements are needed in target enrichment, data analysis and reduction of false positive variants, MPS holds considerable promises for mutations/variants analysis of highly heterogeneous monogenic disorders in clinical practice.

P-Techno-288

High Resolution Melting as a method for mutation screening in complex genes

Heinecke K.[1], Gehrig A.[1], Müller-Reible C. R.[1]
[1]Institut für Humangenetik, Würzburg, Germany

High Resolution Melting analysis (HRM) is a highly automatable technology for heteroduplex analysis of DNA fragments. The melting profile of patient DNA is compared to that of a control DNA. These profiles differ if the patient DNA sequence differs from the control by as little as a single nucleotide. In brief, the examined exon is amplified by PCR in the presence of a saturating intercalating fluorescent dye like EvaGreen that fluoresces only if it is bound to double stranded DNA. After amplification has been completed, the temperature is increased in small steps. Depending on the GC composition of the amplicon, the double-strands separate into single strands at a specific temperature and the dye is released, leading to a sharp decrease in fluorescence. Since the dye saturation is rather homogeneous along the amplicon, HRM allows the differentiation between hetero- and homozygous alleles, as the latter also show differences in their melting profiles. Using the Qiagen RotorGene Q, we have established the HRM conditions for three complex genes: RYR1, BRCA1 and BRCA2 and present data from our validation studies.
The ryanodine receptor RYR1 is the major calcium release channel of skeletal muscle. Dominant and recessive point mutations in RYR1 are the cause for a number of congenital myopathies, e.g. Central Core Disease (CCD), Multi-Minicore Disease (MMD) and related structural myopathies, and for Malignant Hyperthermia (MH), a pharmacogenetic disposition to anesthetic complications. In MH susceptible people, volatile anesthetics and succinylcholine can induce an uncontrolled release of calcium leading to a hypermetabolic, life-threatening crisis. The RYR1 gene is located on chromosome 19 and consists of 106 exons.
BRCA1 and BRCA2 are the major genes for familial predisposition to breast- and ovarian cancer with 22 and 26 coding exons, respectively. Thousands of individual mutations have been described in these genes, thus making a comprehensive analysis a challenge.
We have validated the 41 HRM amplicons of BRCA1 by 121 positive controls with known mutations or SNPs and detected all of them with only 1.6 % false-positives (sensitivity = 97.5 %, specificity = 98.4 %). For RYR1, most exons could be analysed as single amplicons. Larger exons, especially exon 91, have been splitted into smaller fragments. Of the 104 positive controls, all were detected by HRM with only 0.4 % false-positives (sens. = 97.1 %, spec. = 99.6 %). The validation for BRCA2 is ongoing.
In conclusion, High Resolution Melting analysis has been shown to be accurate and reliable for mutation screening of patient samples in large genes. Its advantages are: (1) single-tube assays, (2) highly automatable sample preparation, (3) easy and rapid data analysis, (4) low cost per sample and (5) sensitivity and specificitiy values which compare well to other mutation scanning methods and even to sequencing.

P-Techno-289

INTERSNP: Genome-wide Interaction Analysis Guided by A Priori Information

Herold C.[1], Steffens M.[1], Meesters C.[1], Brockschmidt F.[2,3], Baur M.[1,4], Becker T.[1]
[1]Institute of Medical Biometry, Informatics and Epidemiology - University of Bonn, Bonn, Germany, [2]Department of Genomics - Life & Brain Center - University of Bonn, Bonn, Germany, [3]Institute of Human Genetics, University of Bonn, Germany, [4]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Genome-wide association studies (GWAS) have lead to the identification of hundreds of genomic regions associated with complex diseases. Nevertheless, a large fraction of their heritability remains unexplained. Interaction between genetic variants is one of several putative explanations for the "case of missing heritability" and, therefore, a compelling next analysis step. However, genomewide interaction analysis (GWIA) of all pairs of SNPs from a standard marker panel is computationally unfeasible without massive parallelization. Furthermore, GWIA of all SNP triples is utopian. In order to overcome these computational constraints, we present a GWIA approach that selects combinations of SNPs for interaction analysis based on a priori information. Sources of information are statistical evidence (single marker association at a moderate level), genetic relevance (genomic location) and biologic rel-

evance (SNP function class and pathway information). We introduce the software package INTERSNP that implements a logistic regression framework as well as log-linear models for joint analysis of multiple SNPs. Automatic handling of SNP annotation and pathways from the KEGG database is provided. In addition, Monte-Carlo simulations to judge genome-wide significance are implemented. We introduce various meaningful GWIA strategies that can be conducted using INTERSNP. Typical examples are, for instance, the analysis of all pairs of nonsynonymous SNPs, or, the analysis of all combinations of three SNPs that lie in a common pathway and that are among the top 50,000 single-marker results. We demonstrate the feasibility of these and other GWIA strategies by application to a GWAS data set and discuss promising results.
Availability: The software is available at http://intersnp.meb.unibonn.de

P-Techno-290
Evaluation of the 250 K SNP array for molecular karyotyping in patients with mental retardation
Hofmann K.[1], Göhring I.[1], Hoyer J.[1], Ekici A. B.[1], Reis A.[1], Rauch A.[2]
[1]Institute of Human Genetics, Erlangen, Germany, [2]Institute of Medical Genetics, Schwerzenbach- Zurich, Switzerland

Molecular karyotyping, i. e. genome wide copy number analysis using high- resolution array systems, were shown to uncover microaberrations in about 10% of patients with mental retardation or multiple congenital anomalies of unknown origin.
We explored the use of Affymetrix 250K Nsp GeneChip® arrays in 126 patients with unexplained mental retardation or multiple congenital anomalies for genome wide copy number analysis. 69 patients were additionally analysed with the 6.0 GeneChip Array® which contains 1.8 M probes.
Using the Nexus Copy Number (Version 3.1) software, a total of 1303 CNVs were detected with a mean of 11 CNVs per patient. The average size of copy number changes were 512 kb and 21 SNPs. 38 of 119 CNVs, which had a size of at least 100 kb or which were indicated by at least 5 markers could be confirmed by FISH or MLPA. 14 of these aberrations arose de novo and recurrent microdeletion syndromes were found in 7 cases (16p11.2, 15q13.3, 17p13.3). While none of the potential aberrations smaller than 100 kb indicated by less than 20 markers could be verified, aberrations larger than 1 Mb or containing more than 50 SNPs nearly always proofed true.
69 patients were additionally analysed with the 6.0 microarray. Of 13 gains indicated by the 250 K array, only 2 indicated by 17 and 231 SNPs were also seen with the 6.0 array. The remaining 11 non-reproducible duplications contained 11-76 markers. On the other hand, only 22 of 76 CNVs larger than 1 Mb indicated by 6.0 arrays have been also seen with the 250K array. We therefore conclude that the 250 K array is only capable to detect microaberrations larger than 1 Mb with sufficient reliability and specificity.

P-Techno-291
Exome sequencing of patients with Kabuki syndrome
Hoischen A.[1], Gilissen C.[1], Wieskamp N.[1], Arts P.[1], van Lier B.[1], van der Vliet W.[1], Buckley M.F.[1], van Bon B.[1], de Vries B.[1], Scheffer H.[1], Brunner H.G.[1], Veltman J.A.[1]
[1]Department of Human Genetics Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Up to half of all rare genetic diseases remain unexplained at the molecular level. Identification of the causative genes in these disorders has been difficult because of their low frequency (not allowing association studies), de novo occurrence (not allowing linkage analysis), and lack of frequent occurring structural genomic variants (not allowing microarray-based CNV approaches). The recent development of massive parallel sequencing technology, combined with whole exome enrichment, now for the first time allows the unbiased analysis of all coding sequences in the human genome. We apply this strategy by capturing and sequencing the exome of three patients with Kabuki syndrome. For each patient we capture 34 Mb of genomic sequence using the Roche NimbleGen Exome capture array representing ~180,000 human exons and 550 miRNAs. The captured fragments are processed by the Roche 454 FLX Titanium, resulting in up to 1Gb uniquely map-able data per patient. More than 85% of the captured sequence can be mapped near or on the target sequence, indicating efficient enrichment. We find up to 7100 exonic variations per patient compared to the reference genome including 0.2% nonsense, 34% missense mutations and 13.9% small insertions and deletions. These numbers are consistent with those reported on the sequencing of other individual genomes. Array based genotyping confirms on average 98% of the SNP variations, indicating the genotyping accuracy of this novel approach. After exclusion of all known SNPs, variations are classified based upon information from splice sites, amino acid changes, evolutionary conservation, protein truncating potential, and protein structure predictions. Currently we are in the process of validating the high ranking mutations by Sanger sequencing, testing for de novo occurrence, and testing disease candidate genes in a cohort of more than 50 Kabuki patients.
To even generate higher coverage we have now implemented the Applied Biosystems SOLiD sequencer for exome sequencing. We compared data generated by both sequencing platforms, and compared different enrichment techniques for human whole exome re-sequencing.

P-Techno-292
Translational control of DDX3X and DDX3Y transcripts by microRNA 3′ UTR binding
Jaroszynski L.[1], Jurek C.[1], Zimmer J.[1], Vogt PH.[1]
[1]Unit Molecular Genetics and Fertility Disorders; Dept Gynaecological Endocrinology andFertility Disorders; University Women Hospital,Heidelberg, Germany

Human DDX3X and DDX3Y genes belong to the highly conserved PL10-like DEAD-box RNA helicase family. Its members are characterised by presence of nine conserved sequence motifs organised in two domains and specific N- and C- terminals of variable size. The function of these RNA helicases – mainly unwinding of short stretches of duplex RNA – requires a tight control of their cellular quantities because they are necessary for many cellular processes, including RNA processing, its nuclear export, its stability, and its translation with additional involvement in ribosome biogenesis (Cordin et al., 2005; in: Gene 367: 17-37). Interestingly, although DDX3X and DDX3Y transcripts were found in all human tissues and in leukocytes, only DDX3X transcripts are translated ubiquitously, whereas translation of DDX3Y transcripts is completely inhibited in leukocytes and all non-testis tissues. Moreover, in testis tissue DDX3X protein is restricted to post-meiotic germ cells whereas DDX3Y proteins were found only in pre-meiotic germ (Ditton et al. 2004; in: Hum Mol. Genet. 13: 2333-2341). This suggests a tight cellular control of the translation of DDX3X and DDX3Y transcripts not only in germ cells, but also in all other human tissues with distinct cellular compositions.
Non-testis DDX3X and DDX3Y transcripts have similiar long 3′UTR extensions (ca. 2.5kb), but only the DDX3X transcripts were found to be translated (Ditton et al., 2004). We, therefore, wanted to know whether there are distinct "cis" and "trans" elements binding specifically to the 3′UTR of DDX3X or DDX3Y transcripts supporting the translation of only DDX3X transcripts but inhibiting the translation of DDX3Y transcripts.
It is well known that the translational control of eukaryotic transcripts by their 3′UTR sequence can be mediated by the binding of distinct microRNAs abundantly expressed in each human cell but with distinct profiles (Chekulaeva & Filipowicz, 2009; in: Curr. Opin. Cell Biol. 21: 452-460). We, therefore, analysed whether a different binding pattern

of microRNAs along the 2.5 kb long 3´UTR sequence of DDX3X and DDX3Y transcripts can probably explain their distinct translational capacity.
Using the TargetScan screening program for the analysis of microRNA binding sites and the miRNA databases containing all human microRNA sequences we found indeed a number of microRNA binding sites present in the 3´UTR sequence of DDX3X and DDX3Y transcripts. Some of them were only found in the DDX3Y 3´UTR. Most of these miRNAs displayed expression in all human tissues analysed. Interestingly, the identified 3´UTR binding sites were mainly located downstream of the internal polyadenylation sites located in their proximal 3´UTR and activated specifically only in testis tissue.
We, therefore, set out to clone the different lengths of the DDX3Y and DDX3X 3´UTR sequence in the pGL4.13 LUC expression reporter vector downstream of the luc2 gene and analysed comparatively their translational capacities in 293T cells. We found translational repression only with those reporter constructs which contain the long 3´UTR. When we analysed their translational capacity in cells inhibiting DICER activity (essential for the cellular microRNA processing pathway) translational repression seemed to be released.
We, therefore assume, that the binding of microRNAs not only to the 3´UTR of DDX3Y transcripts, but also to that of DDX3X transcripts, contribute to the translational capacity of both transcripts not only in testis tissue, but also in all other human tissues and in leukocytes, thereby tightly controlling ubiquitously the cellular DDX3X and DDX3Y protein amount.

P-Techno-293
RNAi to identify genes modifying lysosomal cholesterol storage in Niemann-Pick Type C disease
Runz H.[1], Kern L.[1], Blattmann P.[1], Honarnejad S.[1], Kranz A.L.[2], Erfle H.[3], König R.[2], Pepperkok R.[4]
[1]Institute of Human Genetics, Heidelberg, Germany, [2]DKFZ, Heidelberg, Germany, [3]Bioquant RNAi Screening Facility, Heidelberg, Germany, [4]EMBL, Heidelberg, Germany

Lysosomal storage disorders (LSDs) are variable on a clinical as well as on a molecular level. For the rare autosomal-recessive cholesterol-storage disease Niemann-Pick Type C (NPC) it is believed that in addition to mutations in the two known disease-causing genes NPC1 and NPC2 further factors have an impact on disease manifestation and clinical course. We have developed and applied an integrative functional genomics strategy that allows a systematic identification of novel regulators of lysosomal cholesterol storage in cell models of NPC disease. For this, candidate genes were identified by genome-wide gene expression profiling of healthy and diseased cultured cells. The impact of these genes on lysosomal cholesterol storage was then tested by targeted siRNA knock-down experiments. With this strategy, a small number of genes were identified as functional regulators of cellular cholesterol homeostasis, several of which appear to specifically affect cholesterol egress from endo-/lysosomal compartments. With this, our strategy is a valuable tool for disease gene identification in conditions like LSDs that present with a phenotype in cells.

P-Techno-294
MutationTaster - Rapid evaluation of the disease-potential of sequence alterations
Schwarz JM.[1], Rödelsperger C.[1], Schuelke M.[1], Seelow D.[1]
[1]Charité - Universitätsmedizin Berlin, Berlin, FRG

With the advent of Next Generation Sequencing, researchers all over the world face the question of how to handle and process an enormous wealth of data obtained by each sequencing run. While software for the alignment of the sequence snippets and the detection of alteration exist, there is a lack of suitable tools for automatic classification of the alterations that will be revealed. Currently available prediction tools are mainly designed to only evaluate amino acid exchanges or other single aspects which account for the 'severity' of a mutation. Moreover, these tools are not able to handle hundreds or thousands of queries in a reasonable time. Given the huge number of synonymous and non-exonic alterations that are guaranteed to be discovered by Next Generation Sequencing approaches (recent studies suggest more than 1,000 alterations per Mbp, even after applying filters to high coverage data), we felt the need for a fast and comprehensive automatic solution.
We have therefore developed MutationTaster, a web-based application aimed at the prediction of the disease potential of DNA sequence alterations in humans. MutationTaster incorporates many different biological analyses such as amino acid exchanges, evolutionary conservation, the loss of protein features or of splice-sites and hence allows a better distinction between harmless and disease causing alterations. Moreover, it can also analyse intronic or silent alterations.
The prediction is generated by a naïve Bayes classifier which was trained with more than 320,000 valid SNPs and more than 50,000 known disease mutations, leading to a ratio of correct predictions of >85%. The evaluation of a single alteration usually takes less than 0.3 seconds, making MutationTaster an ideal tool for the analysis of the vast numbers of alterations found by Next Generation Sequencing. For this task, we additionally offer alignment software which handles raw data from all common deep sequencing platforms as well as scripts for batch analysis of the alterations found. Besides, we designed an intuitive web interface for the query of single alterations.
To our knowledge, MutationTaster is the first comprehensive application covering the whole process from raw Next Generation data to the analysis of the detected alterations.
The software is freely available at http://www.mutationtaster.org.

P-Techno-295
HomozygosityMapper - an interactive approach to homozygosity mapping
Seelow D.[1], Schuelke M.[1], Hildebrandt F.[2], Nürnberg P.[3]
[1]Charité - Universitätsmedizin Berlin, Berlin, FRG, [2]University of Michigan, Ann Arbor, USA, [3]Universität zu Köln, Köln, FRG

Homozygosity mapping is a common method for mapping recessive traits in consanguineous families. In most studies, applications for multipoint linkage analyses are applied to determine the genomic region linked to the disease. Unfortunately, these are neither suited for very large families nor for the inclusion of tens of thousands of SNPs. Even if less than 10,000 markers are employed, such an analysis may easily last hours if not days.
Here we present HomozygosityMapper, a web-based approach to homozygosity mapping. SNP positions and HapMap allele frequencies are stored in a database and are regularly updated. Users can upload their own SNP genotype files generated by Affymetrix or Illumina devices without any modifications. Within a few minutes, HomozygosityMapper analyses the data, detects homozygous stretches and presents the results in intuitive graphical interfaces: The homozygosity in affected individuals is visualised genome-wide with the ability to zoom into single chromosomes and user-defined chromosomal regions. The software can also display the underlying genotypes in all samples with a comprehensible colour-code to quickly inspect the region and manually adjust its borders. It is integrated with our candidate gene search engine, GeneDistiller (http://www.genedistiller.org), so that users can interactively determine the most promising gene.
Users can at any point restrict access to their data or make it public, allowing HomozygosityMapper to be used as a data repository for homozygosity mapping studies. Data can also be shared with selected co-operation partners, hence allowing the joint analysis of the disease. Besides, the application can be employed ‚on the fly', without registration and permanent storage of the data.

HomozygosityMapper was extensively validated and did not fail to identify the disease regions, albeit in a much a shorter time than conventional linkage software and with a greater robustness against genotyping errors. Since its publication in summer 2009, HomozygosityMapper has caught great attention from researchers from all over the world. By autumn 2009, it had more than 130 registered users. More than 360 different projects, featuring more than a billion of genotypes, had been analysed and permanently stored.
New developments comprise the integration of further species such as cattle, dog, and mouse.
HomozygosityMapper is available at http://www.homozygositymapper.org/.

P-Techno-296
Development of a Protocol for Enrichment of Specific Genomic Target Regions Suitable for Sequencing on a Roche GSflex System
Walter M.[1], Bauer C.[1], Honegger A.[1], Kick B.[1], Bauer P.[1], Riess O.[1], Bonin M.[1]
[1]Institute of Medical Genetics, Tübingen, Germany

Current high throughput sequencing technologies produce several gigabases of sequence information within days. However, the capacity is still too limited to sequence complex eukaryotic genomes routinely. Several capturing technologies and several sequencing platforms are available. We developed protocols to generate sequencing libraries from genomic DNA which are compatible with Roche GSflex sequencing technology. Reduction of sample complexity was performed on febit Biochips and the Geniom RT Analyzer which allows tight control of hybridization and washing conditions. Efficiency of target enrichment was estimated by real-time PCR and Sanger sequencing of cloned fragments prior to high-throughput sequencing. Different library preparation protocols both with single and dual rounds of enrichment were evaluated in terms of overall enrichment, coverage, sequence bias, accuracy and possibility for introducing multiplex identifier.

P-THERAPY FOR GENETIC DISEASES

P-Therap-297
Directed exon skipping as a cancer prophylactic in the hereditary cancer syndrome, Nijmegen Breakage Syndrome
Digweed M.[1], Hildebrand G.[1], Rothe S.[1], Salewsky B.[1], Sperling K.[1]
[1]Institut für Humangenetik, Charité - Universitätsmedizin Berlin, Germany

In the hereditary cancer disorder Nijmegen Breakage Syndrome (NBS), average age at malignancy is 9.7 years and average age at death from malignancy is 10.7 years. Over 90% of NBS patients are homoallelic for a 5bp deletion in the NBN gene. This hypomorphic mutation leads to a partially functional carboxyterminal fragment of the protein nibrin, p70-nibrin. Complete absence of nibrin is embryonically lethal in mammals. We have previously shown that another rare mutation leads to alternative splicing of NBN-mRNA, which then lacks exon 6 and exon 7, leading to an internally deleted but functionally even more active fragment, p80-nibrin. This protein is associated with a milder phenotype, particularly with respect to cancer occurrence.
This situation resembles in some respects that of the genetic disorder muscular dystrophy where truncated Dystrophin or internally deleted Dystrophin lead to strikingly different phenotypes. Molecular therapy for muscular dystrophy has been successfully explored by using antisense oligonucleotides to artificially direct exon skipping. In order to explore the potential of antisense oligonucleotide directed alternative splicing of NBN, antisense phosphorothioate 2'-O-methyl ribose oligonucleotides were synthesized for exonic splice enhancers in Exon 6 and Exon 7. Antisense oligonucleotides were transfected into patient cells using a cationic liposome for delivery and mRNA was subsequently isolated over a 7 day period. RT-PCR was used to detect the alternatively spliced mRNA lacking exons 6 and 7. Alternative splicing occurred rapidly and levels of Ex6Ex7-del mRNA were sustained over several days. The PCR products were isolated and sequeced to verify that they indeed originated from the wild type and Ex6Ex7-del mRNA.
The expression of p80-nibrin in the antisense oligonucleotide-transfected cells was examined by immunoprecipitation using specific antibodies. A protein of the expected size reacting specifically with antibodies directed against nibrin was indeed expressed with a kinetic paralleling that of the alternatively spliced mRNA. In order to exclude non-specific effects of oligonucleotide transfection, cells were treated in parallel with oligonucleotides representing the same sequence in the sense orientation: p80-nibrin was produced exclusively after transfection with the antisense oligonucleotides. Thus, the major hypomorphic mutation in NBS can be converted in cells in vitro into a milder splice variant producing a protein fragment with significant activity. The method can now be tested in vivo in appropriate mouse models with the aim of establishing whether it could potentially become the basis of a cancer prophylactic for NBS patients.

P-Therap-298
A novel Corepressor Inhibits Prostate Cancer Cell Growth
Gerlach C.[1], Reeb C.[1], Heinssmann M.[1], Ceraline J.[2], Baniahmad A.[1]
[1]Institute of Human Genetics and Anthropology, Jena University Hospital, Germany, [2]Laboratoire de Cancérologie Expérimentale et de Radiobiologie, Université Louis Pasteur de Strasbourg, France

Aims: We designed a highly specific corepressor to inhibit the androgen receptor and mutant receptor. The human androgen receptor (AR) promotes prostate cell proliferation and also prostate cancer (PCa) growth, which is one of the most often diagnosed malignancies in men. PCa is treated through the ablation of androgens and application of anti-androgens (AR antagonists), which function by recruiting Corepressors (CoR) such as Alien or SMRT to the AR that lead to transcriptional silencing on epigenetic level. The therapy with androgen ablation and treatment with anti-androgens is at first successful, but eventually prostate cancer proliferation becomes independent of androgens. Crucially, the AR can also be activated in a ligand-independent manner through the signal transduction machinery, such as membrane receptor tyrosine kinases, like Her/neu or protein kinase A (PKA). Interestingly, activation of these signalling pathways inactivate CoR. Thus, these activation pathways may significantly contribute to the androgen refractory prostate cancer proliferation.
Methods: We designed a novel AR CoR by fusing a strong repression domain to the peptide aptamer 524, that interacts highly specific with the antihormone-bound AR and the binding is resistant to PKA signaling.
Results: The transactivation of human AR and accordingly the expression of the endogenous PSA gene was inhibited. Importantly we could show that this new CoR also inhibits the growth of a PCa cell line.
Conclusion: Taken together these data suggest that AR interacting peptides fused to a strong transcriptional silencing domain are potent, specific and PKA independent CoR of the AR.
Therefore, these novel CoRs represent a novel peptide based strategy to treat PCa, also in the androgen-independent and refractory stages.

P-Therap-299
Cell-based examination of stop codon readthrough as a potential therapeutic strategy for genetic diseases: the example of Fanconi Anemia
Heinrich T.[1], Friedl R.[1], Hoehn H.[1], Schindler D.[1]
[1]Department of Human and Medical Genetics, University of Wuerzburg, Germany

Therapeutic options for the treatment of genetic diseases are rare. Depending on the disorder a considerable percentage of alleles (up to 70%) carry nonsense mutations. These mutations produce premature

termination codons (PTCs) generating mRNA isoforms with truncated reading frames leading to mRNA decay or a shortened and nonfunctional protein product. Aminoglycoside antibiotics – apart from their antimicrobial activity – can induce translational readthrough of PTCs. This observation prepared the ground for therapeutic approaches aiming at the development of drugs that promote ribosomal readthrough of premature but not normal termination codons. This way, the reconstitution of sufficient levels of – at least partially – functional proteins might be achieved. A recently reported candidate readthrough agent is PTC124. Currently, several clinical studies explore the potential of PTC 124 for treatment of diseases like cystic fibrosis and Duchenne muscular dystrophy.
Our initial experiments using bivariate ethidium bromide - Hoechst33258/BrdU flow cytometry examined the effects of PTC124 (custom synthesized from commercially available precursors) in cell lines derived from patients carrying biallelic nonsense mutations in various of the Fanconi anemia (FA) genes (FANCA, FANCC, FANCE, FANCJ). FA is an inherited genome instability disorder characterized by progressive bone marrow failure and strongly increased cancer risk. In this pilot series of experiments, there was no significant difference in cell cycle distributions (FA cells typically show G2 phase arrest) between PTC124-treated and untreated FA cells.
Therefore we are currently establishing a different read-out system utilizing plasmid constructs. We developed a dual reporter system consisting of the fluorescent protein AcGFP and renilla luciferase (rluc). Both genes are separated by a linker that allows for the incorporation of different sequence contexts. In the absence of in-frame stop codons a fusion protein is generated retaining both fluorescence and rluc-mediated luminescence activity. We introduced stop codon - containing DNA sequences derived from FA patients carrying nonsense mutations. This approach offers the possibility to assess stop codon readthrough caused by PTC124: the vector design provides a tool for quantifying AcGFP fluorescence as a measure of transfection efficiency and rluc-mediated luminescence as a measure of stop codon readthrough. Using this construct for the transfection of HEK293 cells our preliminary experiments investigated the effect of PTC124 in the case of a FANCJ nonsense mutation (UGAC) that had been introduced into the vector. The cells were cultured in the presence of different concentrations of PTC124 (0.2 µM, 1 µM, 5 µM, 20 µM, 100 µM; DMSO served as control) and were assayed after cell lysis for fluorescence and luminescence activity. Our analysis revealed a low level of ribosomal readthrough which was independent of the PTC124 dose applied. So far, it is not possible to unequivocally assign this readthrough effect to the action of PTC124 since there were no significant differences between PTC124- and DMSO-treated cells. Further studies involving additional sequence contexts are currently in progress, addressed to clarifying the effect of termination suppression mediated by PTC124.

P-Therap-300
Uptake of PNA (peptide nucleic acids) modified by COX VIII presequence peptide into mitochondria of cultured cells
Lamla M.[1], Seliger H.[1], Kaufmann D.[2]
[1]AG CFIBS University Ulm, Ulm, Germany, [2]Institute of Human Genetics University Ulm, Ulm, Germany

Single base pair alterations in the mitochondrial genome (mtDNA) are found in mitochondria of patients with mitochondriopathies, in cancer cells and aged cells in human. We are interested in developing a method for targeting a specific mtDNA sequence in the mitochondria in vitro by oligomers consisting of peptide nucleic acids (PNA). PNAs are synthetic structural homologues of nucleic acids in which the negatively charged phosphate-sugar backbone of the polynucleotide is replaced by an uncharged polyamide backbone consisting of achiral N-(2-aminoethyl) glycine units. The resulting decrease in electrostatic repulsion allows the formation of a PNA–DNA hydrogen-bonded double helix, which is more stable than the one formed by DNA–DNA interaction. PNA is used for targeting chromosomal genes binding DNA and RNA in a sequence-specific manner in cells both in vitro and in vivo. PNA can be designed to target selectively mtDNA containing a point mutation. A limitation for this approach is the transport of the PNA into the mitochondria. Here we investigate the uptake of COX VIII presequence peptide modified PNA (peptide-PNA) into isolated mitochondria from HeLa cells and cultured HeLa cells in comparison to triphenylphosphonium cation modified PNA (TPP-PNA). The isolated mitochondria internalize the peptide-PNA predominantly into the mitochondrial matrix, as detected in subfractionation experiments. This uptake depends on the proton gradient of the mitochondria. The peptide-PNA was processed intramitochondrially, where the peptide was cleaved. This kind of peptide-PNA uptake and processing was also found in cultured HeLa cells using Chariot for transfection and in isolated mitochondria of fibroblasts. The uptake of TPP-PNA into the isolated HeLa mitochondria occurred faster than the uptake of peptide-PNA. The majority of the transfected TPP-PNA was found in the inner membrane fraction, in contrast to the experiments using peptide-PNA. Our study indicates that these modifications of PNA influence their intramitochondrial localization, the amount taken up by mitochondria, the velocity of uptake and the intramitochondrial processing. In the following, we will investigate if COX VIII presequence peptide modified-PNA represents a tool to influence the replication of mutated mtDNA in cells.

P-GENETIC COUNSELLING, EDUCATION, GENETIC SERVICES, PUBLIC POLICY

P-Counse-301
Predictive genetic testing and the right to know and not to know
Bobbert M. (Institute for History and Ethics, Heidelberg, Germany)

Predictive genetic testing (postnatal) can touch upon the core areas of an individual´s personality. It can therefore be generally argued that as far as the own genetic constitution is concerned every individual is entitled to a "right to know" as well as a "right not to know". In many cases physicians have a pre-decisive function which is important for the individual right to know or not to know: they decide first, whether to inform a patient about the possibility of genetic testing. These predecisions are not so easy to make especially if no therapeutic measures are available to treat a disease or if one person´s right to know might collide with another person´s right not to know. It might also be difficult to decide an appropriate approach if a disorder displays only low penetrance or highly variable expressivity.
In medical practice and ethics the right not to know needs well founded arguments. Against a right not to know Chadwick (1997) holds a duty to know - because of the responsibility to lead one´s life on the basis of maximal information in order to make appropriate life decisions. Also Wachbroit (1998) says that we are obliged towards others and thus should be informed about our health risks. Other say that a fundamental understanding of human freedom protects different forms of living with health risks and self-understandings (Widmer 1994). Newly the right not to know is very much defended by Hildt (2007) and Kollek/Lembke (2008). In behalf of children Davis (1997) holds for a right of children to an open future whereas Savalescu (2001) argues for far reaching right to know of parents.
Physicians must give good reasons for pro and cons of predictive genetic testing if they consult patients in this regard. The arguments for a right not to know are controverse and must be profiled against a duty of information about health constitution and health risks. Special ethical questions come up if children might be predictively tested. In which cases should parents be asked for substitute decision making?
Central arguments for and against a right not to know are presented and discussed. It will be shown in what kind of cases the right not

to know should be taken very serious and in what kind of cases the right not know is not relevant. This will be done by commenting some clinical cases from an ethical perspective. A physician´s competence of balancing arguments for and against predictive genetic information guarantees an appropriate consultation prior to a possible test.

P-Counse-302
EuroGentest Clinical Utility Gene Cards – Expert Documents for the Evaluation of Genetic Tests
Dierking A.[1], Javaher P.[1], Schmidtke J.[1]
[1]Institute of Human Genetics, Hannover, Germany

Clinical utility refers to the ability of genetic test results, either positive or negative, to provide information that is of value in the clinical setting. From many different perspectives this is the most important but on the other hand also the most difficult aspect to define. A major challenge is to balance clinical validity, clinical utility and cost-benefit issues. In some cases a test is performing superbly in the laboratory, but is not viable from the clinical or economical point of view. On the other hand some tests are limited in their validity, but nevertheless have great impact on patient and family management. It is therefore important that (1) the specific requirements for a test with regard to clinical validity are defined in the context of their impact on the clinical setting and (2) that the laboratory genetic test is only one of the components of an overall evaluation and/or intervention.
Therefore, the current activities of EuroGentest concentrate on the establishment and administration of clinical utility guidelines ("clinical utility gene cards, CUGCs") which include criteria such as clinical, economic and psychological measures of possible benefit as well as possible harm. The focus of this work lies on Mendelian, mostly rare, disorders. Prototypic CUGCs are presented.

P-Counse-303
Genetic knowledge in the German population
Dinkel A.[1], Berth H.[2], Borkenhagen A.[3], Ronel J.[1]
[1]Department of Psychosomatic Medicine and Psychotherapy, München, Germany, [2]Department of Medical Psychology and Medical Sociology, Dresden, Germany, [3]Department of Medical Psychology and Medical Sociology, Leipzig, Germany

Background: Genetic susceptibility testing for hereditary diseases is developing rapidly. Along with its rapid advancement, critical issues concerning the further development of gene technology have been raised by scientists as well as the lay public. In order to adequately assess the benefits and limits of gene technology, people require genetic knowledge. So far, most studies on genetic knowledge have used clinical samples. In our study, we were interested in the genetic knowledge of the German population.
Methods: We conducted a representative survey of the German general population (N = 2.512). Genetic knowledge was assessed using the Genetischer Wissensindex (GeWi), a 12-item true-false questionnaire with known reliability and validity. Aditionally, we asked about attitudes towards genetic testing and whether the participants (or family members) were personally affected by some kind of genetic disorder.
Results: Correct answers to the individual genetic knowledge items ranged from 50 % to 71 %. 4 percent of the respondents gave 10 or more correct answers. The GeWi mean score was 5.0 (SD = 2.4). In univariate analyses, educational level and religious affiliation were associated with genetic knowledge. Personal experiences with genetic diseases did not influence the level of genetic knowledge. Participants who thought that gene tests would do more harm than good showed a higher level of genetic knowledge.
Discussion: This study documents for the first time the level of genetic knowledge in the German population. The results support the role of educational level in genetic knowledge. Furthermore, general attitudes towards genetic testing were associated with genetic knowledge. Consequently, the question arises whether it would be advisable to educate the public about genetics („public health genomics").

P-Counse-304
Capacity Building for the Transfer of Genetic Knowledge into Practice and Prevention: Health Care Needs Assessment for Medical Genetic Services in Middle- and Low-Income Nations
Nippert I.[1], Kristoffersson U.[2], Schmidtke J.[3], Kent A.[4], Christianson A.[5], Raouf R.[6], Barreiro C.[7]
[1]Universitaetsklinikum Muenster, Muenster, Germany, [2]Lunds University, Lund, Sweden, [3]Medizinische Hochschule Hannover, Hannover, Germany, [4]Genetic Interest Group, London, United Kingdom, [5]University of the Witwatersrand, Johannesburg, South Africa, [6]Ministry of Health, Cairo, Egypt, [7]Hospital de Pediatría SAMIC, Buenos Aires, Argentina

Background: CAPABILITY (http://www.capabilitynet.eu) is a 3-year model project developed by the European Network of Excellence: Genetic Testing in Europe - Network for test development, harmonization, validation and standardization of services (EuroGentest) (http://www.eurogentest.org) and by leading experts from emerging economies: Argentina, Egypt and South Africa, the latter being currently engaged in major development projects to integrate genetic services in primary care and prevention in their countries.
CAPABILITY Objectives:
• identify priorities for capacity building for genetic services by a systematic health care needs assessment (HCNA) and
• validate the HCNA approach by means of demonstration projects.
Methods: CAPABILITY has formalised a HCNA for medical genetic services that middle- and low-income nations can utilise for implementing genetic services. Key elements of the HNCA are the development of strategic aims; an evaluation of existing services and the environment in which they function; a review of epidemiology, effective interventions, opinions of consumers and professionals, available resources and possible contraints. Analysis of these elements enables the production of a HCNA. The approach is currently assessed by demonstrations projects in Argentina, Egypt and South Africa.
In Conclusion: A systematic HCNA will assist nations with emerging economies to marshal and allocate their limited resources when developing medical genetic services.
Funded by: EC Contract no.: FP6-037275.

P-Counse-305
Human Genetics, the Law and the Public.
Petermann H. (Institute for Ethics History and Theory of Medicine, Muenster, Germany)

Since the „Gendiagnostikgesetz" (genetic diagnostik law) passed the German parliament in 2009 the discussion about genetic diagnostics ame to an end.
Together with „Stammzellgesetz" (stem cell law) and „Embryonenschutzgesetz" (embryo protection law) three acts build the frame for research in human genetics in Germany. But there are still more questions than answers are given
General problem oft he discussion on medical topics ist he understanding of term. There is no universal validity for terms like „Embryo" and „Stem cell". Nonetheless legislative regulations and research projects recurr on those. For example, „Embryo" describes in different countries different states oft he human development. „Embryo" is an imprecise term.
In the ethical discussion the argument of potentiality needs clarity and general validity oft he term „Embryo". On the other hand new developments in stem cell research cause further problems in understanding.
Report in media fort he lay public like journals and newspapers are for many people the only source to gain information about human genet-

ics. Although most oft he topics are need for understanding a basic knowledge in natural sciences. Articles illustrate risks and benefits. But also the selection of topics influences the reader's opinion. They create pictures and ideas of future possibilities.
SPIEGEL online published on October 20, 2009: „Noch mehr und noch schneller: Stammzellforscher können seit wenigen Jahren normale Körperzellen in Alleskönner-Stammzellen verwandeln. Allerdings war das ganze mühselig und ineffizient. Nun gelang es Forschern, die Ausbeute auf das 200-Fache zu steigern – durch Zugabe von drei einfachen Chemikalien."
Politicians are influenced by the general opinion oft he public. If results of research cause fear of abuse they try to regulate this in laws like in the „Embryonenschutzgesetz". The question is whether there will be soon a regulation of adult stem cell research.
The conclusion is that there is a need to influence the public debate on human genetics.

P-Counse-306
The APC Open Variation Database
Vogt S.[1], Spier I.[1], Wöllner K.[1], Propping P.[1], Hoffmann P.[2], Nöthen MM.[1], Friedl W.[1], Aretz S.[1]
[1]Institute of Human Genetics, University of Bonn, Germany, [2]Department of Genomics, University of Bonn, Germany

Background: The amount of genetic variation data is continuously increasing. Locus-specific databases (LSDB) are a valuable source of information on genetic variants, especially to evaluate the clinical relevance of unclassified variants in molecular diagnostics. However, currently most of the data is neither systematically collected nor made available for clinicians and researchers. Moreover, sufficient phenotype information is often lacking. Since most identified mutations and their consequences cannot be published in regular journals, the scientific community has to collaborate through structured information handling.
The Human Variome Project (HVP) represents a challenging and long-standing international initiative that aims to collect, catalogue, curate, characterise and make accessible all human genetic variation affecting human health in a standardised quality proved way and support the implementation of the necessary infrastructure. This ambitious effort includes the global collection of mutations in all human genes and the associated phenotypes.
APC database: Familial adenomatous polyposis (FAP) is caused by germline mutations in the tumour suppressor gene APC. The Institute of Human Genetics at the University of Bonn has access to one of the largest polyposis cohorts worldwide. To contribute to the HVP we founded an APC mutation database in the easy-to-use Leiden Open Variation Database (LOVD) format which aims to supply a role in internationally collecting and sharing of variant data combined with phenotypic information (www.lovd.nl/APC). Submitted variants will become public after curation and annotation conform to current mutation nomenclature.
The present content consists of data extracted from all our published and non-published FAP families (n>1550) and provide both comprehensive genetic and clinical information. However, due to privacy protection the latter is currently not available for the public but can be given upon request to professionals such as researchers and health care providers.
Future activities: We plan to soon add much more data extracted from published literature and we will approach all laboratories performing APC diagnostics to submit their unpublished variants. With these efforts and future collaborations we aim to maintain a valuable up-to-date online resource of APC data. The project is supported by the Deutsche Krebshilfe.

P-POPULATION GENETICS, GENETIC EPIDEMIOLOGY, EVOLUTIONARY GENETICS

P-Popul-307
Genetic Screening in Europe
Javaher P.[1], Nyoungui E.[1], Kääriäinen H.[2], Kristoffersson U.[3], Nippert I.[4], Sequeiros J.[5], Schmidtke J.[1]
[1]Department of Human Genetics of Hannover Medical School, Hannover, Germany, [2]Department of Medical genetics of University of Turku, Turku, Finland, [3]Department of Clinical Geneticsof University Hospital, Lund, Sweden, [4]Department of Human Genetics of Universitaetsklinikum Muenster, Muenster, Germany, [5]UnIGENe and CGPP IBMC – Institute for Molecular and Cell Biology and ICBAS of University Porto, Porto, Portugal

Genetic screening has been defined as any kind of test performed systematically for the early detection or exclusion of a genetic disease, or to determine whether a person carries a gene variant, that may produce disease in his or her offspring. In comparison to „genetic testing", the term „genetic screening" should be reserved for the explicit and systematic application of a dignostic genetic test across a whole population of asymptomatic people (population screening), or a subset of a population such as pregnant women (prenatal/antenatal screening) or newborn infants (neonatal screening). This survey intends to present the current (2006-2008) status of genetic screening and the organisation of genetic screenin programmes in selected European countries as a background for future attempts of harmonizing standards and procedures of genetic screening, an explicit aim of the European Network of Excellence, EuroGentest (www.eurogentest.org). It bulids on a first comprehensive assessment of genetic screening programmes in Germany by the European Society of Human Genetics, starting with a workshop of experts in 1999, the production of a background documentation in 2000, and a final report in 2003.

P-Popul-308
Analysis of three Microsatellite Markers (D5S818, D7S870 and D13S317) in Romanian population and their genetic relationship with other European populations
Rodewald A.[1], Kroll A.[2], Cardos G.[3], Tesio C.[4], Banica D.[5]
[1]Institute of Human Biology, University of Hamburg, Germany, [2]Institute of Human Biology, University of Hamburg,Germany, [3]Victor Babes National Institute, Bucharest, Romania, [4]Biological Faculty, University of Bucharest, Romania, [5]Marius Nasta Institute, Bucharest, Romania

We report on analysis of 3 different DNA-polymorphisms (Microsatellites D5S818, D7S870 and D13S317) in a sample of 200 individuals from Bucharest, Romania, as a part of a more complex study, in order to elucidate the genetic structure of Romanian population and to show their genetic relationship with other European human populations.
Genomic DNA was isolated from whole blood samples and amplified by PCR. Allele assignment was performed by capillary electrophoresis by ABI 3100 Analyzer. Our results were compared with similar data of a Romanian population sample from Prahova Valley and other European human populations.
The genetic relationship between populations was evaluated based on both Nei's genetic distance and Principal Component Analysis by Phylip Package (version 3.6) and Statistical Package for the Social Sciences Software.
Our results revealed no significant difference in the allele frequencies of the three microsatellite markers between the panmictic population of Bucharest and the slight isolated population from Prahova Valley.
Genetic distance analysis and PCA showed closer genetic kinship to Greek population, as well as Slavic population from Poland.
Intercultural changes and intense trading activities beteewn old human populations from Romania (Thracians) and Greek population groups,

who established colonies on the west coast of the Black Sea (nowadays East-Romania) during the 7th-8th century, may explain our findings. The Slavic influence may be the result of migrations of Slavic groups across the Charpatian-Danube regions during the 6th-9th centuries. This data can also be used for paternity and forensic analyses in Romanian population.

P-Popul-309

The C15orf2 gene in the Prader-Willi/Angelman syndrome region is undergoing positive selection

Wawrzik M.[1], Lich C.[1], Buiting K.[1], Horsthemke B.[1]
[1]Institut für Humangenetik, Universitätsklinikum Essen, Germany

The proximal long arm of human chromosome 15 contains a cluster of imprinted genes, which are expressed from either the paternal or the maternal allele only. In 2000 we have identified an intronless gene in this region, C15orf2, which is biallelically expressed in testis but paternally expressed in fetal brain. The gene, which does not have an orthologue in rodents, encodes an 1156-amino-acid protein of unknown function. Two genome-wide phylogenetic studies, in which the ratios between nonsynonymous and synonymous single nucleotide polymorphisms (SNPs) in different species were calculated, reported evidence for positive selection of C15orf2 in primates (Nielsen et al. 2006; Kosiol et al., 2009). In population genetic-based searches for partial (Voight et al., 2006) or complete selective sweeps (Williamson et al., 2007), however, C15orf2 did not feature prominently. For investigating this question further, we have used a novel approach, which is based on complex C15orf2 haplotypes. For detecting signs of recent positive selection at this locus, we investigated SNPs, which have low mutation rate, and short tandem repeats (STRs), which have a high mutation rate. By genotyping patients with a deletion 15q11q13 we were in the position to determine the haplotypes experimentally. We analysed three nonsynonymous SNPs and two STRs in a total of 182 probands. We observed four different SNP haplotypes, which occur at different frequencies. In the absence of recent positive selection we would have expected that each SNP haplotype shows a high degree of STR variation. However, this is only the case for the two SNP haplotypes of intermediate frequency (T-G-G and T-C-C). Only the low frequent, apparently ancestral halpotype (C-G-G) and the most frequent haplotype (T-G-C) show very little STR variation. Remarkably, 92% of the chromosomes with the T-G-C haplotype, which represent almost 50% of all chromosomes 15 in our population, have the same STR alleles. It appears as if this SNP haplotype has so recently expanded in the population that it has not yet accumulated many STR mutations. This finding is compatible with the assumption that C15orf2 is still undergoing positive selection.

Autorenregister

C

D

H

S

T

U

V

W