Selected Presentations

SEL Selected Presentations

SEL 1

ZFYVE27, a novel spastin binding protein is mutated in hereditary spastic paraplegia (SPG30).

Mannan A.(1), Krawen P.(1), Sauter S.(1), Boehm J.(1), Agnieszka C.(1), Neesen J.(1), Engel W.(1)

1) Institute of Human Genetics, Goettingen, Germany

Mutation in spastin is the principal cause for autosomal dominant form of hereditary spastic paraplegia (AD-HSP). Spastin is suggested to be involved in vesicular cargo trafficking processes by interacting with microtubule and components of vesicles transport machinery, however a comprehensive function of spastin is not yet elucidated. To characterize the molecular function of spastin, we used the yeast two-hybrid approach to identify new interacting partners of spastin. Here, we report ZFYVE27, a novel member of FYVE finger family of protein as a specific binding partner of spastin. In vivo co-immunoprecipitation experiments in mammalian cells validated the interaction between spastin and ZFYVE27 as observed in yeast. Our intracellular studies revealed a striking co-localization of ZFYVE27 with spastin in vesicle like structures. Spastin mediates its interaction with ZFYVE27 through its N-terminal region containing a MIT domain. More importantly, we report a German family with AD-HSP (SPG30) in which ZFYVE27 is mutated. Sequence analysis of this gene in affected and unaffected members of this family revealed segregation of a missense mutation G105V in all affected patients. The mutated ZFYVE27 protein shows aberrant intracellular pattern in tubular structure and its interaction with spastin is severely affected. We postulate that this specific mutation in ZFYVE27 affects neuronal intracellular trafficking in the longest axons of the CNS, which is consistent with the pathology of HSP.
Note: In conference exciting result on molecular mechanism of spastin and preliminary characterization of other spastin binding proteins will also be presented.

SEL 2

Hyperactive K-Ras signalling causes disorders of the noonan spectrum

Kratz C.(1), Schubbert S.(2), Rowe S.(2), Böll S.(1), Klein C.(1), Bollag G.(3), van der Burgt I.(4), Musante L.(5), Wehner L.-E.(6), Nguyen H.(3), West B.(3), Rauch A.(7), Niemeyer C.(1), Shannon K.(2), Zenker M.(7)

1) University of Freiburg, Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Freiburg, Germany
2) University of California at San Francisco, Department of Pediatrics, San Francisco, United States of America
3) Plexxikon Inc., Berkeley, United States of America
4) University Medical Center Nijmegen, Department of Human Genetics, Nijmegen, Netherlands
5) Max-Planck Institute for Molecular Genetics, Berlin, Germany
6) University of Göttingen, Institute of Human Genetics, Göttingen, Germany
7) University of Erlangen-Nuremberg, Institute of Human Genetics, Erlangen, Germany

Noonan syndrome (NS; MIM 163950) is a dominant disorder characterized by short stature, facial dysmorphism and cardiac defects. In ~50% of the cases NS is caused by heterozygous mutations in *PTPN11*, which encodes the SHP-2 protein tyrosine phosphatase. SHP-2 relays growth signals from activated cell surface receptor complexes to downstream effectors, including Ras. *RAS* genes encode signal switch molecules that regulate cell fates by cycling between inactive GDP-bound (Ras-GDP) and active GTP-bound (Ras-GTP) states. Cancer-associated somatic mutations lead to substitutions at codons G12, G13, or Q61 that impair the intrinsic Ras GTPase and confer resistance to GTPase-activating proteins (GAPs). We discovered novel *de novo* germ line *KRAS* mutations that introduce V14I, T58I, or D153V amino acid substitutions in 5 unrelated individuals with Noonan syndrome (NS) and a P34R alteration in a patient with cardiofaciocutanous (CFC) syndrome (MIM 115150), a disorder that has many overlapping features with NS. Recombinant V14I and T58I K-Ras proteins display aberrant biochemical properties, with intermediate levels of intrinsic GTP hydrolysis and responsiveness to GTPase activating proteins relative to wild-type and oncogenic K-Ras proteins. T58I and V14I K-Ras render primary hematopoietic progenitors hypersensitive to growth factors and deregulate signal transduction in a cell lineage specific manner. These studies establish germ line *KRAS* mutations as a cause of human disease, demonstrate distinct functional properties of two mutant alleles, and provide strong evidence that the NS spectrum is due, in large part, to hyperactive Ras.

SEL 3

Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) is caused by mutations in the sodium/phosphate cotransporter gene SLC34A3

Lorenz-Depiereux B.(1), Benet-Pages A.(1), Eckstein G.(1), Tenenbaum-Rakover Y.(2), Wagenstaller J.(1), Tiosano D.(3), Albers N.(4), Lichtner P.(1), Schnabel D.(5), Hochberg Z.(6), Strom T.M.(7)

1) GSF National Research Center, Institute of Human Genetics, Neuherberg, Germany
2) Ha'Emek Medical Center, Pediatric Endocrine Unit, Afula, Israel
3) Meyer Children's Hospital, Haifa, Israel
4) Children's Hospital, Osnabrück, Germany
5) Charité CVK, Department of Pediatric Endocrinology, Berlin, Germany
6) Meyer Children's Hospital and Technion - Israel Institute of Technology, Haifa, Israel
7) GSF National Research Center and Klinikum rechts der Isar der TUM, Institute of Human Genetics, München, Germany

Hypophosphatemia due to isolated renal phosphate wasting is a heterogeneous disorder of phosphate homeostasis. Three well defined Mendelian hypophosphatemias have been reported: (i) X-linked hypophosphatemia (XLH), (ii) autosomal dominant hypophosphatemic rickets (ADHR), and (iii) hereditary hypophosphatemic rickets with hypercalciuria (HHRH). The genes mutated in XLH and ADHR have been identified as PHEX (The HYP Consortium, Nat. Genet. 11, 1995) and FGF23 (The ADHR Consortium, Nat. Genet. 26, 2000), respectively. HHRH is a rare autosomal recessive form that is characterized by reduced renal phosphate reabsorption, hypophosphatemia and rickets. It is distinct from other forms of hypophosphatemia by increased serum level of 1,25-dihydroxyvitamin D resulting in hypercalciuria. Using SNP array genotyping, we mapped the disease locus in 2 consanguineous families to chromosome 9q34. The candidate region contained a renal sodium/phosphate cotransporter gene, SLC34A3. Sequence analysis revealed disease-associated mutations in 5 families including 2 frameshift and 1 splice site mutation. Loss of function of the SLC34A3 protein presumably results in a primary renal tubular defect, compatible with the HHRH phenotype. We also show that the circulating phosphaturic factor FGF23, which is increased in XLH and carries activating mutations in ADHR, has normal to low normal serum levels in HHRH patients, further arguing for a primary renal defect. Identification of the gene mutated in HHRH adds a new protein, the sodium/phosphate cotransporter SLC34A3, to the proteins involved in the regulation of phosphate homeostasis.
We found no mutation in PHEX, FGF23 and SLC34A3 in 4 families with autosomal recessive inheritance of hypophosphatemia, suggesting further heterogeneity. Carrying out a genome-wide scan in these families should result in the identification of more genes involved in phosphate metabolism.

SEL 4

Johanson-Blizzard syndrome: Multiple congenital abnormalities caused by a defect of the ubiquitin ligase UBR1

Zenker M.(1), Mayerle J.(2), Fiedler A.(1), Lerch M.M.(2), Reis A.(1)

1) Institute of Human Genetics, Erlangen, Germany
2) Ernst-Moritz-Arndt University, Department of Gastroenterology, Endocrinology and Nutrition, Greifswald, Germany

Johanson-Blizzard syndrome (JBS; OMIM 243800), an autosomal recessive multisystem disorder, comprises a unique combination of congenital abnormalities, including exocrine pancreatic insufficiency, hypo-/aplasia of the alae nasi, scalp defects, imperforate anus, deafness, hypothyroidism, dental anomalies, genitourinary malformations, and variable mental retardation. We now report positional cloning the underlying defect. After mapping the disease locus to 15q14-21.1 we identified mutations in the gene *UBR1* as the cause underlying JBS. *UBR1* encodes a homonymous ubiquitin ligase of the N-end rule pathway, an ubiquitin-dependent proteolytic pathway whose substrates include proteins with destabilizing N-terminal residues. The vast majority of JBS-associated *UBR1* alleles represented truncating mutations, corresponding to complete lack of UBR1 protein expression as demonstrated in tissues of JBS patients. We present detailed clinical and molecular data of previously reported and newly ascertained JBS patients, indicating genotype phenotype correlation with particular respect to the cognitive function in JBS patients, that may range from normal to severe impairment. We also provide evidence that the pancreatic defect in JBS, one of the most constant features, is caused by in-

Workshops

trauterine-onset destruction of acinar cells and probably related to impaired zymogen excretion. Moreover, we discuss possible pathogenetic implications in the light of the knowledge that the protein encoded by the JBS gene is involved in intracellular targeted protein degradation. We propose that each of the features of JBS is related to overexpression / increased half-life of certain protein(s) that are specific substrates of UBR1. Identification of these pathogenetically relevant UBR1 substrates will not only lead to an understanding of the unique JBS phenotype, but may also have implications for other, more common disorders.

Workshops

W1 Cancer Genetics I

W1 01

Whole genome submegabase resolution array CGH analysis of AML/MDS patients with complex karyotypes involving 11q amplification.

Zatkova A.(1), Wandehack M.(2), Schoch C.(3), Jotterand M.(4), Storlazzi C.T.(5), Surace C.(5), Mannhalter C.(6), Andersen M.K.(7), Johansson B.(8), Fonatsch C.(1), Wimmer K.(1), Ullmann R.(2)

1) Abt. für Humangenetik, KIMCL, MUW, Wien, Austria
2) Max Planck Institute for Molecular Genetics, Department Ropers, Berlin, Germany
3) Medizinische Klinik III, Klinikum Grosshadern, Ludwig-Maximilians-Universität München, München, Germany
4) Service de génétique médicale, Unité de cytogénétique du cancer, Maternite – CHUV, Lausanne, Switzerland
5) Department of Pathological Anatomy and Genetics, Section of Genetics, University of Bari, Bari, Italy
6) KIMCL, MUW, AKH, Wien, Austria
7) Department of Clinical Genetics, Section of Hematology/Oncology, Rigshospitalet Copenhagen, Copenhagen, Denmark
8) Department of Clinical Genetics, University Hospital, Lund, Sweden

Acute myeloid leukemia or myelodysplastic syndrome (AML/MDS) patients with complex aberrant karyotypes (CAK) typically share a later onset of disease, specific gene expression profiles and poor prognosis. In 40-60% of AML/MDS with CAK, amplification of chromosome region 11q23.3, harbouring the mixed lineage leukaemia gene (MLL) locus, is observed. Recently, we have shown that in addition to this MLL core amplicon, independent sequences in 11q13.5 and/or 11q23-24 were co-amplified within the same cytogenetic markers in 60% and 90% of cases, respectively. Here we present our data on 12 AML/MDS cases with amplification of the MLL locus as obtained by array CGH using a whole genome, submegabase resolution tiling set consisting of more than 36,000 BAC clones. Our results revealed an astonishing complexity of rearrangements, including the delineation of several novel regions frequently co-amplified with the MLL gene. Loss of genomic sequences within chromosome arms 5q, 17p, 7q, and 20q, were observed in 11/12, 9/12, 5/12, and 4/12 cases, respectively. The precise definition of shortest regions of overlap in these recurrently deleted regions of 5q, 7q and 17p lays the ground for uncovering genes possibly involved in this and other leukaemias. This study represents one of the first efforts to fully characterize gains and losses on a whole genome basis with submegabase resolution in a distinct group of leukaemia patients. It proves the potential of array CGH to detect non-random copy-number aberrations responsible for neoplastic transformation that have been masked under complex karyotypes.

W1 02

Different DNA repair response of human and chimpanzee cells to cisplatin-induced DNA damage

Weis E.(1), Haaf T.(1), Galetzka D.(1)

1) Institute for Human Genetics, Johannes Gutenberg University, Mainz, Germany

Humans and chimpanzees differ in their susceptibility to diseases including certain types of cancer, degenerative diseases, virus infections, and malaria. Because the cellular response to DNA damage is likely to play an important role for development and progression of cancer and many other diseases, we were interested to find possible differences in DNA repair between humans and chimpanzees. To this end, EBV-transformed lymphoblastoid cells of five humans and five chimpanzees were treated with the DNA-strand breaker cisplatin. We have developed a sensitive and rapid DNA-immuno-dot-blot assay to quantify the cellular DNA repair response to cisplatin treatment. Binding of an antibody, which has a higher affinity for single-stranded (ss) DNA than for double-stranded DNA, correlates with the amount of ssDNA intermediates, which are generated transiently during processing of the damaged DNA for repair. The total amount of DNA in a given dot was quantified by methylene blue staining of the membrane. Both human and chimpanzee cells showed a biphasic response to the induced DNA damage. The first portion of DNA strand breaks was repaired during the “fast” phase of DNA repair with a peak of ssDNA intermediates at 1 h after DNA damage. This fast phase of DNA repair was identical in humans and chimpanzee. The second portion of DNA strand breaks was repaired during the “slow” phase of DNA repair. Human cells responding with the slow system showed a second peak of ssDNA intermediates at 6 h after cisplatin treatment, whereas chimpanzee cells display this second peak significantly earlier at 5 h. In addition, we have used a customized cDNA chip with several hundred DNA-repair-related genes to study DNA repair gene expression in the same cisplatin-treated cells. So far we have identified few genes which are expressed at different levels during the first hours after DNA damage in chimpanzee cells and may explain the different repair kinetics in humans and chimpanzees.

W1 03

Interaction of Werner and Bloom syndrome genes with p53 in familial breast cancer

Burwinkel B.(1), Wirtenberger M.(1), Bernd F.(1), Klaes R.(2), Rita K S.(3), Wappenschmidt B.(4), Meindl A.(5), Arnold N.(6), Weber B.H.(7), Niederacher D.(8), Bartram C.R.(2), Hemminki K.(1)

1) DKFZ, Molecular Genetic Epidemiology, Heidelberg, Germany
2) University Heidelberg, Institute of Human Genetics, Heidelberg, Germany
3) University Cologne, Division of Molecular Gynaeco-Oncology, Cologne, Germany
4) University Cologne, Cologne, Germany
5) University Munich, Munich, Germany
6) University Kiel, Kiel, Germany
7) University Regensburg, Regensburg, Germany
8) University Düsseldorf, Düsseldorf, Germany

Mutations in genes of the three human RecQ helicases WRN, BLM and RECQ4 lead to rare autosomal recessive diseases, the Werner, the Bloom and Rothmund-Thomson syndromes, associated with premature aging and cancer predisposition. We tested the hypothesis whether frequent polymorphic, non-conservative amino acid exchanges in WRN and BLM act as low-penetrance breast cancer risk factors. RECQ4 was not investigated, since a SNP search in this gene did not reveal any amino acid exchanging polymorphisms. Additionally, we examined whether p53PIN3, a recently reported 16 bp insertion/duplication resulting in reduced TP53 expression, is associated with an increased familial breast cancer risk. Genotyping analyses, performed on 816 BRCA1/2 mutation-negative German familial breast cancer patients and 1012 German controls, revealed a significant association of WRN Cys1367Arg with familial breast cancer (OR = 1.27, 95% CI 1.06-1.53) and an increased familial breast cancer risk of TP53 MspI 1798G>A which is completely linked with p53PIN3 (OR = 2.15, 95% CI = 1.12-4.11). WRN Cys1367Arg, located in the C-terminus, the binding site of TP53, is predicted to be probably damaging. The joint effect of the WRN and p53 variants resulted in a strong dose-dependent risk enhancement (Ptrend = 0.0007). Our results suggest that WRN Cys1367Arg might interfere with the binding of TP53 to WRN and therefore lead to an attenuated apoptotic function of TP53, which has been previously reported for Werner syndrome cells. Consequently, carriers of additionally reduced TP53 mRNA levels due to p53PIN3 would be at an even higher risk for developing breast cancer. In conclusion, this study indicates the importance of inherited variations in the WRN and TP53 genes for familial breast cancer susceptibility.

W1 04

How many patients do we have to test in BC/OC families?

Fischer C.(1), Sutter C.(1), Klaes R.(1), Voigtländer T.(1)

1) Universität Heidelberg, Institut für Humangenetik, Heidelberg, Germany

Genetic testing for the breast and ovarian cancer susceptibility genes BRCA1 and BRCA2 is widely used for women with family history of the disease. Mutation carriers can be offered intensified screening, surgical and chemoprevention.

The disease risk of daughters of mutation carriers displaying no mutation is comparable to that the of the general population.
If no mutation is found in the proband tested initially the cancer could be sporadic or due to other genes but the other affected relatives could still carry BRCA1/2 mutations. Therefore, at the Genetische Polikinik in Heidelberg an additional patient is tested if the proband is tested negative and the family belongs to the high risk group.
In order to answer the question whether and for which family constellations more than one patient should be tested we evaluated the Heidelberg sample of the German Breast Cancer Consortium. It consists of 517 families in which at least one patient was completely screened for BRCA1/2 mutations. In 112 families at least two persons were tested with the following result: in 8 families two patients were negative, in 7 families we observed a mutation in one patient and no mutation in the other, in two families we found a negative result and an unclassified variant (UV) and in one family we observed a mutation and an UV in another. It is generally accepted that in BRCA1/2 positive families all patient should be tested. In the absence of a germline BRCA1/2 mutation we recommend further testing of affected family members if they still have a high heterozygote risk even without the proband. We could have missed the mutation in at least seven families randomly selecting the wrong proband.

W1 05

A new microarray-based diagnostic tool for resequencing the Breast Cancer genes (BRCA1&2)

Bonin M.(1), Altug-Teber O.(1), Hering R.(1), Riess O.(1)
1) Institut für Humangenetik, Medizinische Genetik, Tuebingen, Germany

It has been 10 years since the BRCA1 gene was first identified. During this decade, genetic testing for breast cancer susceptibility has been incorporated into the practice of oncology. In this process, the identification of families at the highest hereditary risk for cancer has served as a model to test strategies for prevention or early detection of breast malignancies. Here we report the first microarray-based resequencing analysis of the complete BRCA1 and BRCA2 genes. For this purpose, we used the oligonucleotide-microarray technology and designed a CustomSeq-Array for the coding region of both genes. 20 unrelated patients and control persons were analysed. All exons of each sample were amplified by PCR using specific primers, pooled, labelled, fragmented, and hybridised to the BreastCancer-CustomSeq array. In addition all samples were confirmed by conventional sequencing procedure. All analysed BRCA mutations could be detected by the new diagnostic system. The BreastCancer-Array provides base calls at more than 99.5 accuracy which is comparable to capillary sequencing. Replicate experiments demonstrated a reproducibility of more than 99.95%. We conclude that array-based sequencing technology has the capability to efficiently and cost-effectively generate large-scale resequencing data of genes. The technology is in particular applicable to large genes with numerous different mutations, like BRCA, but is also utilized for highly heterogenous diseases. Furthermore CustomSeq arrays deliver a complete sequence within 48 hours which opens a revolutionary new era of sequence-based diagnostics.

W1 06

German HNPCC Consortium - molecular and clinical data of the largest HNPCC cohort worldwide

Holinski-Feder E.(1), Engel C.(2), and the German HNPCC-Consortium (München, Bonn, Dresden, Heidelberg, Düsseldorf and Bochum)
1) Medizinisch Genetisches Zentrum, München, Germany
2) Institut f. Med. Informatik, Statistik und Epidemiologie, Leipzig, Germany

HNPCC is clinically defined by familial clustering of colorectal cancer and other associated tumors. Part of HNPCC is the Lynch-Syndrome, characterised by mutations in DNA MMR genes, mainly MLH1, MSH2 and MSH6. The study cohort of the German HNPCC-Consortium (funded by the German Cancer Aid) encompasses 2697 unrelated patients for the evaluation of many questions regarding HNPCC. 69% of the Amsterdam families and 33% of the Bethesda families have MSI-H tumors. Of the patients with MSI-H tumors, 44% revealed pathogenic mutations in the MMR genes including 219 different mutations, 15% had unclassified variants, 25% of the patients had either large genomic deletions or one of the two frequent mutations in MSH2,c.942+3A>T or MLH1,c.1489_1490insC. Of the mutation carriers, 50% fulfilled the Amsterdam criteria, the remaining fulfilled either the Bethesda- or the Amsterdam criteria without age restriction. As esp. the Bethesda criteria apply to very many patients, a logistic regression model was worked out to allocate patients to alternative pathways with MSI- or IH-analysis of the tumor in the first line. Genotype-phenotype correlation revealed the highest tumor risk and the lowest age of onset for MLH1 mutation carriers and the lowest tumor risk and highest age of onset for MSH6 mutation carriers. Tumors not involving the gastrointestinal tract are most frequently found in MSH6 mutation carriers. Disease modifying SNPs for HNPCC mutation carriers were analysed for CyclinD, RNASEL, p53 and MTHFR. Significant clinical differences were found between Amsterdam positive families with and without microsatellite instability, which is a lower age of onset and more syn- and metachronous tumors compared to patients with MSS tumors, which have a distal tumor localisation and a higher incidence of adenomas. These data show, that HNPCC includes at least two entities with clinical and molecular differences. This will have implications for surveillance and for cancer research.

W2 Clinical Genetics

W2 01

Detection of heterozygous SALL1 deletions by quantitative real time PCR proves the contribution of a SALL1 dosage effect in the pathogenesis of Townes-Brocks syndrome

Kohlhase J.(1), Steinmann K.(1), Albrecht B.(2), Bottani A.(3), Devriendt K.(4), Leipoldt M.(1), Borozdin W.(1)
1) Institut für Humangenetik und Anthropologie, Universität Freiburg, Freiburg, Germany
2) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany
3) Division of Medical Genetics, Geneva University Hospitals, Geneva, Switzerland
4) Institute for Human Genetics, University of Leuven, Leuven, Belgium

Townes-Brocks syndrome (TBS) is an autosomal dominantly inherited disorder characterized by ear, anal, limb and renal malformations, and results from mutations in the gene SALL1. All SALL1 mutations previously found in TBS patients create preterminal termination codons. In accordance with the findings of pericentric inversions or balanced translocations, TBS was initially assumed to be caused by SALL1 haploinsufficiency. This assumption was strongly contradicted by a Sall1 mouse-knock out, because neither hetero- nor homozygous knockout mutants displayed a TBS-like phenotype. A different mouse mutant mimicking the human SALL1 mutations, however, showed a TBS-like phenotype in the heterozygous situation, suggesting a dominant-negative action of the mutations causing TBS. We applied quantitative real time PCR to detect and map SALL1 deletions in 240 patients with the clinical diagnosis of TBS, who were negative for SALL1 mutations. Deletions were found in three families. In the first family, a 75 kb deletion including all SALL1 exons had been inherited by two siblings from their father. A second, sporadic patient carried a de novo 1.9 – 2.6 Mb deletion including the whole SALL1 gene, and yet another sporadic case was found to carry an intragenic deletion of 3384 bp. In all affected persons, the TBS phenotype is rather mild as compared to the phenotype resulting from point mutations. These results confirm that SALL1 haploinsufficiency is sufficient to cause a mild TBS phenotype but suggest that it is not sufficient to cause the severe, classical form. It therefore seems that there is a different contribution of SALL1 gene function to mouse and human embryonic development.

W2 02

Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Rapid testing by DNA methylation assay

Mitter D.(1), Buiting K.(1), Eggeling F.(2), Kuechler A.(2), Liehr T.(2), Mau-Holzmann U.A.(3), Prott E.-C.(1), Wieczorek D.(1), Gillessen-Kaesbach G.(1)
1) Institut für Humangenetik, Essen, Germany
2) Institut für Humangenetik und Anthropologie, Jena, Germany
3) Institut für Anthropologie und Humangenetik, Tübingen, Germany

Uniparental disomy (upd) describes the inheritance of both homologous of a pair of chromosomes from one parent. Maternal uniparental disomy for chromosome 14 [upd(14)mat] is associated with a characteristic phenotype including pre- and postnatal growth retardation, hypotonia, early feeding difficulties, motor delay, precocious puberty and truncal obesity. Patients with upd(14)mat show features overlapping with Prader-Willi syndrome (PWS) and are probably under-diagnosed. Upd(14)mat is often described in carriers of Robertsonian translocations involving chromosome 14, but also found in patients with a normal karyotype. We analysed 33 patients with low birth weight, feeding difficulties and consecutive obesity in early childhood in whom PWS was excluded by methylation analysis at the SNRPN locus. Upd(14)mat was detected in 4 of 33 patients (12%). One of these patients in addition shows gonosomal mosaicism. In addition, we present seven patients diagnosed with upd(14)mat including two patients with a normal karyotype, one patient with a de novo Robertsonian translocation (14;21), one patient with a familial Robertsonian translocation (13;14), two patients with a marker chromosome 14 and one patient with gonosomal mosaicism. For rapid testing of upd(14)mat we used a PCR based methylation assay at the MEG3 promoter and confirmed our results by microsatellite testing. We therefore recommend screening for upd(14)mat in patients with low birth weight, growth retardation, hypotonia with neonatal feeding difficulties, motor delay, small hands and feet, precocious puberty and truncal obesity as well as in patients with a PWS like phenotype presenting with low birth weight and obesity.

W2 03

Cerebellar hypoplasia and quadrupedal locomotion in humans as a recessive trait mapping to chromosome 17p

Türkmen S.(1), Demirhan O.(2), Hoffmann K.(1), Diers A.(3), Zimmer C.(4), Sperling K.(5), Mundlos S.(1)
1) Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, Berlin, Germany
2) Faculty of Medicine, Cukurova University, Department of Medical Biology and Genetics, Adana, Turkey
3) Charité, Abteilung für Neuropädiatrie, Berlin, Germany
4) TU München, Abteilung für Neuroradiologie, München, Germany
5) Institut für Humangenetik, Charité,Universitätsmedizin Berlin, Berlin, Germany

Congenital hereditary non-progressive hypoplasia of the cerebellum is a rare condition, frequently associated with other neuropathology such as lissencephaly. Clincally, the condition is associated with variable degrees of mental retardation, microcephaly, seizures, and movement disorders due to ataxia. In severe cases, patients are unable to ambulate independently, but nevertheless use bipedal locomotion. Here we present a family with 7 affected of which 5 never learned to walk on two legs and have fully adapted to quadrupedal palmigrade locomotion. They show signs of cerebellar ataxia and are mentally retarded. MRI analysis demonstrated hypoplasia of the cerebellum and the cerebellar vermis as well as a small nucleus dentatus, and a thin corpus callosum but no other malformations. We show, by a genome-wide linkage scan, that QL is a recessive trait linked to chromosome 17p. Our findings have implications for understanding the neural mechanism mediating bipedalism, and, perhaps, the evolution of this unique hominid trait.

W2 04

The phenoytpe of autosomal dominant proximal spinal muscular atrophy can seldom be caused by mutations of the LMNA gene

Rudnik-Schöneborn S.(1), Botzenhart E.(1), Eggermann T.(1), Senderek J.(1), Wirth B.(2), Schoser B.(3), Zerres K.(1)
1) Institut für Humangenetik, RWTH Aachen, Aachen, Germany
2) Institut für Humangenetik, Universität Köln, Köln, Germany
3) Friedrich-Baur-Institut, LMU München, München, Germany

Autosomal dominant proximal spinal muscular atrophy (AD-SMA) is a rare disease with largely unknown molecular defects. The clinical spectrum of neuromuscular diseases caused by LMNA gene mutations ranges from Emery-Dreifuss muscular dystrophy, limb girdle muscular dystrophy, neurogenic muscle atrophy to mere cardiomyopathy. This lead us to hypothesize that the LMNA gene is a good candidate for families with AD-SMA. We screened 18 families (one index patient each) for mutations of the LMNA gene. Ages of onset ranged from 2-40 years, and affected subjects showed marked intrafamilial variability. None had cardiac problems at the time of diagnosis, and SMA 5q had been excluded.
Only one family (~5%) showed a non-sense mutation (Q493X) of the LMNA gene causing a truncation of the protein product. This German woman had first walking difficulties from 39 years and was diagnosed proximal SMA at the age of 45 years. CK activity was normal, EMG was unspecific but muscle biopsy gave evidence of neurogenic atrophy with scattered atrophic fibers in small groups, target fibers and some moth-eaten fibers. Follow-up examination at the age of 53 revealed a slow progression without peroneal weakness or contractures. At that time, a coronary heart disease and cardiac conduction defects were diagnosed, and the patient was provided with a pace maker. From the patient's family history it was known that her mother has had walking difficulties from age 40 and died at the age of 54 years from a heart attack. Medical reports were not available. Other relatives with a muscular problem were denied, but several maternal relatives died of heart attacks of unknown reasons between 40 and 65 years of age.
We conclude that LMNA gene mutations can rarely mimic AD-SMA in particular in cases with long intervals between muscle weakness and cardiomyopathy. Identification of LMNA mutation carriers is important for medical care and genetic counselling given the high risk for cardiac complications.

W2 05

Functional proof and further clinical evidence of the pathogenic relevance of TBX1 missense mutations

Zweier C.(1), Sticht H.(2), Campbell C.(3), Rauch A.(1)
1) Institute of Human Genetics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
2) Department of Bioinformatics of the Institute of Biochemistry, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany
3) Department of Biochemistry, University at Buffalo, Buffalo, NY, United States of America

Deletion 22q11.2 syndrome is the most frequent known microdeletion syndrome. It is associated with a highly variable phenotype including DiGeorge- and Shprintzen syndromes. From studies in several mouse models, haploinsufficiency of the T-box transcription factor TBX1, which is located within the common deletion interval, was suggested to cause the phenotype.
Nevertheless, to date only 3 patients from Japan were described to have point mutations of TBX1 in association with five of the major features of 22q11.2 deletion. We report the first Caucasian patient with a TBX1 missense mutation within the T-box, associated with the typical facial gestalt, short stature and developmental delay. While all features are known to be associated with the 22q11.2 deletion syndrome, short stature and developmental delay were not previously reported in individuals with TBX1 mutations. The mutation in our patient was inherited from his father, who also showed short stature and facial anomalies. To prove the functional relevance of this mutation we tested our novel and the three published mutations in a transcriptional reporter assay. While the published truncating mutation showed remarkable reduction of TBX1 transcriptional activity, the published and our novel missense mutation showed significantly increased activity. This finding is supported by homology modelling of the mutant protein, which shows new electrostatic interactions. Although unexpected, our results with similar phenotypes caused by reduced and increased activity of TBX1 are in accordance with data from mouse models, showing the same phenotypic spectrum in both, TBX1 under- and overexpression. We therefore provide the first functional evidence for the pathogenic relevance of TBX1 missense mutations.

W2 06

A new, X-linked endothelial corneal dystrophy

Schmid E.(1), Lisch W.(2), Philipp W.(1), Lechner S.(3), Göttinger W.(1), Schötzer-Schrehardt U.(4), Müller T.(5), Utermann G.(3), Janecke A.(3)
1) Department of Ophthalmology, Innsbruck Medical University, Innsbruck, Austria

2) Department of Ophthalmology, City Hospital of Hanau, Hanau, Germany
3) Department of Medical Genetics, Molecular and Clinical Pharmacology,Innsbruck Medical University, Innsbruck, Austria
4) Department of Ophthalmology, University of Erlangen-Nürnberg, Erlangen, Germany
5) Department of Pediatrics, Innsbruck Medical University, Innsbruck, Austria
We describe the clinical spectrum, the histopathologic findings obtained from one corneal button, and the genetic mapping of an X-linked endothelial corneal dystrophy (XECD). We examined a total of 60 members of a family with this dystrophy at the slitlamp. Light and electron microscopic findings of the corneal button were recorded following one male patient's penetrating keratoplasty. A panel of 25 microsatellite markers covering the X chromosome was typed in genomic DNA from 50 family members. The data were analyzed using the ALLEGRO program to obtain two-point and multipoint likelihood of the odds (LOD) scores and to generate haplotypes. A total of 35 trait carriers were identified in 4 generations of the family. Nine male patients demonstrated severe corneal opacifications: 2 congenital corneal cloudings in form of ground glass, milky appearance and 7 subepithelial band keratopathies combined with moon crater-like endothelial changes. 22 female and 4 male patients disclosed only moon crater-like endothelial alterations. No instance of male-to-male transmission of the disease was encountered in the family. Light and electron microscopy disclosed focal discontinuities and degeneration of the endothelial cell layer and marked thickening of Descemet's membrane. Multipoint analysis showed linkage with a maximum LOD score of 10.90 between markers DXS8057 and DXS1047. To the best of our knowledge, this represents the first fully documented report of X-linked inheritance of an endothelial corneal dystrophy. Late subepithelial band keratopathy is a landmark of XECD. A locus for this corneal dystrophy maps to Xq25.

W3 Complex Diseases and Genetic Epidemiology

W3 01

Copy number variation of NCF1 gene is associated with rheumatoid arthritis but not with psoriatic arthritis

Hüffmeier U.(1), Burkhardt H.(2), Thiel C.(1), Lascorz J.(1), Böhm B.(2), Holmdahl R.(3), Schulze-Koops H.(2), Reis A.(1)
1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany
2) Institute for Clinical Immunology and Rheumatology, Department of Internal Medicine III, University Erlangen-Nuremberg, Erlangen, Germany
3) Section for Medical Inflammation Research, Lund University, Lund, Sweden
Mutations in any of four genes encoding different subunits of the superoxide-generating phagozyte NADPH oxidase cause chronic granulomatous disease (CGD), a rare autosomal recessive disorder. In about 20 % of cases loss of one of the genes, NCF1, located in the Williams-Beuren-syndrome critical region on 7q11.23, occurs due to unequal crossing-over with one of the two neighbouring NCF1 pseudogenes. In a rat model of arthritis a missense polymorphism of Ncf1 was associated with arthritis severity and shown to reduce the oxidative burst of the NADPH complex. We hypothesized that lower copy number of NCF1 might be a susceptibility factor in the complex genetics of arthritic disease. To determine copy number variation we developed a quantitative PCR multiplex assay based on Taqman technology. Performance was robust in the majority of DNAs tested but as in previous quantitative methods DNA quality was critical. Three different study groups - 199 rheumatoid arthritis (RA) patients, 375 psoriatic arthritis patients (PSA), 282 healthy controls - were screened in quadruplicates. Interestingly, copy number varied between one and five gene copies. In all three cohorts we observed a higher frequency of heterozygous deletion carriers (1.1-8.7 %) than expected from previously reported estimations of heterozygous carriers of CGD (0.2 %or 1:500). Fisher's exact test revealed a highly significant difference between RA patients and controls: 8.8 % vs. 0.7 % ($p < 8.75 \times 10\text{-}4$), while frequency of NCF1 deletion carriers in PSA patients was similar to controls. Our observation of reduced NCF1 copy number as a susceptibility factor for rheumatoid arthritis is based on relatively small patient numbers and therefore needs independent confirmation. If confirmed our findings provide evidence of an involvement of NADPH oxidase in the pathogenesis of rheumatoid arthritis.

W3 02

Genes involved in vitamin D regulation are associated with asthma and allergy

Wjst M.(1), Altmüller J.(1), Faus-Kessler T.(2), Braig C.(1), Bahnweg M.(1), Andre E.(1)
1) GSF Forschungszentrum, Institut für Epidemiologie, München-Neuherberg, Germany
2) GSF Forschungszentrum, Institut für experimentelle Genetik, München-Neuherberg, Germany
The general introduction of rickets prophylaxis in newborns may play a role in allergic sensitization. In vitro studies show that the main vitamin D metabolite calcitriol suppresses dendritic cell maturation and consecutive development of Th1 cells. Due to the ubiquitous exposure in supplemented baby food -which makes randomized clinical trials currently impossible- we asked if an already diseased population may have inherited genetic variants in the vitamin D turnover or signalling pathway.
Segregation analysis showed 25-OH-D3 (calcidiol) serum levels as a polygenic trait in asthma families ($p<10^{7}$). Lod score peaks for calcidiol as a QTL were observed on chromosome 1, 2 , 5, 6 and 17 where only the peak on chromosome 2 (LOD 3.4) statistical significance. SNPs in several calcitriol regulated genes turned out to be associated with total IgE levels. A frequent 5-point haplotype of the calcitriol degrading enzyme CYP24 is associated with asthma (P=0.0035), total IgE (P=0.0063) and calcidiol serum levels (P=0.0062).
This may be a first entry point into effects of a polygenic inherited vitamin D sensitivity that may affect also other metabolic and immunological mediated diseases.

W3 03

Association of apolipoprotein E (APOE) polymorphisms with age-related macular degeneration in a large German case-control study

Fritsche L.G.(1), Milenkovic A.(1), Freitag S.(2), Keilhauer C.(3), Lichtner P.(4), Meitinger T.(4), Krawczak M.(2), Weber B.H.F.(1)
1) Institut für Humangenetik, Universität Regensburg, Regensburg, Germany
2) Institut für Medizinische Informatik und Statistik, Christian-Albrechts-Universität, Kiel, Germany
3) Universitäts-Augenklinik, Würzburg, Germany
4) Institut für Humangenetik, GSF-Forschungszentrum für Umwelt und Gesundheit, München-Neuherberg, Germany
Introduction: Age-related macular-degeneration (AMD) is the leading cause of blindness in developed countries. Apolipoprotein E (APOE) alleles, particularly e2 and e4 (e = epsilon), have consistently been suggested to be associated with AMD although the majority of studies have not found significant results probably due to weak genetic effects and small sample sizes. Our aim was to investigate the role of the e-alleles, additional single nucleotide polymorphisms (SNPs) at the APOE locus, and their corresponding haplotypes in a large and well-characterized AMD case-control study group.
Methods: We collected 794 unrelated AMD patients and 612 controls matched for age, gender and ethnicity. Two SNPs defining the e-alleles (eSNPs) and common SNPs with minor allele frequencies > 0.2 were selected from the APOE locus. SNP typing was done using the MALDI-TOF Sequenom Technology. Haplotype blocks were defined by confidence intervals according to Gabriel et al. (Science 296:2225-9, 2002). Haplotypes were estimated using FAMHAP. Single-locus and multi-marker haplotype association tests were conducted using the statistics software R.
Results: Four common SNPs at the APOE locus were identified. SNP analysis using common genetic models revealed, besides the known causative and protective eSNPs, a previous unknown protective, intronic SNP significantly associated with AMD (p = 0.00506, 0.00364, 0.00259, respectively). A haplotype block spanning four SNPs was detected which contained four common haplotypes (freq. > 5%). The APOEe3 allele segregates in two haplotypes while the e2 and e4 alleles were each found on a single separate haplotype. Three haplotypes which each exclusively carry one of the above-mentioned SNPs possess a statistically significant association with AMD (p = 0.00445, 0.00444, 0.0042, respectively).
Conclusions: The e2 and e4 alleles as well as a highly frequent, intronic SNP are significantly associated with AMD, the latter under a recessive disease model.

W3 04

Insights from the distribution of genome-wide SNP-QTLs for cardiac repolarization (QT-interval) and their interaction in men and women

Pfeufer A.(1), Arking D.E.(2), Jalilzadeh S.(1), Akyol M.(1), Perz S.(3), Post W.(2), Kao W.H.L.(2), Ikeda M.(2), Lin S.(2), Cutler D.J.(2), Illig T.(4), Wichmann H.-E.(4), Marban E.(2), Spooner P.M.(2), Chakravarti A.(2), Meitinger T.(1)

1) Institute of Human Genetics, TU Munich and GSF Research Center, Munich, Germany
2) Johns Hopkins Medical Institutes, Baltimore, MD, USA, Baltimore, United States of America
3) Institute for Medical Informatics, GSF Research Center, Munich, Germany
4) Institute for Epidemiology, GSF Research Center, Munich, Germany

Aim: To identify QTLs of cardiac repolarization - measured as QT-interval (QT) - we performed a genome-wide SNP association study using 100k arrays. QT is normally distributed, its heritability is ≥0.3 and - if unregular – predisposes to sudden death. Candidate gene studies suggested that some of its QTLs display strong interaction with gender. QTc_RAS is QT corrected for covariates heart rate, age and gender.

Method: From the population-based KORA S4 survey (n=3,966 after exclusion criteria, including n=2,007 women) 103/103 women were selected from QTc_RAS distribution extremes (<7.5th and >92.5th percentile, 385.7±7.7 ms and 444.8±3.6 ms) to avoid gender as confounder and were genotyped for 88.500 SNPs with CR>0.85 and MAF>0.025 (phase I). 60 most significant SNPs (p<10-4) were genotyped in additional 200/200 women (phase II). 7 SNPs significant at (p<5*10-3) in combined phase I and phase II data where screened in entire S4 (phase III). Analytical Strategy: Small effect sizes of QT-QTLs identified by us and others (<0.05 explained variance) demanded for optimized analytical strategies: we compared eight different test statistics to select loci for confirmation from phase I data: The case control allelic model, three case control genotypic models (dom, rec, log add), the truncated measures (ANOVA) allelic model and three truncated measures genotypic models. None of the models gave a clear advantage in predicting true and false positive QTLs in larger datasets.

Results: We identified seven QTLs of QTc_RAS, the largest one (CAPON Gene) explaining 0.013 of trait variance. QTLs between males and females differed significantly. Effects were generally weak making multiple locus analysis depended on large datasets. Unravelling the genuine architecture of human QTLs and their genetic and nongenetic interactions will enable us to devise more efficient strategies to confirm entire spectra of QTLs for any given trait to reasonable levels of statistical certainty.

W3 05

Genetic architecture of the *APM1* gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1727 healthy Caucasians

Heid I.M.(1), Wagner S.(2), Gohlke H.(1), Iglseder B.(3), Mueller J.C.(4), Cip P.(2), Ladurner G.(3), Reiter R.(2), Stadlmayr A.(2), Illig T.(1), Kronenberg F.(5), Paulweber B.(2)

1) GSF-Institut für Epidemiologie, Neuherberg, Germany
2) First Department of Internal Medicine, Paracelsus Private Medical University Salzburg, Salzburg, Austria
3) Department of Neurology, Paracelsus Private Medical University Salzburg, Salzburg, Austria
4) Clinic 'Rechts der Isar', Technical University Munich, Munich, Germany
5) Division of Genetic Epidemiology; Innsbruck Medical University, Innsbruck, Austria

Reports on variants of the adiponectin *(APM1)* gene showed numerous, but inconsistent associations with parameters of the metabolic syndrome, which is a cluster of risk factors related to cardiovascular disease and type 2 diabetes. We performed a systematic investigation based on fine-mapped SNPs highlighting the genetic architecture of *APM1* and its role in modulating adiponectin plasma concentrations in a particularly healthy population. Genotyping 53 SNPs (average spacing of 0.7 kb) in the *APM1* gene region in 81 Caucasians revealed a two-block LD structure and enabled comprehensive tagSNP selection. We found particularly strong association with circulating adiponectin for 11 out of the 18 tagSNPs genotyped subsequently in the 1727 well-phenotyped subjects up to a difference of 3 μg/ml adiponectin concentration (increased levels: 14811, p<0.000001; -11388 p<0.000001; 45, p=0.0006; 276, p=0.00007; 712, p<0.000001; Y111H, p=0.04; 3639, p=0.001; 4545, p=0.01; decreased levels: 10066, p<0.000001; 8564, p=0.00002; and -450, p=0.0003). Haplotype analysis provided a thorough differentiation of adiponectin levels with 9 out of 17 showing significant association. The variants in the *APM1* explained 8% of the adiponectin variance. No significant association was found for any of the parameters of the metabolic syndrome. We thus present the first study based on fine-mapped *APM1* SNPs showing strong associations of *APM1* variants with adiponectin plasma concentrations in healthy subjects. The revealed two-block structure may point towards two causal regions, one including the promoter SNPs and a second the relevant exons. Our data on healthy subjects further suggests that the modulation of adiponectin concentrations by *APM1* is not merely a concomitant effect in the course of disease but most apparent already in a healthy status.

W3 06

How does the number of affected family members influence the presence of risk alleles in complex diseases?

Seis D.(1), Boehringer S.(1)

1) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany

Study designs in complex diseases often involve nuclear families. These are usually ascertained by a certain scheme, e.g. requiring at least one affected offspring. Collecting all the remaining sibs in a family is often a reasonable way to increase sample size and to glean more information about the mode of inheritance. However, it is well known that certain statistics can be biased by such a strategy, for example the TDT, if these sibs are assumed to be independent, conditional on parents. We have therefore undertaken extensive simulations to explore the phenotype distribution of offspring. In our simulations, the number of contributing loci range of two to hundreds, thereby reflecting most plausible genetic models of complex disorders. Models of independent effects, interaction effects, and various modes of inheritance are all considered. We show that under a wide variety of heterogeneity models, small effects of many alleles yield phenotype distributions that can be well approximated by a binomial distribution, i.e. sibs can be assumed to be conditionally independent. On the other hand, stronger effects of a few alleles can cause deviations from the binomial. When this occurs, we can use the phenotype distribution to detect heterogeneity and to stratify the sample to increase power in many settings. Thus, the phenotype distribution of offspring in nuclear families harbours valuable information that can be used to optimize studies and to learn more about the phenotype itself.

W4 Neurogenetics

W4 01

Mutations in SIL1 cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy

Senderek J.(1), Krieger M.(1), Stendel C.(1), Breitbach-Faller N.(2), Harting I.(3), Bergmann C.(1), North K.(4), Muntoni F.(5), Renault F.(6), Topaloglu H.(7), Voit T.(8), Weis J.(9), Schröder J.M.(9), Ebinger F.(10), Zerres K.(1)

1) Department of Human Genetics, Aachen University of Technology, Aachen, Germany
2) Department of Pediatrics, Esslingen Community Hospital, Esslingen, Germany
3) Department of Neuroradiology, University of Heidelberg, Heidelberg, Germany
4) Institute for Neuromuscular Research, Children's Hospital at Westmead, Sydney, Australia
5) Dubowitz Neuromuscular Centre, Imperial College, London, United Kingdom
6) Unité de Neurophysiologie, Hôpital d'Enfants Armand-Trousseau, Paris, France
7) Department of Pediatric Neurology, Hacettepe University, Ankara, Turkey
8) Department of Pediatrics and Pediatric Neurology, University Hospital Essen, Essen, Germany
9) Department of Neuropathology, Aachen University of Technology, Aachen, Germany
10) Department of Pediatric Neurology, University Children's Hospital, Heidelberg, Germany

We, with others, have recently shown that mutations in the SIL1 gene cause Marinesco-Sjögren syndrome. SIL1 (also called BAP, BiP-associated protein) acts as a nucleotide exchange factor for the Hsp70 chaperone BiP (also called

GRP78), which is a key regulator of the main functions of the endoplasmic reticulum. Marinesco-Sjögren syndrome (MSS, OMIM 248800) is an autosomal recessive neurodegenerative disorder characterized by cerebellar ataxia, congenital or infantile-onset cataracts, myopathy, mental retardation, short stature, skeletal abnormalities and hypogonadism. We studied consanguineous MSS families to narrow the known candidate region on chromosome 5q31. We identified the gene encoding SIL1 in the region of apparent homozygosity and found 12 independent SIL1 mutations in individuals with MSS. Our data suggest that MSS is caused by loss of SIL1 function, as most of the disease-causing mutations are predicted to prematurely truncate the protein. Identification of SIL1 mutations implicates MSS as a disease of endoplasmic reticulum dysfunction and suggests a role for this organelle in multisystem disorders.

W4 02

Molekulare und klinische Charakterisierung der kongenitalen myasthenen Syndrome (CMS)

Abicht A.(1), Müller J.S.(1), Baumeister S.K.(1), Hoellen F.(1), Schara U.(2), Huebner A.(3), Lochmüller H.(1)
1) Friedrich-Baur-Institut, Neurologische Klinik und Poliklinik der Ludwig-Maximilians-Universität, München, Germany
2) Zentrum für Neuropädiatrie, Städtische Kliniken, Neuss, Germany
3) Abteilung für Kinderheilkunde, Technische Universität, Dresden, Germany

Kongenitale myasthene Syndrome (CMS) bilden pathogenetisch und klinisch eine heterogene Gruppe von Erkrankungen, bei denen eine hereditäre Störung der neuromuskulären Erregungsübertragung vorliegt. Ursächlich sind unterschiedliche genetische Defekte im Bereich der neuromuskulären Endplatte. Ein Patientenkollektiv von über 300 CMS Patienten aus Deutschland und anderen europäischen Ländern wurde klinisch charakterisiert und molekulargenetisch analysiert. Die Untersuchung von sieben synaptisch exprimierten Genen (Gene der AChR-Untereinheiten, RAPSN, COLQ und CHAT) führte bei einem Großteil der Patienten (>60%) zur Identifizierung des zugrunde liegenden molekularen Defekts. Ein Großteil der Mutationen betrifft das Gen der AChR epsilon-Untereinheit (CHRNE). Dort ließ sich in über 50% der südosteuropäischen Patienten die Mutation 1267delG nachweisen, was sich auf einen Founder-Effekt der Roma zurückführen ließ und deren Migration aus dem indischem Raum vor ca. 800 genetisch Jahren belegen konnte. Die Mutation N88K des RAPSN Gens kodierend für das postsynaptische Protein Rapsyn wurde als häufigste CMS-Ursache bei mitteleuropäischen Patienten (ca. 20%) ausgemacht und auf einen alten indoeuropäischen Founder zurückgeführt. Klinisch auffällig bei RAPSN N88K Patienten waren häufige krisenhafte Verschlechterungen des Krankheitsbilds, die mit einer respiratorischen Insuffizienz einhergehen und darüber hinaus - bei einigen Patienten - ein spät manifestierender Phänotyp mit einer Erstsymptomatik im Erwachsenenalter. Bei weiteren CMS Patienten wurden Mutationen im synaptisch exprimierten COLQ-Gen sowie im Gen kodierend für die präsynaptische Cholinacetyltransferase (CHAT) identifiziert und charakterisiert. Die Analyse von Geno- und Phänotypen der Patienten erbrachte wichtige Informationen zu klinischen Besonderheiten, Therapiemöglichkeiten und Prognose der einzelnen CMS-Unterformen. Erstmals konnten Daten zu Vorkommen und Häufigkeit einzelner CMS Mutationen gewonnen werden.

W4 03

Evidence of in vivo increase of SMN RNA and protein in SMA carriers and patients treated with valproic acid

Brichta L.(1), Hölker I.(1), Haug K.(2), Klockgether T.(3), Wirth B.(1)
1) Institute of Human Genetics, Institute of Genetics and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany
2) Institute of Human Genetics, University Bonn, Bonn, Germany
3) Department of Neurology, University of Bonn, Bonn, Germany

Proximal spinal muscular atrophy (SMA), an autosomal recessively inherited motoneuron disorder, leads to death in childhood in about half of all patients. While the disease is caused by homozygous absence of the survival motor neuron gene 1 (SMN1), each patient retains 1-4 SMN2 copies. They are almost identical to SMN1, but exon 7 is skipped in 90% of SMN2 transcripts. The encoded protein is not fully functional. Using fibroblasts from SMA patients, we have previously demonstrated that therapeutic doses of valproic acid (VPA), an antiepileptic drug, increase full-length (FL) SMN2 mRNA/protein levels in vitro by enhancing SMN2 transcription and promoting exon 7 inclusion. These findings opened an exciting perspective for a causal SMA therapy.
Here, we provide a first proof of principle of an in-vivo activation of a causative gene by VPA, a histone deacetylase (HDAC) inhibitor, in a human inherited disease. Ten SMA carriers with 1 SMN1 and 1-3 SMN2 copies were enrolled in a VPA pilot trial. Drug treatment revealed increased FL-SMN mRNA/protein levels in blood from 7/10 probands. In a subsequent investigation of peripheral whole blood from 20 SMA type I-III patients treated with VPA in individual experimental curative approaches, FL-SMN2 mRNA levels were found to be increased in 7 patients, whereas 13 presented unchanged or decreased transcript levels. Difficulties in developing effective clinical biomarkers are pointed out and a strategy is suggested how to monitor drug response in treated patients. This will be an essential tool required for discriminating between responders and non-responders to VPA treatment.

W4 04

Clinical and molecular characterization of 5 probands with periventricular nodular heterotopia resulting from an FLNA mutation

Hehr U.(1), Uyanik G.(2), Hehr A.(1), Spranger S.(3), Oehl-Jaschkowitz B.(4), Janssens S.(5), Reardon W.(6), Winkler J.(2)
1) Zentrum für Humangenetik, Regensburg, Germany
2) Universität Regensburg, Klinik und Poliklinik für Neurologie, Regensburg, Germany
3) Praxis für Humangenetik, Bremen, Germany
4) Praxis für Humangenetik, Homburg/Saar, Germany
5) Universitair Ziekenhuis, Centrum voor Medische Genetica, Gent, Belgium
6) Our Lady's Hospital for Sick Children, Dublin, Ireland

Periventricular nodular heterotopia (PNH) is a genetically heterogeneous brain malformation, resulting from defective initiation of neuronal migration with accumulation of neurons along the walls of both lateral ventricles, often accompanied by epileptic seizures. Most sporadic as well as familial female patients carry a heterozygous FLNA mutation (Xq28) predicted to result in loss of function due to protein truncation. In addition, distinct FLNA missense mutations or in frame deletions disrupt the development of craniofacial and long bones as well as additional organ systems, resulting in Otopalatodigital syndrome and overlapping phenotypes.
Here we report the clinical and molecular data of 5 independent patients (4 females, 1 male) with PNH resulting from a previously unreported FLNA mutation. 3 females with classical bilateral PNH were demonstrated to carry a heterozygous truncating FLNA mutation. In addition, we observed a heterozygous intragenic deletion in a 39 year old female patient with PNH including at least the FLNA coding region after exon 47. A hemizygous FLNA splice mutation was identified in exon 13 in a boy presenting with mild PNH, but dysmorphic features reminiscent of Cerebro-fronto-facial syndrome and severe constipation. Interestingly, an X-linked form of intestinal pseudo-obstruction has already been described with tentative evidence of linkage to Xq28, the location of FLNA. Utilization of a newly created donor site was demonstrated to result in lack of the 3´part of exon 13 with frameshift and premature protein truncation. We postulate that due to the demonstrated alternative generation of both normal and aberrant FLNA mRNA this patient retains sufficient FLNA function to circumvent the lethal outcome usually associated with loss of function mutations in males. Our findings further expand the phenotypic spectrum associated with FLNA mutations and our understanding of the FLNA function during normal and abnormal embryonal development.

W4 05

Disruption of AE3 confers seizure susceptibility in mice

Hentschke M.(1), Kurth I.(1), Wiemann M.(2), Hentschke S.(1), Seidenbecher T.(3), Hermans-Borgmeyer I.(4), Jentsch T.J.(4), Gal A.(1), Hübner C.A.(1)
1) Institut für Humangenetik, Universitätskrankenhaus Hamburg-Eppendorf, Hamburg, Germany
2) Institut für Physiologie, Universität Essen, Essen, Germany
3) Institut für Physiologie, Universität Münster, Münster, Germany
4) ZMNH, Universität Hamburg, Hamburg, Germany

Anion-exchangers (AE) exchange intracellular bicarbonate for extracellular chloride and thus usually lower intracellular pHi. AE3, encoded by Slc4a3, is predominantly expressed in the heart and brain. Though the function of AE3 in vivo is largely unknown, the variant Ala867Asp has been associated with common subtypes of idiopathic generalized epilepsy. To explore, whether AE3 itself may be involved in the pathogenesis

of seizures, we generated an AE3-knockout mouse model by targeted disruption of Slc4a3. AE3-knockout mice were apparently healthy and neither showed gross histological nor behavioral abnormalities. However, the seizure threshold of AE3-knockout mice exposed to bicuculline or pentylenetetrazole was dramatically reduced and seizure-induced mortality significantly increased compared to wild-type littermates. Nevertheless, no spontaneous seizures or spike wave complexes were found in electrocorticograms of wake adult AE3-knockout mice. In hippocampal CA3 neurons, disruption of AE3 reduced the ability to regulate alkalotic shifts of the intracellular pH. Moreover, the intraneuronal alkalosis upon withdrawal of extracellular chloride was abolished, indicating that AE3-knockout mouse pyramidal neurons were devoid of sodium-independent chloride-bicarbonate-exchange. Neuronal hyperexcitability was also confirmed by electrophysiological analysis of hippocampal slices of AE3-knockout mice. These findings strongly support the hypothesis that AE3 itself modulates seizure susceptibility in humans and are of great relevance for understanding the role of intracellular pH in epilepsy.

W4 06

Functional analysis of LRRK2 — a protein kinase associated with autosomal dominant late-onset Parkinson disease

Gloeckner C.J.(1), Kinkl N.(1), Schumacher A.(1), Braun R.J.(1), O'Neill E.(2), Meitinger T.(1), Kolch W.(2), Prokisch H.(1), Ueffing M.(1)
1) GSF-Research Center, Institute of Human Genetics, Munich-Neuherberg, Germany
2) The Beatson Institute for Cancer Research, Glasgow, United Kingdom

Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) have been recently identified in families with autosomal dominant late-onset Parkinson disease (PD). The LRRK2 protein consists of multiple domains and belongs to the Roco family, a novel group of the Ras/GTPase superfamily. Besides the GTPase (Roc) domain it contains a predicted kinase domain, with homology to MAP kinase kinase kinases (MAP-KKK). Using cell fractionation and immunofluorescence microscopy we show that LRRK2 is localised in the cytoplasm and is associated with cellular membrane structures. The purified LRRK2 protein demonstrates autokinase activity. We found that the disease associated I2020T mutant causes a significant increase in autophoshorylation of about 40% in comparison to wild-type protein *in vitro*. This suggests that the autosomal dominant trait of PD associated LRRK2 mutations is caused by an increase in kinase activity and/or an altered substrate, rather than by a loss of function. Whether all LRRK2 mutations have the same effect on the kinase activity is now under investigation.

Literature: A. Zimprich, S. Biskup, P. Leitner, P. Lichtner, M. Farrer, S. Lincoln, J. Kachergus, M. Hulihan, R.J. Uitti, D.B. Calne et al. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*, **44**, 601-607.
C.J. Gloeckner, N. Kinkl, A. Schumacher, R.J. Braun, E. O'Neill, T. Meitinger, W. Kolch, H. Prokisch and M. Ueffing (2005). The Parkinson disease causing LRRK2 mutation I2020T is associated with increased kinase activity. *Hum. Mol. Genet.*, **in press**.

W5 Developmental Genetics and Imprinting

W5 01

Differentiation of stem cells to male germ cells

Nolte J.(1), Drusenheimer N.(1), Rathsack K.(1), Lee J.H.(1), Michelmann H.W.(2), Wulf G.(3), Elliott D.J.(4), Zechner U.(5), Haaf T.(5), Meinhardt A.(6), Engel W.(1), Nayernia K.(1)
1) Institut für Humangenetik, Göttingen, Germany
2) Universitätsklinikum, Gynäkologie, Göttingen, Germany
3) Universitätsklinikum, Hämatologie und Onkologie, Göttingen, Germany
4) Institute of Human Genetics, Newcastle upon Tyne, United Kingdom
5) Institut für Humangenetik, Mainz, Germany
6) Universität Giessen, Anatomie und Zellbiologie, Giessen, Germany

Germline stem cells are immature cells in the reproductive system that can proliferate and mature into sperm and eggs. Fascinating breakthroughs have been achieved in recent years in regard to our knowledge and to novel applications on spermatogonial stem cells, the adult germline stem cells in testis. These cells provide the basis for spermatogenesis throughout adult life by undergoing self-renewal and by providing progeny that differentiate into sperm. We developed different strategies for establishment of male germ cells from embryonic and adult stem cells. In a first approach we report about an in vitro generation of a germ cell line (SSC1) from the pluripotent teratocarcinoma cells by a novel promoter-based sequential selection strategy and show that the SSC1 cell line form mature seminiferous tubule structures, and support spermatogenesis after transplantation into recipient testes. In a second approach we derivated spermatogonial stem cells from embryonic stem cells and showed that the established cell lines are able to undergo meiosis in vitro. In a third approach the derivation of male germ cells from bone marrow stem cells will be reported.The merits of these strategies will be presented, along with remaining hurdles that might imped transfer of stem cell technology to the clinic as a treatment for male infertility.

W5 02

Functional consequences of NSDHL mutations explain the ichthyosiform skin phenotype in CHILD syndrome

Riemann J.(1), Leveleki L.(1), König A.(2), Debus M.(1), Bornholdt D.(1), Rölle P.(1), Happle R.(2), Grzeschik K.-H.(1)
1) Institut für allg. Humangenetik, Philipps-Universität Marburg, Marburg, Germany
2) Klinik für Dermatologie und Allergologie, Philipps-Universität Marburg, Marburg, Germany

CHILD syndrome (MIM 308050), is an X-linked dominant, male-lethal trait characterized by an strictly lateralized ichthyosiform nevus as well as ipsilateral hypoplasia of the body. The trait is caused by mutations in NSDHL encoding a 3ß-hydroxy-steroid dehydrogenase functioning in the cholesterol biosynthetic pathway.
The CHILD phenotype appears to be caused by loss of function because it can be associated with nonsense- and missense mutations as well as with deletions eliminating several exons or the complete gene. However, missense mutations observed in patients exchange amino acids of NSDHL located outside the predicted functional domains (co-factor binding site, catalytically active site, transmembrane helix). Since the affected amino acids are highly conserved the protein should have a functional role other than the catalytic one. To identify functional consequences of these missense mutations, particularly for the ichthyotic skin phenotype, we generated human NSDHL transgenes reflecting changes observed in patients and, for comparison, missense-mutations induced at other sites. In transfected cells, wild type protein is localized at the surface of lipid droplets (LDs) and on the ER, CHILD-specific mutant NSDHL is misplaced and the LDs are clumped. In the erg26ts yeast strain, which is mutated in the orthologous gene, wild type human NSDHL can complement the metabolic defect. Mutant NSDHL has lost this capability and is even detrimental to yeast cell proliferation, indicating a dominant negative effect.
The findings suggest, that both a block in cholesterol biosynthesis and disturbed intracellular traffic might cause the phenotype. Both could affect transmission of cellular signals, in particular sonic hedgehog (SHH) signals which are required during skin development. To corroborate this notion we demonstrate by histochemical studies similar expression changes in epidermal proteins of NSDHL deficient mice as known from SHH-ko mice.

W5 03

Replication timing and spatio-temporal organization of imprinted and non-imprinted chromosome regions during mouse preimplantation development

May A.(1), Zechner U.(1), Großmann B.(1), Haaf T.(1)
1) Universitätsklinikum Mainz, Institut für Humangenetik, Mainz, Germany

Asynchronous (allele-specific) replication, a cytogenetic hallmark of imprinted chromosome regions, is maintained throughout development. However, considering that in the mouse and many other species the male and female pronuclei do not replicate synchronously, the observed replication asynchrony of imprinted genes in early mouse embryos is not surprising. To reveal at which time during preimplantation development the non-imprinted regions switch from asynchronous to synchronous replication, we performed a comparative replication timing analysis of two imprinted genes (Snrpn, Peg3) and two non-imprinted control regions in 1-cell, 2-cell, 4-cell, 8-cell, and morula stage embryos, using two color interphase FISH with region-specific BAC probes. Non-imprinted regions exhibited asynchronous replication patterns in 1-cell and 2-cell embryos. However, a transition from asynchronous to synchronous replication occurred between the 2-cell and morula stages. Imprinted genes maintained their asynchronous replication patterns throughout preimplantation development, as expected. Parent-specific methylation marks are protected from genome-wide demethylation waves after fertilization. To find out whether a specific higher-order nuclear organization is important for protection, we also

studied the arrangement and the nuclear localization of imprinted gene clusters and non-imprinted controls in early mouse embryos. Preliminary evidence suggests a higher percentage of colocalizations of imprinted genes from two different clusters on mouse chromosome 7, compared to non-imprinted control regions with comparable physical distance in 1-cell and 4-cell embryos.

W5 04

The AS-SRO element of the 15q imprinting centre contains an enhancer that can be blocked by the transcription factor TIEG1

Zogel C.(1), Gärtner-Rupprecht B.(2), Spangenberg C.(2), Prawitt D.(2), Kaiser F.(1), Buiting K.(1), Horsthemke B.(1)
1) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany
2) Kinderklinik, Universität Mainz, Mainz, Germany

In the majority of patients with a chromosome 15 imprinting defect (ID) causing Angelman syndrome (AS), the defect is a primary epimutation that occurred spontaneously in the absence of a DNA mutation. We have recently investigated whether common DNA sequence variants in the bipartite imprinting centre (IC), which overlaps the *SNRPN* promoter/exon 1 region, are associated with an increased susceptibility to imprinting defects. We found two polymorphisms in the critical IC region for AS (the AS-SRO) that appear to modify the risk of an imprinting defect on the maternal chromosome. It is possible that these polymorphisms lie within the binding site of a maternal imprinting factor. To identify such a factor, we used a fragment of the AS-SRO (AS2) containing one of the two polymorphisms as a bait in a yeast one-hybrid screen. In a human ovary cDNA library we identified, among others, three independent partial cDNA clones for the TGFβ-inducible early growth response protein 1 (TIEG1). In a luciferase reporter gene assay we analyzed the effect of TIEG1 on the AS-SRO. The AS2 fragment was cloned 5´ to the SV40 promoter in the pGL3-Control reporter plasmid. After transient transfection of COS-7 cells we observed enhanced expression of the reporter gene. Co-transfection of TIEG1 significantly decreased the luciferase activity. We conclude that the AS-SRO contains an enhancer that can be blocked by TIEG1. Enhancer blocking may play a role in the establishment of the maternal imprint.

W5 05

Silver-Russell and Beckwith-Wiedemann syndrome: Two opposite (epi)genetic disturbances of the same chromosomal region

Eggermann T.(1), Meyer E.(1), Schönherr N.(1), Mavany M.(1), Eggermann K.(1), Wollmann H.(2)
1) Universitätsklinikum, Institut für Humangenetik, Aachen, Germany
2) Universitätskinderklinik, Pädiatrische Endokrinologie, Tübingen, Germany

Silver-Russell syndrome (SRS) is a heterogeneous syndrome which is characterised by severe intrauterine and postnatal growth retardation and typical dysmorphic features. Until recently, the clinical diagnosis could be confirmed only in about 10% of cases who carried a maternal uniparental disomy of chromosome 7 (UPD7) or cytogenetic aberrations. Now first SRS patients with (epi)genetic mutations in 11p15 affecting the telomeric imprinting domain (ICR1) have been published. Interestingly, opposite mutations are associated with Beckwith-Wiedemann syndrome (BWS). However, the general significance of epigenetic mutations in 11p15 for the aetiology of SRS remained unclear We screened a cohort of 51 SRS patients for epimutations in ICR1 and KCNQ1OT1 by methylation-sensitive Southern-Blot analyses. ICR1 demethylation could be observed in 16 out of 51 SRS patients, corresponding to a frequency of approximately 31%. Changes in methylation at the KCNQ1OT1 locus were not detected. Combining these data with those on maternal duplications in 11p15, nearly 35 % of SRS cases are associated with detectable (epi)genetic disturbances in 11p15. We now also have to consider a general involvement of 11p15 alterations in growth retarded patients with only minor or without further dysmorphic features. Altogether, SRS and BWS may be regarded as two diseases caused by opposite (epi)genetic disturbances of the same chromosomal region displaying opposite clinical pictures. With the identification of ICR1 methylation defects in more than 30% of SRS patients, molecular testing of SRS is significantly improved: analysis of ICR1, maternal UPD7 and conventional karyotyping allows the detection of genetic defects in more than 45% of SRS patients.

W5 06

Expression profiling of uniparental mouse embryos is inefficient in identifying novel imprinted genes

Ruf N.(1), Dünzinger U.(2), Galetzka D.(2), Bähring S.(1), Haaf T.(2), Nürnberg P.(3), Luft F.C.(1), Zechner U.(2)
1) Max-Delbrück-Centrum, Berlin, Germany
2) Institute of Human Genetics, Johannes Gutenberg University, Mainz, Germany
3) Cologne Center for Genomics, University of Cologne, Köln, Germany

Imprinted genes are expressed from only one allele in a parent-of-origin-specific manner. We here describe a systematic approach to identify novel imprinted genes using Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP), a highly accurate method to detect allele-specific expression differences.
We analyzed 66 candidate imprinted transcripts from a recent mouse microarray study that map to known imprinted chromosomal regions. Up to now, three novel imprinted transcripts encoding putative nonprotein-coding RNAs have been identified on the basis of monoallelic expression in d11.5 p.c. (C57BL/6J x Cast/Ei)F1 embryos and adult tissues of hybrid mice. The results were confirmed in informative embryos derived from the reciprocal cross. Intriguingly, a vast majority of the analyzed transcripts showed no imprinting-associated expression in F1 embryos. These data strengthen the view that a large fraction of non-imprinted genes is differentially expressed between parthenogenetic and androgenetic embryos and question the efficiency of expression profiling of uniparental embryos to identify novel imprinted genes.
The three novel imprinted transcripts are located in close vicinity of the known imprinted genes Usp29, Lit1 and Gtl2. By RT-PCR experiments, we showed that the newly identified imprinted transcript on proximal mouse chromosome 7 is transcribed independently from Usp29. In addition, we detected a transcript in the orthologous region on human chromosome 19q13.4 that is monoallelically expressed and also transcribed independently from the human USP29 gene.

W5
W6

W6 Prenatal Diagnosis, Reproductive Medicine

W6 01

Women with advanced maternal age and without additional female factors for ICSI benefit most from aneuploidy testing in polar bodies

Grossmann B.(1), Lambrecht A.(1), Schwaab E.(2), Khanaga O.(3), Schorsch M.(3), Hahn T.(3), Haaf T.(1)
1) Institut für Humangenetik, Johannes Gutenberg Universität, Mainz, Germany
2) Praxis für Humangenetik, Wiesbaden, Germany
3) Kinderwunschzentrum, Wiesbaden, Germany

Five-color FISH on polar bodies can detect aneuploidies for chromosomes 13, 16, 18, 21, and 22 in fertilised eggs. The transfer of embryos without detectable maternal aneuploidies for the tested chromosomes can be expected to increase the implantation rate and decrease the number of spontaneous abortions. Another benefit is prevention of a pregnancy with trisomy 13, 18, or 21. We performed first and second polar body diagnoses on more than 5500 fertilised eggs from more than 1000 ICSI cycles because of advanced maternal age, recurrent spontaneous abortions and/or more than three previously failed ICSI cycles. Women were classified into groups according to their age and different female factors including endometriosis, tubular occlusion, and recurrent spontaneous abortions. Women who received polar body diagnosis were compared to matched controls in order to find out which group(s) benefited from aneuploidy screening and which didn't. In couples with only male indication for ICSI and women aged 40 years and older the pregnancy rate increased from 8% in the control group to 12% after aneuploidy screening, whereas the rate of abortions decreased from 67% to 39%. Evidently, this group benefited most from aneuploidy screening. In women younger than 35 years polar body diagnosis neither increased the implantation rate nor decreased the rate of spontaneous abortions. Therefore, we recommend aneuploidy screening only to women from 35 years on. Within this age group couples with only male indication for ICSI had the largest benefit from polar body diagnosis.

W6 02

Aneuploidy testing for chromosoms 1, 2 and 3 in polar bodies increases the pregnancy rate in low pregnancy rate cases

Hahn A.(1), Schwab E.(2), Lambrecht A.(1), Schorsch M.(3), Khanaga O.(3), Haaf T.(1), Grossmann B.(1)
1) Institut für Humangenetik, Johannes Gutenberg-Universität, Mainz, Germany
2) Praxis für Humangenetik, Wiesbaden, Germany
3) Kinderwunschzentrum, Wiesbaden, Germany

Polar body diagnosis (PBD) can unveil maternally transmitted mutations and chromosomal aberrations. Because it is performed within the first hours after impregnation but before pronuclear fusion, it is legal in Germany and other countries where preimplantation diagnosis in the embryo is banned by law. Nearly 50-60% of all first-trimester spontaneous abortions show monosomies and trisomies. Of these up to 90% are caused by faulty maternal meiosis and will be increasingly frequent in advanced maternal age. PBD is therefore used to increase the success rate of intracytoplasmatic sperm injection (ICSI). FISH analysis of chromosomes 13, 16, 18, 21, and 22 in polar bodies is commonly performed to detect numerical aberrations of these chromosomes which are causes of well defined neonatal malformation syndromes or spontaneous abortions in early pregnancy. Here we analysed 57 cycles of 50 women for aberrations of the large chromosomes 1 and 2 or 1 and 3 in addition to standard PBD by five-color FISH. All women had a history of advanced maternal age, recurrent abortions, or multiple unsuccessful ICSI-cycles. Women aged >40yrs who underwent ICSI because of male infertility benefited most. The pregnancy rate increased from 11.8 to 28.5% and spontaneous abortions occurred less frequently. We also noted that numeric aberrations were equally common for all analyzed chromosomes, i.e. some 30%. Because trisomies and monosomies of chromosomes 1, 2, and 3 are very rare in abortions, aneuploidy for these gene-rich chromosomes may already prevent normal implantation. We therefore suggest including FISH of chromosomes 1, 2, or 3 in routine PBD for aneuploidy detection, especially in cases of advanced maternal age, recurrent abortions, and multiple unsuccessful ICSI-cycles.

W6 03

Aneuploidy-screening in single cells by a rapid comparative genomic hybridization protocol for prenatal diagnostics

Landwehr C.(1), Montag M.(2), Weber R.G.(1)
1) Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn, Germany
2) Department of Gynecological Endocrinology & Reproductive Medicine, Rheinische Friedrich-Wilhelms-University, Bonn, Germany

It was the aim of this study to establish and validate a protocol for rapid comparative genomic hybridization (CGH) using DNA from a single cell for the use in prenatal diagnostics. To accomplish this, different parameters of existing protocols were optimized to allow the analysis of small amounts of DNA in the shortest possible period of time. The test-DNA used was from an ovarian adenocarcinoma cell line (COLO-704) with several known chromosomal imbalances. Different protocols for universal DNA amplification were tested. With a modified DOP-PCR protocol sufficient DNA for a CGH experiment could be generated rapidly from 5 pg of COLO-704 DNA, which approx. corresponds to the amount of DNA in a diploid cell. Overall, the duration of a whole CGH experiment could be reduced from approx. 120 to 18 hours. The greatest time reductions were achieved in the duration of DOP-PCR, DNA labeling and hybridization time, which are the most time consuming steps of the technique. To validate this protocol, somatic cells, including lymphocytes and fibroblasts, with known karyotypes (normal, trisomy 18 and 21) were examined. Single cells were microscopically dissected and subjected to the protocol established. CGH-profiles of good quality could be obtained showing the expected result. This indicates that the protocol is reliable in detecting numerical aberrations even of small chromosomes in a single cell. Finally, our aim was to screen the haploid genome of a polar body for genomic imbalances. The DNA from several polar bodies could be amplified successfully. So far, no polar body showed any imbalances. The detection of a Y chromosome loss served as an internal control, since the reference DNA used was from a normal male individual. In summary, a fast protocol for chromosomal CGH was developed that allowed to perform a successful aneuploidy-screen in a single cell within 18 hours. We have started to use this protocol on DNA-arrays to further automate and speed up data analysis.

W6 04

Rapid polar body testing by comparative genomic hybridization

Langer S.(1), Geigl J.B.(1), Clement-Sengewald A.(2), Buchholz T.(2), Speicher M.R.(1)
1) Institut für Humangenetik der TU, München, Germany
2) Zentrum für Polkörperdiagnostik, Planegg, Germany

It is a well-established fact that more than half of all human preimplantation embryos contain aneuploid cells. Therefore, chromosome analyses of polar bodies should help to identify fertilized but aneuploid eggs. A detailed testing will be especially helpful for females who are carriers of balanced translocations. At present, polar body analysis has two constraints: First, the number of chromosomes, which can be simultaneously analysed and second, the limited time frame of about 20 hours to complete analysis according to German legislation. In previous experiments we used our single cell linker adaptor approach to amplify the entire DNA content of a single polar body for a subsequent CGH evaluation. By using this approach we tested 17 polar bodies. As expected, 11 polar bodies revealed chromosomal copy number changes while 6 polar bodies had a normal chromosome count. However, a drawback of the linker adaptor PCR approach is the long duration (31 hours) needed for amplifying the genome of a single cell, which makes this technique ineffective for prenatal diagnosis. Therefore, we changed to a new protocol allowing the amplification of single cell DNA within 5 hours. Careful testing of amplification products revealed that the CGH-profiles with the 5 hour amplification protocol have a similar or even better quality as compared to the conventional linker adaptor PCR approach. For a further acceleration of whole genome polar body analyses we are currently testing the performance of the amplification product on various array platforms. Our goal is to achieve a rapid array based diagnosis with an improved resolution.

W6 05

Distribution of the sex chromosomes in dysgenetic gonads of a patient with mixed gonadal dysgenesis

Röpke A.(1), Kalinski T.(2), Mohnike K.(3), Sel S.(4), Jakubiczka S.(1), Pelz A.-F.(1), Roessner A.(2), Wieacker P.F.(1)
1) Institut für Humangenetik, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
2) Institut für Pathologie, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
3) Klinik für Allgemeine Pädiatrie und Neonatologie, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
4) Universitätsklinik und Poliklinik für Augenheilkunde, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany

In a four week-old child with female external and internal genitalia but clitoris hypertrophy chromosome analysis in lymphocytes revealed a 46,XY karyotype. No deletion of Y chromosomal sequences was detected by PCR analysis of DNA from peripheral blood leucocytes. After gonadectomy because of the elevated risk for gonadal tumor chromosome analysis was performed in gonadal tissue. Chromosome analysis of the left dysgenetic gonad revealed a gonosomal mosaicism with a 45,X cell line in 27 of 50 metaphases by GTG banding. The dysgenetic left gonad demonstrated a significant higher proportion of nuclei carrying a Y chromosome (46.3%) than the streak gonad from the right side (33.9% of the analyzed nuclei). The histomorphological examination of the left gonad revealed immature testicular tissue besides rete-like structures and irregular ovarian type areas with cystic follicular structures. The different tissues of this dysgenetic gonad demonstrated variable proportions of cells with an X and a Y chromosome. Whereas Sertoli cells and rete-like structures revealed a significant higher proportion of XY cells in relation to the whole section of the dysgenetic gonad, almost all granulose-like cells carried no Y chromosome. Theka-like cells and Leydig cells revealed the same proportion of XY/X cells and none of both cell types shows a significant difference to the whole dysgenetic gonad. In contrast to these findings, spermatogonia contained exclusively a XY constellation.

W6 06

The impact of ovarian stimulation on methylation imprints in early mouse development

Winter M.(1), Alexandridis A.(1), May A.(1), Großmann B.(1), Haaf T.(1), Zechner U.(1)
1) Universitätsklinikum Mainz, Institut für Humangenetik, Mainz, Germany

Several studies suggest a relationship between assisted reproductive technologies (ART) and genomic imprinting disorders. Ovarian stimula-

tion which is an integral component of ART may interfere with genome-wide methylation reprogramming, implantation, and fetal development in mice and increase the risk for genomic imprinting disorders in humans. To analyze the impact of superovulation on the epigenome, we examined the methylation patterns of the imprinted genes H19, Snrpn, Igf2r, Lit1, Mest, and Peg3 in 4-cell and morula stage embryos from superovulated and nonsuperovulated C57BL/6J females which were mated with C57BL/6J males. DNA was isolated from pools of ten 4-cell embryos each or single morulae and subjected to bisulphite modification, PCR amplification, subcloning, and sequencing. Up to now, we generated representative data for the differentially methylated regions (DMRs) of Snrpn, H19, and Igf2r in single morulae from nonsuperovulated and superovulated females, respectively. For Snrpn and H19, a significant loss of methylation was found after superovulation. Morulae from nonsuperovulated females displayed average methylation levels of 70% (Snrpn) and 75% (H19), while in morulae from superovulated matings average methylation levels of 9% (Snrpn) and 49% (H19) were observed. In contrast, the DMR of Igf2r showed a slightly increased but rather similar average methylation level in morulae from superovulated females (92%) compared to morulae from nonsuperovulated females (78%). In addition, preliminary evidence suggests that 4-cell embryos from superovulated females also exhibit a loss of DNA methylation at the imprinting control region of the H19 gene. Thus, ovarian stimulation seems to cause an aberrant hypomethylation of at least some imprinted genes. Our findings support the hypothesis that developmental abnormalities associated with ovarian stimulation are due to impaired imprinted gene methylation.

W7 Cytogenetics

W7 01

Parental-origin-determination-FISH (pod-FISH) can distinguish homologues chromosomes

Weise A.(1), Gross M.(1), Horsthemke B.(2), Claussen U.(1), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany

The differentiation of homologues chromosomes (chr) as well as their parental origin can presently be done/determined exclusively by molecular genetic methods using microsatellite or SNP-analysis (Schlötterer et al., 2004). Only in exceptional cases a distinction on chromosomal level is possible – e.g. due to variations within the heterochromatic regions of chrs. 1, 9, 16 and Y or the p-arms of the acrocentric chrs. In the absence of such polymorphisms a distinction of the homologues chrs on a single cell level was not possible until now. Consequently, various questions of scientific and diagnostic relevance could not be answered. Recently detected polymorphisms called LCV or CNP include genomic variations up to several Mb in size (Iafrate et al., 2004, Sebat et al., 2004). These polymorphic variations were discovered by array based sequence comparison of different individuals. Due to the big size of the described polymorphic regions we started to develop a FISH-based approach for an inter-individual differentiation of the homologues chrs inherited from different individuals (parents), called parental-origin-determination-FISH (pod-FISH) technique. pod-FISH probe sets are at present chr-specific and are composed of BAC clones covering in summary 122 regions distributed over the whole human genome. One- up to 5-color pod-FISH probe sets were created, evaluated, optimised and verified on a known heterochromatic polymorphism of chr 16. Moreover, a case with a known UPD 15 was used to show the suitability and reproducibility of the method. This new method will open new doors for diagnostic and scientific fields that could not be questioned by now. E.g. the analysis of single cells will in leukaemia create new diagnostic markers for therapy control after transplantations or to detect maternal contamination in prenatal diagnosis.

Supported in parts by a grant from the university of Jena, Deutsche Krebshilfe (70-3125-Li1) and IZKF / TMWFK (TP 3.7 / B307-04004).

W7 02

Genome-wide array-CGH screening of 52 patients with mental retardation of unknown etiology – Detection of two causative imbalances inherited from a parent and four de novo deletions

Engels H.(1), Ehrbrecht A.(1), Hoischen A.(1), Landwehr C.(1), Ehrler M.(1), Bosse K.(1), Pagenstecher C.(1), Radlwimmer B.(2), Tödt G.(2), Lichter P.(2), Weber R.G.(1)
1) Institute of Human Genetics, Rheinische Friedrich Wilhelms Universität, Bonn, Germany
2) German Cancer Research Center, Department of Molecular Genetics, Heidelberg, Germany

Array-CGH is used for the genome-wide microdeletion/-duplication screening in mentally retarded patients with multiple congenital anomalies (MR/MCA). For the differentiation of causal imbalances from benign copy number variations, *de novo* occurrence is considered the strongest evidence for causality until now. We screened 52 well-characterized patients with unexplained MR (mostly MR/MCA) and normal subtelomere results. Genome-wide Array-CGH was performed with arrays containing 6000 (6k) and 8000 (8k) BACs/PACs/cosmids (average resolution <1Mb). Thresholds used were ±5SD (6k chip) and ±7SD (8k). We verified all outliers by FISH and analyzed parents for all confirmed imbalances. Six most likely causal imbalances were detected (>11% detection rate). FISH breakpoint definition is ongoing. De novo deletions were found in 4 patients with moderate or severe MR, dysmorphisms, neurological and/or brain anomalies (3 patients each) and malformations (1 patient) at 1q24 (3.7Mb), 4p12-p13 (1.1-1.3Mb), 6q11-q13 (8.8-11.0Mb) and 18q21 (0.5-1.4Mb). We will present detailed clinical findings. Interestingly, two *inherited* imbalances were found which we believe to be causative: A 3.7-4.3Mb Xq21 deletion was detected in a slightly dysmorphic 6 year old boy with borderline MR, inguinal and umbilical hernia as well as hypospadia and his healthy mother. The phenotype is most likely caused by the nullisomic form of his deletion as opposed to the heterozygous deletion in the mother. A slightly dysmorphic 12 year old girl who had developmental delay, muscular hypotonia, sleep apnea, frequent infections and microcephaly in childhood showed a 2.6-3.6Mb enhancement in the 22q11.2 DiGeorge syndrome critical region/DGCR also detected in her healthy father and two sibs with learning deficiency or MR. Since (1) DGCR duplications are known to cause MR/MCA, (2) all 3 sibs fit the known clinical spectrum and (3) normal carrier parents have been observed, the enhancement seems causative.

W7 03

Detection of copy number changes in patients with mental retardation using high density SNP microarrays

Wagenstaller J.(1), Spranger S.(2), Heye B.(3), Kazmierczak B.(2), Cohen M.(4), Freisinger P.(4), Meitinger T.(5), Speicher M.R.(5), Strom T.M.(5)
1) GSF-National Research Center for Environment and Health, Institute of Human Genetics, München-Neuherberg, Germany
2) Praxis für Humangenetik, Bremen, Germany
3) Technical University Munich, Institute of Human Genetics, München, Germany
4) Children's Hospital, Technical University Munich, München, Germany
5) GSF-National Research Center for Environment and Health and Technical University Munich, Institute of Human Genetics, München, Germany

Array-based genomic screening is a powerful strategy to identify chromosomal and allelic rearrangements. Among these techniques, whole genome analysis using high-density SNP genotyping oligonucleotide arrays may allow identification of yet unknown microdeletions, microduplications and uniparental disomies. We collected a cohort of 70 children and their parents with unexplained mental and developmental retardation, facial and/or sceletal dysmorphologies, and also other symptoms. The first screening, including high resolution banding analysis and metabolic investigations was unconspicuous. The patients genomic DNAs were analysed using the Affymetrix GeneChip 100K Array, consisting of 116,204 single nucleotide polymorphism (SNP) probes with an average spacing of 23.6 kilobases. Data analysis was performed with dChip and Bioconductor software. Preliminary analysis of 10 families revealed a single de novo deletion of approximately 7 Mb in chromosome 1q31.1-31.3. Rearrangements will be confirmed by LOH, quantitative PCR or fluorescence in situ hybridization (FISH) using BAC clones mapping to the potentially deleted or amplified regions. We were also able to detect deletions at the breakpoint region of 2 children with known translocations/inversions, which were not detected by cytogenetic investigations. Therefore, microarrays provide an efficient way to serve as a tool for precise mapping of regions with allelic imbalances and chromosomal breakpoints and may help to characterize new microrearrangement syndromes, delineate regions of UPD and identify genes involved in chromosomal rearrangement phenotypes. Further studies will also be performed to determine the resolution of different SNP genotyping and tiling path array techniques.

W7 04

Rapid fine mapping of chromosomal breakpoints in patients with apparently balanced translocations

Erdogan F.(1), Tzschach A.(1), Arkesteijn G.(2), Chen W.(1), Krause-Plonka I.(3), Pelz A.-F.(4), Kalscheuer V.M.(1), Muradyan A.(1), Hultschig C.(1), Ropers H.H.(1), Ullmann R.(1)
1) Max Planck Institute for Molecular Genetics, Human Molecular Genetics, Berlin, Germany
2) Inst. of Inf. Diseases and Immunology, Utrecht University, Utrecht, Netherlands
3) Institut für Humangenetik, Klinikum Ernst von Bergmann, Potsdam, Germany
4) Institut für Humangenetik, Otto von Guericke Universität, Magdeburg, Germany

Here we describe an improved protocol for the rapid mapping of chromosomal breakpoints in balanced translocations. This protocol has been employed to study a constitutional balanced translocation, 46,XY, t(1;13)(p22;q14), found in a 24 year old male lymphoma patient and his father, who died from acute myeloid leukemia at the age of 46. Familial history suggests that this aberration predisposes to the development of malignancies. For the analysis of the translocation, a permanent lymphoblastoid cell line was established from the patient. The presence of any additional DNA copy number changes was ruled out by sub-megabase resolution array CGH. About 6,000-20,000 chromosomes were flow-sorted and an aliquot of this DNA was uniformly amplified. Next, we applied array painting, a technique, in which sorted chromosomes are hybridised on DNA arrays. Using our tiling path 36k BAC arrays already enabled us to narrow down the breakpoints to about 150kb in a single experiment. In the next step we generated a sub-array consisting of a set of PCR fragments, which evenly covered the previously determined 150kb fragments. With this sub-array, we have assigned the breakpoints to intervals of less than 4kb, which then could be bridged by long range PCR. Sequencing of the PCR products identified one of the breakpoints to be located within a Mer5C element. The method presented here paves the way for rapid fine mapping of chromosomal breakpoints in large cohorts of patients with balanced translocations.

W7 05

Towards a molecular genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC) by applying microdissected sSMC probes on a 21.5 k BAC array-CGH

Liehr T.(1), Mrasek K.(1), Kosyakova N.(2), Stankiewicz P.(3), Cheung S.W.(3), Cai W.W.(3), Weise A.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russian Federation
3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States of America

Small supernumerary marker chromosomes (sSMC) are present in 0.043% of newborns and about 2.5 million humans are carriers of sSMC. In about 30% of sSMC carriers, an abnormal phenotype is observed. Clinical outcome of sSMC is difficult to predict as various phenotypic consequences can appear. Recently a first genotype-phenotype correlation for sSMC was proposed, based on the differences in euchromatic DNA-content of sSMC characterized by molecular cytogenetics (Liehr et al., 2006, Cytogenet. Genome Res., 112: 23-34). To characterize the size of partial chromosomal trisomies provided by the sSMC presence in more detail, we used array CGH comprised of 21500 BACs covering the whole human genome to analyse 15 sSMC from 14 different individuals. The chromosomal origin, size and shape of each sSMC were analyzed by previous molecular cytogenetic studies. For array CGH, the sSMC of each case was microdissected and the DNA was amplified by DOP-PCR. This amplified DNA derived from sSMC was used as test DNA and DNA of a normal male or female was used as the reference DNA. DNA of sSMC derived from chromosomes 1, 2, 5, 8, 9, 11, 12, 15 (3x), 16, 17, 21 and X (2x) were applied. sSMC-origin and -breakpoints were characterized by FISH before. In 2 cases the FISH results were consistent with the finding from array CGH. In 3 cases only one breakpoint was confirmed while in the remaining cases they had to be corrected after array CGH. In all cases the breakpoints could be narrowed down to one breakpoint-spanning BAC. In conclusion, array CGH using DNA derived from microdissection is a straight forward method to characterize origin and genetic content of a sSMC in a single experiment. Nonetheless, molecular cytogenetic studies will always have to be performed to confirm the array CGH results, to characterize the sSMC-shape, and the level of mosaicism of an sSMC. Array CGH will be helpful for better genotype-phenotype correlation in future.

Supported in parts by DFG 436 RUS 17/109/04.

W7 06

Ulnar-mammary syndrome with dysmorphic facies and mental retardation caused by a novel 1.28 Mb deletion encompassing the *TBX3* gene

Klopocki E.(1), Neumann L.M.(2), Tönnies H.(2), Ropers H.-H.(3), Mundlos S.(1), Ullmann R.(3)
1) Charité Universitätsmedizin Berlin, Institute of Medical Genetics, Berlin, Germany
2) Charité Universitätsmedizin Berlin, Institute of Human Genetics, Berlin, Germany
3) Max-Planck Institute for Molecular Genetics, Berlin, Germany

UMS is a rare autosomal dominant disorder caused by mutations in *TBX3*. The condition is characterized by hypoplasia or aplasia of upper limbs on the ulnar side, mammary glands and nipples, and of apocrine glands in both sexes (MIM #181450). We report on a girl presenting with an ulnar-mammary syndrome (UMS) like phenotype, a dysmorphic facies, and mental retardation. Mutation analysis of *TBX3* and G-banded chromosome analysis from lymphocytes were performed. We used microarray-based comparative genomic hybridization (array CGH) to investigate the patient's genomic DNA for submicroscopic aberrations. No mutation of the *TBX3* gene was detected in our patient and chromosome analysis revealed a normal female karyotype (46,XX). Hybridization of a whole genome tiling path array consisting of more than 36,000 BAC clones revealed an interstitial 1.28 Mb deletion within chromosomal band 12q24.21. The deleted region encompasses one known gene, *TBX3*. The deletion and haploinsufficiency of *TBX3* was confirmed by FISH using BAC clones representing the deletion on the BAC array. To our knowledge this is the first description of *TBX3* haploinsufficiency caused by a genomic deletion in a patient with UMS. We suggest that the UMS phenotype in conjunction with the characteristic facial changes and mental retardation observed in our patient is due to the deletion of *TBX3* and the involvement of neighbouring genes.

W8 Molecular Genetics I

W8 01

Mutations in the gene for ichthyin in patients with autosomal recessive congenital ichthyosis - disease causing or polymorphic?

Eckl K.(1), Kurtenbach J.(1), Nätebus M.(1), Önal Akan A.(1), Küster W.(2), Traupe H.(3), Krieg P.(4), Hennies H.C.(1)
1) Universität zu Köln, Dermatogenetics, Cologne Center for Genomics, Köln, Germany
2) TOMESA Fachklinik, Bad Salzschlirf, Germany
3) Universität Münster, Dept. for Dermatology, Münster, Germany
4) Deutsches Krebsforschungszentrum, Eicosanoids and Tumor Development, Heidelberg, Germany

Autosomal recessive congenital ichthyoses (ARCI) form a group of rare, severe disorders of keratinization. Phenotypes and genetic etiology are both extremely heterogeneous, clinical findings regarding scales and erythema may vary from patient to patient.
The gene coding for ichthyin on 5q33 was recently found to be mutated in patients with ARCI. We have analyzed samples from a total of 186 ARCI patients from non-related families without mutations in either TGM1 or ALOX12B/ALOXE3. Additionally, 144 non-related healthy controls were analyzed. We have found five different mutations in 31 families. One of these was the previously described nonsense mutation R83X. All others were missense mutations: 15 families showed the homozygous mutation A114D, eight patients showed heterozygous A114D. Three patients showed the heterozygous mutation S391L. Nevertheless in 4 and 13, respectively, of 144 control samples we found the mutations A114D and S391L. The mutation A114D was also described previously. Therefore, we have cloned the transcript and expressed various tagged constructs. With a cloned GFP-linked ichthyin we now want to contribute to understanding the unknown structure, localization, and function of the protein. Only such studies into function can help to distinguish between polymorphism and disease causing mutation. This we have done for missense mutations in ALOX12B and ALOXE3, both encoding epidermal lipoxygenases. We have established an in vitro enzymatic activity assay to analyze recombinant mutated ALOX12B and

ALOXE3. Complete mutation analysis in a large group of non-related ARCI patients from Central Europe, India, and Turkey revealed that about 40% of all patients have mutations in TGM1, 12% in ALOX12B and ALOXE3, and up to 14% have base changes in ichthyin. Mutations in ABCA12 were only seen in patients from Northern Africa. As we suppose some of the ichthyin variants to be polymorphisms, 30 to 40% of all ARCI patients have mutations in still unknown genes.

W8 02

Functional characterization of P67S – A novel CFTR mutation leading to atypical cystic fibrosis with severe pancreatic insufficiency

Kraus C.(1), Rauh R.(2), Naehrlich L.(3), Reis A.(1), Korbmacher C.(2)
1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany
2) Institute of Cellular and Molecular Physiology, University Erlangen-Nuremberg, Erlangen, Germany
3) University Hospital for Children and Adolescents, University Erlangen-Nuremberg, Erlangen, Germany

Cystic fibrosis (CF) is the most common lethal genetic disorder in Caucasian populations, characterized by chronic bronchopulmonary infection, pancreatic insufficiency and other systemic complications. Most children are diagnosed before school age. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene resulting in defective chloride conductance in epithelial cells of a number of different organ systems. Mutations lead to dysfunctions of the channel protein and are classified into six classes, based on their functional effect.

Here we report on a 14 y old girl of Turkish ancestry who complained of recurrent abdominal pain in the upper abdomen. Faecal pancreatic elastase (EL-1) levels were below 50µg/g stool and severe exocrine pancreatic insufficiency was diagnosed. Although a sweat test was negative and no signs of a bronchopulmonary disease were found the diagnosis of CF was considered. Molecular analysis revealed a hitherto unknown homozygous P67S missense mutation. The mutation was confirmed in both parents. Another mutation at this residue (P67L) was already described and was found on about 1.4% of Scottish CF alleles. The effect of the mutation was described as “dominant mild” in compound heterozygous patients, based on diagnosis at a later age, lower sweat chloride levels, better nutritional status, and (usually) pancreatic sufficiency. As our patient exhibited a severe pancreatic insufficiency but no lung disease and was homozygous for P67S we investigated the functional effect of P67S in a heterologous expression system using electrophysiological and molecular biological methods. We found that P67S largely reduces cAMP stimulated CFTR chloride currents and channel surface expression without altering the ion selectivity of the channel. Our findings indicate that P67S is a CFTR mutation associated with reduced channel synthesis and/or channel trafficking, resulting in atypical CF with severe pancreatic insufficiency

W8 03

Enrichment of the active HRAS GTPase form causes Costello syndrome

Rosenberger G.(1), Schulz A.L.(1), Heller R.(2), Gillessen-Kaesbach G.(3), Wieczorek D.(3), Meinecke P.(4), Seemanova E.(5), Streubel B.(6), Arici C.(7), Horn D.(8), Schulze A.(9), Zenker M.(10), Kutsche K.(1)
1) Institut für Humangenetik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
2) Institut für Humangenetik, Klinikum der Universität zu Köln, Köln, Germany
3) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany
4) Abteilung für Medizinische Genetik, Altonaer Kinderkrankenhaus, Hamburg, Germany
5) Institute of Biology and Medical Genetics, Charles University, University Hospital, Prague, Czech Republic
6) Klinisches Institut für Pathologie, Allgemeines Krankenhaus Wien, Wien, Austria
7) Genel Cerrahi, Antalya Akdeniz Üniversitesi, Antalya, Turkey
8) Institut für Medizinische Genetik, Charité Berlin, Berlin, Germany
9) Department of Paediatrics, Sonderburg Sygehus, Sonderburg, Denmark
10) Institut für Humangenetik, Universitätsklinikum Erlangen, Erlangen, Germany

Costello syndrome is a rare congenital disorder characterized by postnatal growth deficiency, relative or absolute macrocephaly, coarse face, loose skin, heart abnormalities, and mental retardation. Recently, germline mutations in the *HRAS* gene have been described to cause Costello syndrome. We performed mutation analysis of *HRAS* in 33 patients with Costello syndrome. In 21 patients we identified four different heterozygous missense mutations that affect either codon 12 or 13. Three mutations (p.G12S, p.G12A, p.G13D) have been described, whereas the p.G13C mutation was novel. The p.G12S variant seems to be a recurrent mutation as it was present in 16 patients. Fourteen mutations occurred *de novo*, and in eight families we could demonstrate that the *HRAS* mutation was present on the paternal allele. The 21-kDa HRAS protein belongs to the superfamily of small GTPases, which cycle between an inactive GDP- and an active GTP-bound form. Somatic mutations in codons 12 and 13 were found in tumors and lead to constitutive HRAS activation. By GTPase pull-down assays, we showed an 1.17 to 1.37-fold increased *in vivo* activation of HRAS in fibroblasts of Costello patients. To date, the best characterized effectors of HRAS are the RAF kinases. Activated RAF phosphorylates the MEK1/2 kinases, which then activate the ERK1/2 protein kinases. However, so far we were unable to detect an increase of phosphorylated MEK1/2 or ERK1/2 in Costello fibroblasts compared to normal fibroblasts by using phosphospecific antibodies.

HRAS also participates in regulating the activity of integrins, a large family of transmembrane receptors. Initial immunofluorescence analysis revealed a disturbed distribution of β1 integrin in Costello fibroblasts suggesting a decrease in integrin aggregation and activation. Taken together, our results suggest a novel role of HRAS activation in integrin aggregation that might be involved in the pathogenesesis of Costello syndrome.

W8 04

Revertant mosaicism in Fanconi anemia: a 5bp deletion is compensated by an in cis splice site mutation in a patient of subtype FA-L

Kalb R.(1), Neveling K.(1), Rio P.(2), Hanenberg H.(3), Schindler D.(1)
1) Department of Human Genetics, Würzburg, Germany
2) CIEMAT, Madrid, Spain
3) Department of Pediatric Hematology, Düsseldorf, Germany

11 Fanconi anemia genes are known to date that interact in the recognition and repair of DNA-damage. Eight of the FA proteins assemble into a nuclear complex which is necessary for monoubiquitination of FANCD2 during S phase and in response to DNA damage. The ring finger protein FANCL provides the presumptive E3 ligase function. Here we describe the worldwide second FA patient who has been assigned to subgroup FA-L via retroviral gene complementation. The patient presented with bone marrow failure between ages 9 and 11 necessitating alternate donor hematological stem cell transplantation. Only two years following transplant the patient developed squamous cell carcinoma of the tongue that was successfully removed. Sequencing of fibroblast-derived FANCL cDNA and gDNA revealed a 5-bp deletion (exon 7) and a missense substitution (exon 11) resulting in skipping of exons 10 and 11 at RNA level. Two independently established lymphoid cell lines, however, lacked the typical hypersensitivity to MMC in the cell cycle assay and showed functional monoubiquitination of FANCD2 suggesting a somatic reversion. Sequence analysis of gDNA from LCLs, peripheral blood and bone marrow revealed a heterozygous base substitution at IVS7-2A>G which was not present in fibroblast DNA. The base substitution leads to the loss of the natural splice acceptor and to the use of the following 3´ AG for splicing (located 10 bases downstream of the regular AG and a single base ahead of the 5 bp deletion). Accordingly, the ORF is restored. Functionality of the compensating mutation was proven in FA-L deficient fibroblasts. Retroviral transduction of the reverted cDNA sequence resulted in the loss of MMC sensitivity and, as expected, in FANCD2 monoubiquitination. FANCD2 foci were absent in patient fibroblasts but were restored after transduction with reverted FANCL cDNA. The formation of RAD51 foci was unimpaired suggesting independent functions of RAD51 and FANCD2 in the FA/BRCA caretaker network.

W8 05

Bardet-Biedl syndrome: Interaction of BBS proteins with other factors in centrosomes

Oeffner F.(1), Moch C.(1), Neundorf A.(1), Koch M.(1), Grzeschik K.-H.(1)
1) Philipps-Universität Marburg, Zentrum für Humangenetik, Marburg, Germany

Bardet-Biedl syndrome (BBS) is a rare, pleiotropic disorder characterized by rod-cone dystrophy, postaxial polydactyly, central obesity, renal dysplasia, learning difficulties, and hypogenitalism in males. It is a striking example of locus heterogeneity with at least nine genes (BBS1 – BBS9) linked to the disease. Recently, BBS gained increasing attention: Following an autosomal recessive mode of inheritance in general, the phenotype seems to require three mu-

tations in two genes in some families. The proposed model of "triallelic inheritance" suggests BBS as a link between Mendelian and complex phenotypes. BBS shares clinical features with prevalent traits such as obesity, diabetes and coronary heart disease – thus possibly providing important clues to the understanding of those common diseases. After the cloning and molecular charcterization the BBS genes it appears as if their gene products constitute part of a multisubunit complex causing basal body or cilia dysfunction when mutated.
Here, we report on our efforts to identify new interaction partners of BBS4 applying a yeast-2-hybrid screen:
i) We describe direct interactions between BBS4 and other BBS proteins.
ii) We identify additional potential binding partners interacting with BBS proteins.
iii) We corroborate our yeast-2-hybrid results by co-immunoprecipitation assays.
iv) We identify the cellular localization of these proteins by expressing constructs coding for fusion proteins between GFP or DsRed proteins and BBS proteins in cell cultures.
v) We show by immunohistochemistry, which of the identified potential interacting factors co-localize at the centrosomes.
vi) We validate our approach by confirming the interaction of BBS4 with the pericentriolar protein PCM1 described recently by other groups.
We will discuss the biological relevance of our findings for the molecular pathology of BBS.

W8 06

Identification of the genetic defect in the original Wagner syndrome family

Kloeckener-Gruissem B.(1), Bartholdi D.(2), Abdou M.-T.(3), Zimmermann D.R.(3), Berger W.(1)
1) University of Zurich, Institute of Medical Genetics, Division of Medical Molecular Genetics and Gene Diagnostics, Schwerzenbach, Switzerland
2) University of Zurich, Institute of Medical Genetics, Schwerzenbach, Switzerland
3) University Hospital Zurich, Department of Pathology, Molecular Biology Laboratory, Zurich, Switzerland

The aim of this study was to determine the genetic defect in Wagner syndrome, a rare autosomal dominant disorder belonging to the group of hereditary vitreoretinal degenerations. This disease had been genetically mapped to chromosome 5q14.3. DNA sequence of the 15 exons and their flanking introns of the CSPG2 gene in the progeny of the original pedigree described by Wagner in 1938 revealed several alterations. Only one of these co-segregates in 46 tested family members with the disease phenotype. It is a heterozygous substitution, G to A, of the first nucleotide in intron 8 and disrupts the highly conserved GT splice donor sequence. In blood cells of an index patient the entire exon 8 of CSPG2 is skipped. In addition, an aberrant transcript that lacks the last 21 base pairs of exon 8 accumulates. While these two transcripts were not found in control samples, CSPG2 mRNA containing a normal exon 8/9 splice junction were present in both, patient and control. CSPG2 encodes versican, a large proteoglycan, which is an extra cellular matrix component of the human vitreous. Four naturally occurring variants of versican can be distinguished based on the presence or absence of exon 7 and/or exon 8, in a tissues specific manner. One of the two erroneous transcripts encodes variant V2, which is normally found in the adult central nervous system. It carries a reduced number of chondroitin sulfate side chains. We hypothesize that the ectopic expression of versican V2 variant in the vitreous leads to a partial replacement of the normally synthesized splice variant V1, thereby affecting complex interactions with other extra cellular matrix components. Consequent disturbance of the ultra structural organisation may lead to a liquefaction of the vitreous and render the physiological properties to the observed pathology of vitreoretinopathy. Analysis of versican protein in patients' fibroblasts are underway and may aid in understanding the molecular basis of the Wanger syndrome.

W9 Cancer Genetics II

W9 01

Proficiency testing of the FA/BRCA pathway via FANCD2 immunoblotting

Neveling K.(1), Kalb R.(1), Hader C.(2), Schulz W.A.(2), Hanenberg H.(3), Baumer A.(4), Hoehn H.(1), Schindler D.(1)
1) Institut für Humangenetik, Würzburg, Germany
2) Department of Urology, Düsseldorf, Germany
3) Department of Pediatric Hematology, Düsseldorf, Germany
4) Department of Medical Genetics, Zürich, Switzerland

Monoubiquitination of FANCD2 is a central event in the activation of the FA/BRCA pathway. In response to DNA damage, posttranslational modification of FANCD2 is caused by the assembly of a nuclear core complex of at least 8 FA proteins. The appearance of the larger isoform of the FANCD2 protein (FANCD2-L) can be used as a functional test of the integrity of FA proteins which are part of a nuclear protein complex. A defect in one of the core complex genes causes failure of FANCD2 monoubiquitination such that immunoblotting detects only the smaller FANCD2 isoform (FANCD2-S). In contrast, inactivation of genes that function downstream of FANCD2 (e.g. FANCD1/BRCA2 or FANCJ/BRIP1) has no effect on its ubiquitination. Using retroviral complementation, we assigned more than 400 patients to any of the 11 known FA genes. However, complementation analysis was not informative in 42 patients. Immunoblotting of FANCD2 suggests that 26 of these have a defect in an as yet unknown member of the FA core complex, whereas 16 show proficient FANCD2 monoubiquitination such that their defective gene functions downstream of FANCD2. The proficiency of FANCD2 monoubiquitination was also tested in 22 established bladder carcinoma lines. A single cell line (BFTC 909) proved deficient in FANCD2 monoubiquitation and highly sensitive towards mitomycin C, suggesting a defective FA/BRCA pathway. MMC sensitivity was restored to normal by complementation with the FANCF gene. There was no mutation in FANCF cDNA, but a HpaII-restriction assay and sequencing of bisulfite modified DNA indicated extensive methylation of FANCF CpG islands. FANCF promotor hypermethylation has been previously observed in malignancies suggesting that epigenetic silencing of the FA/BRCA pathway may contribute to tumorigenesis. We conclude that FANCD2 proficiency testing can be used for the functional classification of known and unknown FA genes, and for the detection of defects in the FA/BRCA pathway in tumor cell lines.

W9 02

Specific genomic imbalances in paediatric familial and potential sporadic Pheochromocytomas

Hering A.(1), Guratowska M.(2), Bucsky P.(3), Claussen U.(4), Decker J.(5), Ernst G.(6), Höppner W.(7), Michel S.(4), Neumann H.P.(8), Parlowsky T.(3), Loncarevic I.(1)
1) Institut für Humangenetik und Anthropologie des UKJ, Tumorgenetik, Jena, Germany
2) Jagiellonian University, Department of Anthropology, Cracow, Poland
3) Kinderklinik der Medizinischen Universität, Lübeck, Germany
4) Institut für Humangenetik und Anthropologie des UKJ, Jena, Germany
5) Bioscientia, Ingelheim, Germany
6) Institut für Humangenetik und Anthropologie des UKJ, Cuca, Jena, Germany
7) Institut für Hormon und Fertilitätsforschung, Hamburg, Germany
8) Medizinische Universitätsklinik, Freiburg, Germany

Pheochromocytoma (PCC) in children is rare, genetically not well described, and often related with poor prognosis. We analyzed 14 tumours from children by comparative genomic hybridization (CGH) to detect genomic imbalances. A combinatorial loss of chromatin from chromosomes 3 and 11 was a common feature brought about by either a complete loss of chromosomes 3 and 11, as seen in 6/14 patients (43%), or a loss confined to the p-arms of chromosomes 3 and 11, as seen in 4/14 patients (29%). The combined loss of 3p with 11p was the minimal common deletion in all the 9 patients proven to have a constitutive VHL-mutation, and in one patient with a somatic VHL-mutation detected in the tumour DNA solely, but was absent in the two patients proven to have other familial syndromes (NF1, SDHD). Therefore, the pattern of imbalances in the tumour DNA provides a strong hint for an underlying familial VHL mutation. In children with pheochromocytoma it is highly recommended to check all related predisposing genes accurately, to select those children with true sporadic tumours, which tend to be very rare and might have a more adverse outcome.

W9 03

Proteomic identification of the MYST domain histone acetyltransferase TIP60 as a coactivator of the myeloid transcription factor C/EBPα

Bararia D.(1), Peer Zada A.A.(2), Behre G.(2), Bohlander S.K.(3)
1) Department of Medicine III and GSF, Clinical Cooperative Group Leukemia, University of Munich, Munich, Germany

2) Internal Medicine IV, University of Halle, BMT, Halle, Germany
3) Department of Medicine III and GSF, Clinical Cooperative Group Leukemia, University of Munich, München, Germany
C/EBPα is a lineage instructive transcription factor for granulopoiesis. Conditional expression of C/EBPalpha triggers neutrophilic differentiation, and in C/EBPα knockout mice, no mature granulocytes are present. In the current study we sought to identify interacting proteins of C/EBPα to further gain insight into its function. We applied mass spectrometry based proteomics using C/EBPα fused to glutathione-S-transferase. Proteins which formed complexes with the GST-C/EBPα fusion protein were analyzed by 2-D gel electrophoresis and identified by MALDI-TOF mass spectrometry. A number of novel protein-protein associations were observed. These interactions included the association between C/EBPα and the MYST domain histone acetyl transferase (HAT) TIP60 (Tat-interacting protein 60 kD). This interaction was confirmed both in vitro and in vivo by GST-pulldown on co-immunoprecipitation experiments, respectively. As a consequence of this interaction, TIP60 can potentiate C/EBPα mediated transcription of a reporter gene driven by a minimal CCAAT-binding sites. We further demonstrate that the HAT domain of TIP60 is crucial for this synergistic effect and that a HAT dead mutant of TIP60 acts as a dominant negative over wild type TIP60. The C/EBPα DNA binding region is important for the TIP60 co-activation function. ChIP (chromatin immunoprecipitation) experiments demonstrated that TIP60 was associated with the human C/EBPα promoter in-vivo and that this association resulted in an increase of histone H3 and H4 acetylation. Expression profiling of diverse leukemia subtypes (Affymetrix platform) indicated lower expression of TIP60 in all AML subtypes in comparison to normal bone marrow. These data suggest a crucial role for TIP60 in the process of hematopoietic cell differentiation and leukemogenesis.

W9 04

A RQ-PCR screening assay for RUNX1 copy number changes in childhood B cell precursor ALL

Haas O.A.(1), Moritz A.(1), Stanulla M.(2), König M.(1), Röttgers S.(3), Reichelt C.(3), Harbott J.(3), Strehl S.(1), Schrappe M.(4)
1) CCRI, Vienna, Austria
2) Med. School, Dpt. Ped. Hematol. Oncol., Hannover, Germany
3) Univ. Hosp., Dpt. Ped. Hematol. Oncol., Giessen, Germany
4) Univ. Med. Center Schleswig-Holstein, Dpt. Ped., Kiel, Germany
An increased RUNX1 copy number is a common finding in BCP-ALL. It usually identifies additional copies of chromosome 21, which in turn is a typical feature of hyperdiploid cases. In a much smaller proportion of cases, however, it results from rearrangements that specifically multiply the region 21q22 (intra-chromosomal RUNX1 multiplication; ICRM). This distinct genetic marker, also known as AML1 amplification, designates a specific form of BCP-ALL with a pronounced risk of relapse. Noteworthy, less than 100 such cases have been reported so far worldwide. The apparent rarity results in part also from the fact that at present an ICRM can only be detected with fluorescence in situ hybridization (FISH) and a systematic FISH screening is only conducted in very few treatment trials. To overcome this diagnostic obstacle, we developed a DNA-based real-time polymerase chain reaction (RQ-PCR) screening assay. It is based on the comparative quantification of three regions within RUNX1 at 21q22, PRSS7 at 21q21.1 (as an intra-chromosomal control) and BBS1 at 11q13.2 (as an inter-chromosomal control). The assay was set up and evaluated with DNA from cases with two (normal controls), three (Down syndrome patients) and four (hyperdiploid ALL) chromosomes 21 and put to test on samples from 13 Austrian cases with a previously FISH-verified ICRM. The number of additional RUNX1 copies in these samples was determined to range from 4 to approximately 8. Subsequent screening of 221 BCP ALL samples from the German BFM-ALL trial identified altogether 88 cases with an increased RUNX1 copy number. The respective PCR results were in good accordance with those suggested by DNA-index, cytogenetic or FISH analyses. They prove that such a DNA-based screening technique can reliably identify and delineate RUNX1 overrepresentations in different genetic BCP ALL subforms, such as those with an ICRM, a hyperdiploid or pseudotetraploid karyotype as well as those ETV6/RUNX+ ones with secondary changes.

W9 05

Breakpoints in chromosomal region 5p15 centromeric of the hTERT locus are recurrent in hematological malignancies

Nagel I.(1), Martin-Subero J.I.(1), Harder L.(1), Klapper W.(2), Horsman D.(3), Gascoyne R.D.(3), Dyer M.(4), Martinez-Climent J.A.(5), Stilgenbauer S.(6), Callet-Bauchu E.(7), Siebert R.(1)
1) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
2) Department of Pathology and Lymph Node Registry, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
3) Department of Pathology, British Columbia Cancer Agency, Vancouver, Canada
4) MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom
5) Division of Oncology, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain
6) Department of Internal Medicine III, University of Ulm, Ulm, Germany
7) Service d'Hematologie Biologique, Centre Hospitalier Lyon Sud, Lyon, France
In two B-cell chronic lymphocytic leukemias (B-CLL) and one acute lymphoblastic leukemia we identified a cytogenetically cryptic translocation t(5;14)(p15;q32) by fluorescence in situ hybridization (FISH) using probes for the IGH-locus. Using various BAC- and Fosmid-clones we narrowed down the breakpoint region on chromosome 5p15 to 25kb. The breakpoints were closely centromeric of the hTERT (human telomerase reverse transcriptase) gene, which is an obvious candidate oncogene. Quantitative RT-PCR analysis in one B-CLL with t(5;14) and available RNA revealed overexpression of hTERT in comparison to B-CLL lacking 5p15 aberrations. To determine the frequency of chromosomal aberrations close to the hTERT locus in hematologic neoplasms, we have analyzed additional 68 cytogenetically characterized lymphomas and leukemias with aberrations in 5p14~15 by FISH using a newly established three color break-apart probe for the hTERT locus. By this approach, we identified four cases in which the hTERT locus was translocated to different partners, i.e. 1q21, 7p11, 9q31~33 and 19p13. Interestingly, three of these cases were diagnosed as mantle cell lymphoma (MCL). This led us to further analyze 105 MCLs. Among those, five cases harbored translocations and two cases amplifications affecting the region of interest in 5p15. These data show that chromosomal rearrangements in 5p15 closely centromeric to the hTERT locus are recurrent in B-cell malignancies, predominantly in MCL. Further molecular and functional analyses are underway to investigate the possible involvement of hTERT in these malignancies.

Supported by the „Lymphoma Research Foundation" (New York), the „European MCL Network" (LSHC-CT-2004-503351) and Deutsche Krebshilfe (Verbundprojekt "Molekulare Mechanismen der malignen Lymphome", 70-3173-Tr3/B2)

W9 06

Multiple myeloma (MM): Heterogeneity of chromosome 13q aberrations

Mors T.(1), Cremer F.W.(1), Mazitschek U.(2), Bila J.(2), Buck I.(1), Kartal M.(1), Hose D.(2), Bartram C.R.(3), Goldschmidt H.(2), Jauch A.(1)
1) Institute of Human Genetics, Molecular Cytogenetics, Heidelberg, Germany
2) Internal Medicine V, Subdepartment Myeloma, Heidelberg, Germany
3) Institute of Human Genetics, Heidelberg, Germany
In MM, prognostic evaluation is frequently based on interphase-FISH using a single probe for 13q14. Controversy exists regarding the frequency, extent of deletion, and prognostic significance. CD138+ bone marrow plasma cells enriched by MACS from 305 patients (pts) with newly diagnosed MM were analyzed by interphase-FISH. A deletion 13q14 was found in 145/305 pts (47.5%; major clone: 32.5%; subclone [<60% of nuclei]: 15.1%). The extent of the deleted region was assessed in 72 selected pts using additional probes for 13q12, 13q21, 13q32 and 13q34. Evidence for monosomy 13 was found in 29/32 pts with del(13q14) [major clone], but only in 10/20 pts with del(13q14) in a subclone, and 4/20 pts without del(13q14) showed a del(13q34) in a subclone. The translocation t(4;14) (incidence 14.3%) was more frequent in pts with a del(13q14) in the major clone (30.8%; vs. 9.5 [subclones] and 5.8 % [no del(13q14)]), and in pts with monosomy 13 (34.2%; vs. 5.9% [partial deletions] and 18.8% [no deletion]). In contrast, t(11;14) (incidence 19.3%) was most often observed in pts with del(13q14) in a subclone (30.9%; vs. 8.6% [major clone] and 22.4% [no del(13q14)]), and in pts with no or partial deletion 13q (35.3% [partial del13q] and 43.8% [no deletion] vs. 10.8% [monosomy 13]). A hyperdiploid karyotype, indicated by at least 2 of 3 trisomies of 9q34, 11q23 and 15q22 (n=134 analyzed pts), was seen more frequently in pts without del(13q14), and more frequently in pts with partial deletion than monosomy. Deletion 17p13 was seen in similar frequencies in pts with or without del(13q14). We

conclude that FISH-detected deletion of chromosome 13q14 alone does not define a distinct subgroup of MM, as considerable heterogeneity of the extent of the deleted region, the proportion of cells with the respective aberration, and of associated chromosome aberrations exists. The prognostic meaning of deletions of 13q14 thus has to be evaluated in the context of accompanying chromosome aberrations.

W10 Molecular Genetics II

W10 01

Association of diabetic nephropathy with the carnosinase gene CNDP1: carnosine as a protective factor.

Janssen B.(1), Hohenadel D.(2), Brinkkoetter P.(2), Peters V.(3), de Heer E.(4), Baelde H.(4), Köppel H.(2), Yard B.(2), Zschocke J.(1), van der Woude F.(2)

1) Institute of Human Genetics, Heidelberg, Germany
2) 5th Medical Clinic, Medical Faculty Mannheim, Mannheim, Germany
3) First Department of Pediatrics, University Hospital Heidelberg, Heidelberg, Germany
4) Department of Pathology, LUMC, Leiden, Netherlands

Diabetic nephropathy (DN) is the most important cause of renal insufficiency in the Western world. Apart from blood glucose levels and blood pressure, genetic susceptibility is known to determine the risk for this complication. Multiple studies have shown linkage of DN with a locus on chromosome 18q22.3-q23.We assessed if variants in the serum-carnosinase (CNDP1) gene on 18q22.3 are associated with DN in patients with type 1 and 2 diabetes mellitus and studied the renoprotective properties of the substrate of the CNDP1 gene product: carnosine.

DNA polymorphisms were determined in 154 cases (nephropathy) and 88 controls (diabetes without nephropathy). A functional trinucleotide repeat in exon 2 of the CNDP1 gene, coding for a leucine repeat in the leader peptide of the carnosinase-1-precursor, was found to be associated with nephropathy. The shortest allelic form was more common in the absence of nephropathy (p=0.0021, OR:2.64, CI:1.40-4.99) and associated with lower serum-carnosinase levels. This association has recently been confirmed by American colleagues in European Americans (B. Freedman, pers. communication). In vitro, carnosine inhibited the increased production of fibronectin and collagen type VI, usually occurring in podocytes at high glucose levels (25 mM). It also prevented the increment of TGFß2 expression in mesangial cells at 25 mM glucose.

We conclude that diabetic patients with the short CNDP1 repeat are protected against nephropathy and hypothesise that a longer leucine repeat leads to an increased secretion of enzyme by CNDP1-expressing cells, thereby causing increased hydrolysis of carnosine. Carnosine protects against the adverse effects of high glucose levels on renal cells. The data show that the CNDP1 genotype is a major determinant of susceptibility to DN.

W10 02

Systematic mutational screening of candidate genes in a putative glaucoma locus on chromosome 14q11 in German patients

Fernández-Martínez L.(1), Mardin C.(2), Pasutto F.(1), Kruse F.(2), Reis A.(1)

1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany
2) Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen, Germany

Glaucoma is a clinically and genetically heterogeneous group of ophthalmologic disorders leading to visual impairment and a major cause of blindness worldwide. The most common form is primary open angle glaucoma (POAG), characterised in most cases by late onset and elevated intraocular pressure. In about half of the cases familial aggregation with autosomal dominant inheritance with reduced penetrance is observed. Several loci have been linked to POAG, but until now only 3 genes have been identified, MYOC, OPTN and WDR36, accounting for about the 7% of the cases.

In order to identify new glaucoma predisposing genes in previously described POAG loci, we resequenced 11 genes (ZNF219, RPGRIP1, SALL2, DAD1, OXA1L, MMP14, BCL2L2, BM668528, NRL, ISGF3G and ADCY4) in 46 unrelated German POAG patients. All mapped to a 3,3Mb region in 14q11. Direct sequencing of exons, 5'-3' UTRs and flanking intronic regions lead to the identification of 156 SNPs. In one gene, RPGRIP1, previously unreported missense-variations were identified. Subsequently, we screened further 192 POAG patients and identified altogether 11 novel variants. 8 of them were not present in a group of 94 healthy individuals of comparable age, who had normal ophthalmologic examinations. Moreover, 4 of these variations affect evolutionary conserved amino acid positions among human, mouse, rat, cow and chimpanzee. We are currently performing familial segregation studies, protein structural modelling of these variants and further screening of new POAG patients and controls, in order to establish a potential role of RPGRIP1 in the aetiology of POAG.

W10 03

The distribution of K-IV repeat alleles in Apolipoprotein(a) differs across African, Asian, European and Arctic & Ozeanic populations

Kraft H.G.(1), Schmidt K.(1), Utermann G.(1)

1) Department of Medical Genetics and Molecular and Clinical Pharmacology, Human Genetics, Innsbruck, Austria

The human LPA gene (OMIM 152200) is characterized by extensive copy number variation of a translated Kringle-IV motif (unit size = 5.5 kb) with copy numbers ranging between 11 and 52. The encoded protein, apolipoprotein(a), is found in plasma covalently attached to LDL as lipoprotein(a) (Lp(a)) which is a quantitative genetic trait with extreme variation between individuals and populations. The K-IV size polymorphism explains a large fraction of the concentration variation of Lp(a). In order to understand the reason for the variation of Lp(a) within and between populations we have embarked on a epidemiologic study including 2997 individuals from 16 world populations from Europe (4 populations), Africa (4 populations), Asia (6 populations) and the Artic & Oceania (1 population each). The K-IV size polymorphism was analyzed by PFGE and Southern blotting.

Cumulative frequency plots of K-IV repeat alleles revealed significant differences between populations from the 4 continents. A plot of the frequency differences showed that European and African populations expressed a decrease whereas Asian and Arctic & Ozeanic populations had an excess of mid size LPA alleles. Median K-IV numbers increased and the skewness of the distribution of K-IV repeat alleles decreased from African to European, Asian and Arctic & Ozeanic populations. K-IV alleles of median copy number are associated with the highest Lp(a) concentrations in Africans. We conclude that K-IV alleles coding for high Lp(a) have been preferentially lost following the migration of humans out of Africa.

W10 04

Mutation screening of brain-expressed miRNA in patients with non-syndromic X-linked mental retardation

Chen W.(1), Jensen L.(1), Moraine C.(2), Fryns J.-P.(3), Chelly J.(4), Van Bokhoven H.(5), Gecz J.(6), Ropers H.H.(1), Kuss A.(1)

1) Max Planck Institute for Molecular Genetics, Department of Human Molecular Genetics, Berlin, Germany
2) Services de Génétique (INSERM), Tours, France
3) University Hospital Leuven, Centre for Human Genetics, Leuven, Belgium
4) Institut Cochin de Génétique Moléculaire, Centre National de la Recherche Scientifique (INSERM), Paris, France
5) Radboud University Nijmegen Medical Center, Department of Human Genetics, Nijmegen, Netherlands
6) University of Adelaide, Department of Genetic Medicine,Women's and Children's Hospital and Department of Pediatrics, Adelaide, Australia

MiRNAs constitute an abundant class of small non-coding RNAs that regulate gene expression through sequence-specific base pairing. They have been implicated in controlling developmental timing, programmed cell death, haematopoietic lineage differentiation, insulin secretion and brain morphogenesis. It is therefore conceivable that defects in miRNAs can play an important role in genetic disorders.

Mental retardation (MR) is a very heterogeneous disorder, which is significantly more common in males than in females, suggesting an important role of X-linked genes. However, it has been pointed out that the observed male to female ratio in MR (1.4 for severe and about 1.9 for mild forms) is much too large to be due to disease-causing mutations in X-chromosomal genes alone, and that this observation might point to the existence of X-chromosomal factors with more subtle effects on the IQ. If so, such sequence variants might be expected to be more common in small families than in large pedigrees with numerous affected males. So far, no such sequence variants have been identified, but looking for them in miRNA genes is attractive because of their putative function as genetic modifiers.

In an attempt to identify such modifier genes, or novel causative genes for X-linked MR (XLMR), we have screened all known, brain-expressed X-chromosomal pre-miRNAs for mutations in a co-

hort of 464 mostly small families with non-syndromic XLMR. In total, we identified 7 nucleotide changes affecting 7 different miRNAs. All changes are located outside the mature miRNA sequences; three of them are located within the pre-miRNAs whereas the remaining 4 are close to the pre-miRNAs. None of these is listed as a known SNP in the relevant databases. If found to be specific for XLMR patients, the relevance of these changes will be verified by functional studies. In parallel, mutation screening is being extended to regions of proven XLMR genes that are predicted as targets of miRNAs.

W10 05

MEGAP impedes cell migration via regulating actin and microtubule dynamics and focal complex formation

Yang Y.(1), Marcello M.(2), Endris V.(1), Saffrich R.(3), Fischer R.(2), Trendelenburg M.(2), Sprengel R.(4), Rappold G.(1)

1) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany
2) German Cancer Research Center, Biomedical structure analysis group, Heidelberg, Germany
3) University of Heidelberg, Department of Medicine V, Heidelberg, Germany
4) Max-Planck-Institute for Medical Research, Department of Molecular Neurobiology, Heidelberg, Germany

Over the past several years it has become clear that the Rho family of GTPases plays an important role in various aspects of neuronal development including cytoskeleton dynamics and cell adhesion processes. We have analysed the role of MEGAP, a GTPase-activating protein that acts towards Rac1 and Cdc42 in vitro and in vivo, with respect to its putative regulation of cytoskeleton dynamics and cell migration. To investigate the effects of MEGAP on these cellular processes, we have established an inducible cell culture model consisting of a stably transfected neuroblastoma SHSY-5Y cell line that endogenously expresses MEGAP albeit at low levels. We can show that the induced expression of MEGAP leads to the loss of filopodia and lamellipodia protrusions, whereas constitutively activated Rac1 and Cdc42 can rescue the formation of these structures. We have also established quantitative assays for evaluating actin dynamics and cellular migration. By time-lapse microscopy we show that induced MEGAP expression reduces cell migration by 3.8 fold and protrusion formation by 9 fold. MEGAP expressing cells also showed impeded microtubule dynamics as demonstrated in the TC-7 3x-GFP epithelial kidney cells. In contrast to the wildtype, overexpression of MEGAP harbouring an artificially introduced missense mutation R542I within the functionally important GAP domain did not exert a visible effect on actin and microtubule cytoskeleton remodelling. These data suggest that MEGAP negatively regulates cell migration by perturbing the actin and microtubule cytoskeleton and by hindering the formation of focal complexes.

W10 06

Enhancement of SMN2 gene expression by the DNA demethylating agent 5-aza-2´-deoxycytidine

Hauke J.(1), Schwingel E.(1), Hahnen E.(1), Wirth B.(1)

1) University of Cologne, Institute of Human Genetics, Institute of Genetics, and Center for Molecular Medicine Cologne (CMMC), Cologne, Germany

Spinal muscular atrophy (SMA) is an autosomal recessively inherited α-motoneuron disorder. The disease determining survival motor neuron gene 1 (SMN1) is homozygously deleted/mutated in approximately 97% of all SMA patients. Within the SMA region on chromosome 5q, the SMN genes exist in two almost identical copies, SMN1 and SMN2, which are ubiquitously expressed and encode identical proteins. Since all SMA patients lacking SMN1 carry at least one SMN2 copy, transcriptional activation of the disease modifying SMN2 gene is likely to be clinically beneficial. We and others have shown that histone deacetylase inhibitors such as valproic acid (Brichta et al., 2003) and the highly potent second generation HDAC inhibitors SAHA (Hahnen et al., submitted) and M344 (Riessland et al., submitted) increase SMN2 expression levels in fibroblasts from SMA patients and various neuroectodermal tissues. Here we report for the first time that the DNA demethylating agent 5-aza-2´-deoxycytidine (5-Aza, DecitabineTM) increases SMN2 transcript- and protein levels, indicating that both, histone acetylation and DNA methylation regulate human SMN2 gene activity. Studies were conducted using fibroblasts derived from SMA patients, while the SMN2 gene expression was quantified by real-time PCR and western blot. SMN2 promoter analyses indicated that the human SMN2 gene contains 4 putative CpG islands. Bisulfite treatment of DNA followed by methylation-specific PCR, cloning of PCR products and sequencing revealed methylation of specific cytosine residues within predicted SMN2-CpG islands. The SMN2 methylation pattern of the human SMN2 promoter was also analyzed after treatment with low micromolar concentrations of 5-Aza, giving evidence that SMN2 promoter methylation can be modulated by a pharmacologically active compounds. These results improve the understanding of how expression of the SMN2 gene is regulated and may contribute to the development of an effective treatment of SMA.

W11 Genotype / Phenotype Correlation

W11 01

Syndromes in children with hearing impairment - what kind of screening is effective?

Kohlschmidt N.(1), Zechner U.(1), Limberger A.(2), Haaf T.(1), Keilmann A.(2)

1) Institute of Human Genetics, Mainz University School of Medicine, Mainz, Germany
2) Dept of Communication Disorders, Mainz University School of Medicine, Mainz, Germany

Hearing disorders are among the most common impairments in childhood, affecting some 0.1% to 0.2%. It has long been known that genetic causes play a major role in hearing development. To date some 20 loci are identified for nonsyndromic deafness and over a hundred known genes code for forms of syndromic deafness.

To canalize molecular testing, skilled clinical examination of individuals with hearing disorders by an experienced geneticist is performed in many institutions specialized in hearing disorders. We examined 200 children with varying degrees of hearing impairment who attended a specialist clinic. Routine workup included physical examination, EKG, ophthalmologic examination including fundoscopy, tests for thyroid hormone levels, creatinine, BUN, and complete blood count, and pedigree analysis. In nonsyndromic cases sequencing of GJB2 (connexin26) and SLC26A4 (Pendred syndrome) was routinely performed. In cases of neonatal aminoglycoside treatment, mitochondrial-encoded gene MTRNR1 was analyzed. If a syndrome was diagnosed, specific molecular testing was undertaken when applicable.

Over 90% of the cases were nonsyndromic. Two children had Alport-Syndrome, one child had Long-QT syndrome, two children had Pendred syndrome, one child had Coffin-Lowry syndrome, three had Waardenburg syndrome, one had Treacher-Collins Syndrome, one had Heimler syndrome, four had Goldenhar syndrome, and one girl had Perrault syndrome. One boy had a family history indicating Usher syndrome but no retinal changes, one girl complained about vision loss but was found to simulate. Another girl was diagnosed with Rubinstein-Taybi syndrome but the diagnosis had already been made elsewhere and parents wanted a second opinion.

No case requiring additional treatment or prophylaxis was newly discovered by clinical examination.

W11 02

Genotypes and phenotypes in patients with SHOX deficiency: Clinical indicators of SHOX haploinsufficiency

Rappold G.(1), Niesler B.(1), Crowe B.J.(2), Roeth R.(1), Shavrikova E.P.(3), Quigley C.(3), Ross J.L.(4), Blum W.F.(5)
1) Institut für Humangenetik, Molekulare Humangenetik, Heidelberg, Germany
2) Eli Lilly and Company, Indianapolis, United States of America
3) Pharma Support Inc., St. Petersburg, Russian Federation
4) Thomas Jefferson University, Department of Pediatrics, Philadelphia, United States of America
5) Lilly Research Laboratories, Eli Lilly&Co., Bad Homburg, Germany; and University Children's Hospital Giessen, Bad Homburg/Giessen, Germany

Short stature affects three in a hundred children thus making it to one of the more frequent disorders that require clinical attention during childhood. Despite an assumed extraordinary genetic heterogeneity, mutations in one gene, SHOX, which encodes a homeodomain protein involved in bone growth, are found in up to 4 % of patients with short stature. Haploinsufficiency of the SHOX gene causes short stature with varying phenotypes ranging from isolated short stature to Leri-Weill dyschondrosteosis and Langer dysplasia. In this study, we assessed the association between the genotype and phenotype in a large cohort of short prepubertal children from 14 different countries with an average age of 7.6 years. Screening of 1641 unrelated individuals with sporadic or familial short stature revealed SHOX mutations in 68 (4.2%) individuals. We also performed detailed anthropometric measurements in all children. Whereas mean height was not different between patients with and without SHOX deficiency (-2.57 versus -2.58 SDS), a number of anthropometric measures including short forearm and lower leg, cubitus valgus, Madelung deformity, high-arched palate and muscular hypertrophy differed significantly ($p<0.001$). These phenotypic data were also contrasted to 33 children with Turner syndrome where haploinsufficiency of SHOX is thought to be responsible for the height deficit. Different types of SHOX mutations (48/70.6% classified as complete deletions, 4/5.9% as partial deletions and 16/23.5% as point mutations) were compared to the respective phenotype suggesting a trend towards a more severe phenotype in individuals with homeodomain missense mutations compared to the other types of mutations. Altogether, this study offers a detailed genotype-phenotype comparison in a large cohort of children with short stature and provides clear quantitative indication as to which children call for testing of the SHOX gene.

W11 03

Cell growth regulation by the RMRP gene: From cartilage hair hypoplasia to anauxetic dysplasia

Thiel C.(1), Horn D.(2), Zabel B.(3), Ekici A.(1), Salinas K.(4), Gebhart E.(1), Rüschendorf F.(5), Sticht H.(6), Spranger J.(7), Müller D.(8), Zweier C.(1), Schmitt M.(4), Mortier G.(9), Reis A.(1), Rauch A.(1)
1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany
2) Institute of Medical Genetics, Virchow-Klinikum, Berlin, Germany
3) Children's Hospital, University of Mainz, Mainz, Germany
4) Department of Biochemestry and Molecular Biology, SUNY Upstate Medical University, Syracuse, United States of America
5) Gene Mapping Centre, Max-Delbrück-Centre, Berlin, Germany
6) Institute of Biochemistry, University of Erlangen-Nuremberg, Erlangen, Germany
7) Greenwood Center, Greenwood, United States of America
8) Institute of Medical Genetics, Klinikum Chemnitz, Chemnitz, Germany
9) Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium

The growth of an individual is deeply influenced by regulation of cell growth and cell division, which also contribute to a wide variety of pathological conditions, including cancer, diabetes, and inflammation. Homozygosity mapping in patients with Anauxetic dysplasia, a spondylometaepiphyseal dysplasia characterized by extreme short stature with mild mental retardation, led to the identification of novel mutations in the RMRP gene, which were previously known to cause two milder types of short stature with susceptibility to cancer, cartilage hair hypoplasia (CHH) and metaphyseal dysplasia without hypotrichosis (MDWH) (Thiel et al. 2005 Am J Hum Genet). So far, 4 mutations causing Anauxetic dysplasia, at least 62 mutations causing CCH and MDWH, and 8 SNP's have been identified within and around the RMRP gene. We showed that different RMRP gene mutations lead to decreased cell growth by impairing ribosomal assembly and altering cyclin dependent cell cycle regulation. While the CHH founder mutation affects both pathways intermediately, Anauxetic dysplasia mutations do not affect B-cyclin mRNA levels but severely incapacitate ribosomal assembly via defective endonucleolytic cleavage. Anauxetic dysplasia mutations thus lead to poor processing of rRNAs while allowing normal mRNA processing and therefore genetically separate the different functions of RNase MRP. We now identified two mutations in a further family with Anauxetic dysplasia, a novel deletion c.254_263delCTCAGCGCGG and the missense mutation c.195C>T. Interestingly, this latter mutation has been reported causing CHH and MDWH when compound heterozygous with other mutations. Therefore, clinical heterogeneity is explained by a correlation between the level and type of functional impairment in vitro and severity of short stature or presence of cancer predisposition.

W11 04

Maternal alleles of genes involved in cholesterol transport are modifiers of the Smith-Lemli-Opitz Syndrome

Witsch-Baumgartner M.(1), Utermann G.(1)
1) Dept. of Med. Genetics, Molec. and Clin. Pharmacology, Medical University, Innsbruck, Austria

The Smith-Lemli-Opitz Syndrome (SLOS,MIM 270400) is a malformation syndrome that ranges in clinical severity from minimal dysmorphisms and mild mental retardation to severe congenital anomalies and intrauterine death. SLOS is caused by mutations in the delta7sterol-reductase gene (DHCR7), which impair endogenous cholesterol biosynthesis and make the growing embryo dependent on exogenous (maternal) sources of cholesterol. Previous studies demonstrated a correlation of severity with DHCR7 genotype and maternal ApoE genotype. We have now investigated whether other genes involved in lipid metabolism including apoCIII, LCAT, CETP, LDLR, ABCA1 (ATP-binding cassette transporter A1), and additionally MTHFR, involved in the metabolism of folic acid, may act as modifiers of the severity of the SLOS. SNP genotyping was performed in 68 SLOS patients, their mothers and fathers. Genotypic frequencies of analysed SNPs showed no deviation from Hardy-Weinberg equilibrium in SLOS patients, their mothers and fathers. We tested for correlation between patients´ clinical severity score and gene dose of the rare alleles in the patients and their parents. Neither patients nor paternal genotypes were associated with disease severity. In addition to the previously observed association with DHCR7 genotype and apoE genotypes, only maternal ABCA1 genotypes ($p=0.007$) but no SNPs in other genes showed a significant correlation. The rare maternal K1587 allele in the ABCA1 gene was associated with milder phenotypes. The correlation of maternal ABCA1 genotypes and SLOS severity persisted after stratification for cholesterol concentration ($p=0.041$) and for the DHCR7 genotype of the patient ($p=0.008$). ABCA1 is a transporter of cellular cholesterol across membranes and was found highly expressed in the placenta. We conclude that at least three factors, DHCR7-, ApoE- and ABCA1 genotypes modify the phenotype of the SLOS.

supported by FWF grant no. T161 to M.W.-B.

W11 05

Clinical heterogeneity in patients with Cohen syndrome caused by novel mutations in *COH1*

Horn D.(1), Seifert W.(2), Holder-Espinasse M.(3), Spranger S.(4), Hoeltzenbein M.(5), Rossier E.(6), Kotzot D.(7), Huhle D.(8), Meinecke P.(9), Albrecht B.(10), Dollfus H.(11), Chrzanowska K.H.(12), Hennies H.C.(2)
1) Institute of Medical Genetics, Charité University Medicine of Berlin, Berlin, Germany
2) Cologne Center for Genomics, University of Cologne, Köln, Germany
3) Hôpital Jeanne de Flandre, Génétique médicale, CHRU, Lille, France
4) Practice for Human Genetics, Bremen, Germany
5) Max Planck Institute for Molecular Genetics, Berlin, Germany
6) Department of Human Genetics, University of Ulm, Ulm, Germany
7) Division of Clinical Genetics, Department of Medical Genetics, Molecular and Clinical Pharmacology, Medical University of Innsbruck, Innsbruck, Austria
8) Praxis für humangenetische Beratung und Diagnostik, Wetzlar, Germany
9) Altonaer Kinderkrankenhaus, Clinical Geneticist, Hamburg, Germany
10) Institut für Humangenetik, Universität Duisburg-Essen, Essen, Germany
11) Centre de Référence pour les Affections Génétique Ophtalmologiques, Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

12) Department of Medical Genetics, Children's Memorial Health Institute, Warzaw, Poland

Cohen syndrome (MIM 216550) is a rare autosomal recessive disorder with variability in the clinical manifestations including psychomotor retardation, microcephaly, typical facial dysmorphism, progressive pigmentary retinopathy appearing at mid-childhood, early onset myopia, and intermittent neutropenia. Recently, the Cohen syndrome phenotype was found to be associated with mutations in the gene *COH1* in different populations. The Finnish patients present with a homogeneous phenotype as result of a specific allele and because of identical ethnic background. However, a broad clinical spectrum in non-Finnish cases and the age dependent appearance of some clinical signs may contribute to a delay in making the diagnosis. To determine the variability of the clinical manifestations and the spectrum of mutations associated with this condition, we investigated 24 patients with Cohen syndrome from 16 families, with a wide range of geographical and ethnic origins. In the full-length transcript of *COH1* we identified 25 different mutations, 19 of these were novel, including nine nonsense mutations, eight frameshift mutations, four verified splice site mutations, three larger in-frame deletions, and one missense mutation identified in two unrelated families. In our series of patients, ranging in age from 2.5 to 60 years, we observed marked variability of developmental and growth parameters. Mental retardation and intellectual impairment varied from moderate to severe. Progressive visual disability was a consistent finding, however retinopathy present at school age appears not to be obligatory for the diagnosis of Cohen syndrome. The detailed clinical information and the results of the molecular analysis of *COH1* in these patients enabled us to provide evidence of extended allelic heterogeneity as well as to obtain further information regarding the clinical findings, particularly the facial features, in patients of different ethnic backgrounds.

W11 06

Diagnostic yield of various genetic approaches in patients with unexplained mental retardation

Hoyer J.(1), Guth S.(1), Zweier C.(1), Nürnberg P.(2), Trautmann U.(1), Kraus C.(1), Reis A.(1), Rauch A.(1)

1) Institute of Human Genetics, Erlangen, Germany

2) Cologne Center for Genomics (CCG), Cologne, Germany

The underlying causes of mental retardation are often said to remain unknown in about half of the patients. While subtelomeric aberrations probably account for about 5% of cases, recent studies using array-CGH report of subtle chromosomal rearrangement in up to 25% of these patients. However, as patients involved in these studies were usually selected for morphological anomalies, this figure is likely biased towards a higher yield.

In order to obtain a more reliable view on diagnostic yields of various genetic methods and the diagnostic spectrum in unselected individuals with mental retardation, we reviewed the last 350 patients seen in our genetic clinic for unexplained developmental delay or mental retardation. The deVries scores of these patients ranged from 0 to 8 with a normal distribution around 3. In about 16.4% of patients an etiological diagnosis was established after clinical investigation, high resolution conventional chromosomal studies, subtelomeric screening and targeted molecular genetic testing. The likelihood of any genetic diagnosis being made increased with higher deVries scores. The most common diagnoses were structural chromosomal abnormalities in 5.7%, monogenic disorders in 5.9% and clinical recognizable microdeletion syndromes in 2% of patients. Pathogenic subtelomeric rearrangements accounted for only 0.85% of patients. In those patients without a clear diagnosis, 3.5% of mothers of affected boys showed complete skewing of X-inactivation suggesting X-linked mental retardation. 3 out of 12 patients in whom skin biopsies were taken to analyse for chromosomal mosaicism showed mosaic trisomies. Among the remaining 83.6% of patients with unknown diagnosis we selected 20 for copy number variation analysis using genome wide high density SNP arrays (10 K). In 1 of these 20 patients (5%) we identified a pathogenic deletion of 5 Mb containing 16 genes. Surprisingly, this patient showed no major dysmorphism or growth disturbances.

W12 Functional Genomics / New Technologies

W12 01

Proteohistography — direct analysis of tissue with high sensitivity and high spatial resolution using ProteinChip technology

Ernst G.(1), Melle C.(1), Schimmel B.(1), Bleul A.(1), von Eggeling F.(1)

1) Institut für Humangenetik und Anthropologie, Core Unit Chip Application (CUCA), Jena, Germany

On the proteomic level, all tissues, tissue constituents, or even single cells are heterogeneous, but the biological relevance of this cannot be adequately investigated with any currently available technique. The analysis of proteins of small tissue areas by any proteomic approach is limited by the number of required cells. Increasing the number of cells only serves to lower the spatial resolution of expressed proteins. To enhance sensitivity and spatial resolution we developed Proteohistography (PHG). Laser microdissection was used to mark special areas of interest on tissue sections attached to glass slides. These areas were positioned under microscopic control directly on an affinity chromatographic ProteinChip Array so that cells were lysed and their released proteins bound on a spatially defined point. The ProteinChip System, surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), allows the laser to be steered to up to 215 distinct positions across the surface of the spot, enabling a high spatial resolution of measured protein profiles for the analyzed tissue area. Protein profiles of the single positions were visually plotted over the used tissue section to visualize distribution proteohistologically. Results show that the spatial distribution of detectable proteins could be used as a Proteohistogram for a given tissue area. Consequently, this procedure can provide additional information to both a matrix-assisted laser desorption/ionization (MALDI)-based approach and immunohistochemistry, as it is more sensitive, highly quantitative, and no specific antibody is needed. Hence, proteomic heterogeneity can be visualized even if proteins are not known or identified.

The work was supported by BMBF und IZKF Jena.

W12 02

Comparison of a new MALDI-TOF based re-sequencing method with Sanger sequencing for molecular diagnostics of multiple endocrine neoplasia type 2 (Men2)

Storm N.(1), Vater N.(1), Salazar R.(1), Leuenberger B.(1), van den Boom D.(2), Höppner W.(1)

1) Bioglobe GmbH, Hamburg, Germany

2) Sequenom, Inc., San Diego, United States of America

Introduction: One of the main tasks in molecular genetics is the efficient sequencing of long DNA molecules amplified from disease genes to detect heterozygous or homozygous mutations. The gold standard for diagnostic sequencing is still the electrophoresis based method introduced by Sanger in 1977.

Method: This study was set out to determine the specificity, accuracy and robustness of an alternative technology based on T7/SP6 RNA polymerase promotor tagged primer, in vitro transcription of forward and reverse strand and base specific cleavage of the single strand RNA molecules by RNase. A modified biochemistry allows base-specific cleavage at each of the 4 nucleotides in 4 separate reactions. The generated fragment mixture is analyzed by MALDI-TOF mass spectrometry. Sequencing results from exons 10, 11, 13, 14, 15 and 16 of the RET proto-oncogene, which harbor more than 99% of MEN2 causing mutations were compared between the new MALDI-TOF based technology with the classical Sanger method. Typical mutations for this disease can occure in 1 of 23 codons. 361 samples (213 from MEN2 patients and 148 unaffected controls) were analyzed with both methods.

Results: The MALDI-TOF based sequencing technology displayed 100% concordance with the results obtained by Sanger sequencing with respect to the MEN2 causing mutations. Polymorphisms encountered in the analyzed regions of the RET proto-oncogene were also correctly genotyped.

Conclusion: The novel MALDI-TOF based was able to detect all mutations relevant for molecular diagnosis of MEN2. Furthermore the method was able to detect all SNPs in the analyzed region of the RET proto-oncogene. The procedure is simple, can be scaled down and completely automated. We conclude that the high quality of our data qualifies the new MALDI-TOF based method for diagnostic re-sequencing applications.

W12 03

Molecular diagnosis of Beckwith-Wiedemann syndrome using the Pyrosequencing technology

Wildhardt G.(1), Trübenbach J.(1), Enklaar T.(2), Prawitt D.(2), Zabel B.(2), Holton A.(1), Sancken U.(1), Lentes K.-U.(1), Decker J.(3), Steinberger D.(4)

1) Bioscientia, Zentrum für Humangenetik, Ingelheim, Germany
2) Johannes-Gutenberg Universität, Kinderklinik, Mainz, Germany
3) Bioscientia, Zentrum für Humangenetik, Ingelheim und, Johannes-Gutenberg Universität, Medizinische Klinik und Poliklinik Hämatologie, Onkologie und Pneumologie, Mainz, Germany
4) Bioscientia, Zentrum für Humangenetik, Ingelheim und, Justus-Liebig Universität, Institut für Humangenetik, Giessen, Germany

Beckwith-Wiedemann syndrome (BWS) is a congenital disorder that is characterized by overgrowth and neoplasia. The primary features are macrosomia, macroglossia, abdominal wall defects and predisposition to develop tumors during infancy. Several complex genetic and epigenetic abnormalities have been described in patients with BWS, including uniparental paternal disomy of 11p, abnormal methylation of CpG sites at KCNQ1OT1 (KvDMR) and H19, point mutations in the CDKN1C-gene or large chromosomal rearrangements affecting 11p15. The majority of abnormalities are epigenetic alterations. Up to 50% of sporadic patients exhibit a KvDMR-hypomethylation and for additional 5-10% of cases hypermethylation of H19 associated CpGs has been described.

To determine the methylation status of KvDMR in BWS patients we established a Pyrosequencing™ protocol. The Pyrosequencing™ technology is a real time sequencing method for the analysis of short to medium sized DNA sequences. Four enzymes and specific substrates are used, resulting in fluorescent signals whenever a nucleotide is incorporated into the complementary strand of a DNA template.

The presented data includes 30 control DNA samples and 50 DNAs of BWS patients that have been statistically evaluated by disciminant analysis. The analysis revealed that pathological and normal results can be discriminated highly significant with the pyrosequencing protocol. Compared to Southern blot analysis the Pyrosequencing™ based procedure is a fast, easy and more sensitive method for the quantification of DNA fragments. In addition to the good reproducibility of results and the short turn around time it enables the analysis of CpG sites that were difficult to examine with conventional methods until now.

W12 04

Quantification of the methylation status of the PWS/AS imprinted region using two novel methods

Dikow N.(1), Nygren A.(2), Schouten J.(2), Kraemer N.(3), Hartmann C.(1), Janssen B.(1), Zschocke J.(1)

1) Inst. f. Humangenetik, Heidelberg, Germany
2) MRC-Holland bv, Amsterdam, Netherlands
3) Univ Heidelberg, Paediatrics, Heidelberg, Germany

The majority of methods used for genomic methylation analysis allow the detection of complete absence of either methylated or non-methylated alleles but are usually unable to recognise changes in the proportion of methylated and unmethylated alleles. Identification of such changes by quantitative methylation analysis can have important clinical consequences. We report two novel methods for quantitative methylation analysis, using the chromosome 15q11-13 imprinted region as model. Absence of the non-methylated paternal allele in this region leads to Prader-Willi Syndrome (PWS) whilst absence of the methylated maternal allele results in Angelman Syndrome (AS). A proportion of AS is caused by mosaic imprinting defects which may be missed with standard methods and require quantitative analysis for their detection. We present and compare two novel methods for quantitative methylation analysis in the SNRPN gene. One method, denoted Sequence-based Quantitative Methylation Analysis (SeQMA), involves quantitative comparison of peaks generated through standard sequencing reactions after bisulfite treatment. It is simple, cost-effective and can be easily established for a large number of genes. However, our results suggest that methods based on bisulfite treatment may be intrinsically unreliable for exact quantification of methylation status. The other method is denoted Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) and avoids bisulfite treatment. It detects changes in both CpG methylation as well as copy number of up to 40 chromosomal sequences in one simple reaction. Once established in a laboratory setting, the method is more accurate, reliable and less time consuming.

W12 05

Histone acetylation dependent allelic expression imbalance of *BAPX1* in patients with the oculo-auriculo-vertebral spectrum (OAVS)

Fischer S.(1), Lüdecke H.-J.(1), Wieczorek D.(1), Böhringer S.(1), Gillessen-Kaesbach G.(1), Horsthemke B.(1)

1) Institut für Humangenetik, Essen, Germany

The oculo-auriculo-vertebral spectrum (OAVS, OMIM %164210) is a common birth defect involving first and second branchial arch derivatives. It is characterised by hemifacial microsomia, epibulbar tumours, ear malformation and vertebral anomalies. Although rare familial cases suggest that OAVS has a genetic basis, no genetic defect has been identified so far. In a patient with OAVS, multiple exostoses and a chromosomal translocation t(4;8) we have found that the translocation disrupts the multiple exostoses gene *EXT1* on chromosome 8 and the *RAB28* gene on chromosome 4. To investigate whether *RAB28* plays a role in OAVS, we sequenced this gene in 75 patients, but did not find any mutation. The *BAPX1* gene maps 76.4 kb proximal to the chromosome 4 breakpoint. As shown in mice, zebrafish and frog, the homeobox protein BAPX1 plays an important role in the development of the first and second branchial arch and other craniofacial structures. Thus, *BAPX1* is a good positional and functional candidate gene for OAVS. We screened 105 patients for *BAPX1* mutations. In 12 patients we found six different rare nucleotide variants, three with and three without an effect on the amino acid sequence. In two families the amino acid changes were also found in non-affected relatives. By quantitative primer extension analysis of fibroblast RNA we observed a strong allelic expression imbalance (sAEI) in three of six patients, but not in nine normal controls (Fisher's exact test, P = 0.044). sAEI was *de novo* in one patient and inherited in the other two. Prolonged cell culture or treatment with the histone deacetylase inhibitor Trichostatin A led to reactivation of the downregulated allele. We propose that epigenetic dysregulation of *BAPX1* plays an important role in OAVS. Such a mechanism can explain many of the genetic and phenotypic peculiarities of OAVS, e.g. the rareness of familial cases and the occurrence of discordant MZ twins.

W12 06

Identification and functional characterization of novel factors regulating cellular cholesterol metabolism

Runz H.(1), Gilbert D.(2), Wirkner U.(3), Wiemann S.(4), Zschocke J.(1), Pepperkok R.(3)

1) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany
2) Institute of Zoology, University of Heidelberg, Heidelberg, Germany
3) EMBL, Cell Biology/Biophysics Programme, Heidelberg, Germany
4) German Cancer Research Center (DKFZ), Heidelberg, Germany

Hypercholesterolemia is an important risk factor for atherosclerosis and coronary heart disease. Current pathophysiological models of hypercholesterolemia assume a tight association of environmental as well as genetic factors, many of which a yet unknown. For the identification of genes predisposing for hypercholesterolemia, we are trying to establish combined expression profiling and microscope-based functional screening approaches that will allow us to systematically identify new candidate genes which are regulated by cholesterol and fatty acids and themselves are involved in regulating cellular lipid metabolism. Central feature of our approach is the use of microscopic cDNA- and RNAi-based functional cell arrays, a high-content screening microscopy platform and automated image analysis software. Altogether, this technology allows gain-of-function and loss-of-function studies in cultured cells with a high throughput and up to a genome-wide scale. Our study aims towards a more comprehensive understanding of the molecular basis of cellular sterol regulation, with our methodology being suitable for addressing a wide range of biological and medical questions.

Poster

P001

Study of MYH gene in Polish population

Skrzypczak M.(1), Plawski A.(2), Heinritz W.(1), Slomski R.(2), Froster U.(1)
1) University of Leipzig - Medical Faculty, Institute of Human Genetics, Leipzig, Germany
2) Polish Academy of Sciences, Institute of Human Genetics, Poznan, Poland

Familial Adenomatous Polyposis (FAP), an autosomal dominantly inherited predisposition for colorectal cancer, is characterized by the occurrence of numerous polyps in the large bowel. In about 50% of all patients, FAP is caused by germline mutations in the APC gene. A variant of FAP with a milder phenotype and inherited in an autosomal recessive manner, is caused by mutations in the base excision repair gene MYH. The aim of our study was to determine the prevalence of MYH mutations in Polish FAP patients without detectable APC mutations. We have tested 96 patients from the DNA bank from the Institute of Human Genetics of the Polish Academy of Sciences in Poznañ. All the FAP patients had no mutations in the APC gene (including 12 cases with attenuated FAP). Following PCR, fragments of the MYH gene were screened for mutations using heteroduplex analysis and SSCP. Exons 7 and 13 were analyzed for two common mutations using DHPLC. According to the pre-screening results selected fragments were sequenced. Additionally, we have performed position analysis using enzyme digestion in a case with compound heterozygosity. Two of the most frequent mutations of the MYH gene (Y165C and G382D) that were found in this gene occurred in the heterozygotic system in 13% of the patients. In the examined group 9 cases with the G382C substitution, 4 cases with the Y165C and 5 cases with the V22M were recorded. In two brothers with classical FAP we detected compound heterozygosity for Y165C and a novel missense mutation (G169D). Using restriction enzymes we could provide evidence for transposition of these mutations. Biallelic MYH mutations were found to be responsible for FAP in only a small proportion of patients compare to 12% in the Germane population. This might be explained by difficult clinical selection criteria in the Polish population. With the methods used we can exclude neither complex APC or MYH mutations nor mutations in other genes possibly related to the FAP phenotype.

P002

Identification of 40 homozygous deletions in Hodgkin lymphoma cell lines using six different array-CGH platforms

Giefing M.(1), Martin-Subero J.I.(2), Nieländer I.(2), Arnemann J.(3), Hansmann M.L.(3), Küppers R.(4), Siebert R.(2)
1) University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany and, Institutes of Human Genetics, Polish Academy of Sciences, Poznan, Poland
2) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
3) Institute of Pathology, University Hospital, Frankfurt / M, Germany
4) Institute for Cell Biology (Tumor Research), University Hospital, Essen, Germany

Several Tumor Suppressor Genes (TSG) like p16 or RB1 have been identified through mapping of homozygous deletions in cancer cells. In order to identify putative TSG loci involved in the pathogenesis of cHL we performed array-CGH to six different platforms to scan for homozygous deletions in this neoplasm.
DNA from four Hodgkin lymphoma cell lines (L428, HDLM2, KMH2 and L1236) was analyzed with six array-CGH platforms with a resolution ranging from 10Mb – 30kb. Four BAC/PAC (Abbott / Vysis - GenoSensor Array 300; Spectral Genomics - Spectral Chip 2600; Integra-Gene - Integra Chip; BCCRC, Canada - tiling-path SMRT) and two oligonucleotide platforms (Agilent - Human Genome CGH 44A; Affymetrix - GeneChip 100k Set) were used. Candidate regions of homozygous losses were verified by PCR.
Altogether 40 regions of homozygous deletions were identified by at least one of the platforms and verified by PCR. The size of these regions ranged from approximately 50 to 500kb. Previously described aberrations like clonal IGH and TCR rearrangements leading to homozygous loss through locus recombination served as positive control. Only 3 of the homozygous deletions were detectable by one or more BAC/PAC platforms. In contrast the Agilent - Human Genome CGH 44A platform detected 37 / 40 homozygous deletions. Genes located in the homozygously deleted regions can be grossly ascribed to three groups: (I) potential TSG, (II) genes involved in the NFkB pathway that is known to be constitutively activated in Hodgkin lymphoma and (III) genes involved in chromatin structure and remodeling.

Supported by a Collaborative Experimental Scholarship for Central & Eastern Europe - Federation of European Biochemical Societies (FEBS) and a PhD fellowship of the President of the Polish Academy of Sciences.

P003

Problems of HNPCC out-patient management in Germany

Kunstmann E.(1), Bach K.(1), Epplen J.T.(1)
1) Human Genetics Ruhr-University, Bochum, Germany

Introduction: Patients with diagnosis of colorectal or endometrial cancer before the age of 45 are suspicious of hereditary non-polyposis colorectal cancer (HNPCC). In daily routine, the differentiation of HNPCC from sporadic cancer is difficult, although the consequences are severe.
Methods and cohort: A standardised interview was conducted with 36 gastroenterologists and 36 gynecologists to evaluate the management of HNPCC patients in private practice in Germany. Statistical analyses were done with SPSS 12.0.
Results: Care of tumour-patients is a fundamental component of the daily work for gastroenterologists and gynecologists. Yet identification of patients at high risk for HNPCC causes difficulties: 91% of the gastroenterologists and 50% of the gynecologists take care of patients at risk for HNPCC (Bethesda criterion B4; cancer before the age of 45). Testing for microsatellite instability should have been done in these patients in order to diagnose HNPCC or exclude this diagnosis. But 36% of gastroenterologists and 50% of the gynecologists did not consider diagnosis of HNPCC. Surprisingly part of the gastroenterologists working in private practice for more than 11 years, did not seem to be familiar with the clinical feature HNPCC. Surveillance recommendations have not fully been taken into account. Gastroenterologists rarely refer female patients to transvaginal ultrasound. Gynecologists normally extend surveillance recommendations (mammography 97%, tumor markers 91%), but only half of them refer patients to colonoscopy.
Conclusions: Awareness for patients fulfilling the Amsterdam or Bethesda criteria should be strengthened in gastroenterologists and gynecologists. Otherwise patients at risk and their families will not enter the recommended surveillance program. In case genetic diagnostics and counselling of the index patient is not initiated, family members do not have the opportunity of predictive testing.

P004

Recurrent chromosome rearrangements in ovarian cancer

Arnold N.(1), Weimer J.(1), Martin-Subero J.I.(2), Siebert R.(2)
1) Department of Gynecology and Obstetrics, UKSH Campus Kiel, Kiel, Germany
2) Institute of Human Genetics, UKSH Campus Kiel, Kiel, Germany

A central aim in cancer research is to identify altered genes that play a causal role in cancer development. Many such genes have been identified through the analysis of recurrent chromosomal rearrangements that are characteristic of leukemias, lymphomas, and sarcomas. In contrast to leukemias in malignat epithelial tumours disease specific aberrations make up less than 5% of the cases as reported in the Mitelman database. Up to now, technical deficiencies prevent the detection of recurrent aberrations as in solid tumours the morphology of the chromosomes are mostly poor. New methods in the field of cancer research promise a solution to the problem as exemplified in recent reports. Here we report the application of a novel strategy for the reliable identification of recurrent chromosomal aberrations in solid tumours. We employed FISH-MD in combination with array hybridization to identify chromosomal breakpoints in ovarian cancer cells. These breakpoints were subsequently detected in touch preparation and paraffin embedded tissues of primary tumours by four-colour Interphase-FISH. With this strategy we characterized two recurrent marker chromosomes in a primary cell culture derived from an ovarian cancer. One marker showed breakpoints in chromosome bands 5q14, 12q22, 17q21.2 and 20q11.1, the other in 11q13 and 19p13.2. Based on the array results, breakpoint flanking BACs were identified and used for Interphase-FISH experiments. Interphase-FISH provided evidence for the second marker being present in 73% of the original cell line, which is comparable to its occurrence in the analyzed metaphases (60%). Remarkably, our FISH-assay indicates the presence of a similar marker in 60% of cells of another ovarian cancer sample suggesting the aberration to be recurrent. Fine mapping of the breakpoints is underway to disclose potential

cancer-associated genes involved in the rearrangement.

P005

Cryptic rearrangements are detectable in ~40% of AML-cases with normal routine banding karyotype – a molecular cytogenetic pilot study on 40 cases

Gross M.(1), Mkrtchyan H.(2), Glaser M.(1), Karst C.(1), Claussen U.(1), Wedding U.(3), Höffken K.(3), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Department of Genetics and Laboratory of Cytogenetics, State University, Yerewan, Armenia
3) Klinik und Poiklinik für Innere Medizin II, Jena, Germany

Cytogenetic studies of acute leukemia revealed that the majority of patients display acquired chromosome aberrations. However, in 40-50% of acute myeloid leukemia (AML) cases specific chromosomal rearrangements cannot be detected after routine banding cytogenetics. Even though the introduction of molecular cytogenetics in the last decades facilitated routine tumor cytogenetic analysis, high mitotic index and chromosomes of good quality have still importance, especially in the initial chromosome analysis. Nonetheless, the characterization of cryptic rearrangements still remains problematic. Thus, FISH-banding techniques like the multitude multicolor-banding (mMCB, Weise et al., 2003, Cytogenet Genome Res) were developed to overcome these difficulties. mMCB studies were performed on 30 of in summary 40 studied, according to routine cytogenetics, karyotypically normal AML cases. In one of the studied cases a cryptic deletion in 5qter was detected. 22 of the 40 cases were further studied by subtelomere- and subcentromeric probes (subCTM-FISH, Gross et al., 2006, Cytogenet Genome Res); subtelomeric deletions in 9qter, 11pter, 12pter and 13qter were found in 11 cases. Additionally, 25 of the in summary 40 studied cases were analyzed by microdissection based comparative genomic hybridization (micro CGH) using the protocol described in Karst et al. (2005, Int J Onc). Applying this approach, cryptic copy number alterations were detectable in 5 cases. Microclones with monosomie 7 and trisomy 7, 17 and 18 were detected by that approach and confirmed by interphase FISH. In summary, this pilot study shows that cryptic chromosomal aberrations can be detected in ~30% of the in vitro proliferating and in ~10% of the in vitro non-proliferating cell fraction of according to banding cytogenetics normal AML-cases.

Supported by Deutsche Krebshilfe (70-3125-Li1), DFG (436 ARM 17/2/04), UICC (ICR/05/030) and IZKF/TMWFK (TP 3.7 / B307-04004).

P006

Multicolor-FISH applied to resolve complex chromosomal changes in a case of T-ALL (FAB L2)

Pittasch K.(1), Mkrtchyan H.(2), Glaser M.(1), Gross M.(1), Wedding U.(3), Höffken K.(3), Karst C.(1), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Department of Genetic and Laboratory of Cytogenetics, State University, Jerewan, Armenia
3) Klinikum für Innere Medizin II, Jena, Germany

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease that has its origin in precursor cells of the B or T lineage, or even in more primitive cells with multilineage potential. Like other malignancies, ALL is suggested to develop by a stepwise accumulation of stable genetic abnormalities, involving oncogenes and tumorsuppressor genes, leading in the end to an uncontrolled cellular growth and arrested differentiation. Even though there has been a rapid increase in knowledge of T-ALL pathogenesis in recent years, the characterization of acquired cytogenetic alterations in aberrant leukemia is still of high scientific impact. Here we present the results of the molecular cytogenetic characterization of a complex aberrant T-ALL (FAB L2) karyotype, which appeared in an only 18 year old patient. The aberrant karyotype 45,XY,-4,-9,-11,-17,-18,+mar1-5[cp8]/46,XY[30] was detected in bone marrow cells of the patient suffering from T-ALL since two years. After application of spectral karyotyping (SKY) the karyotype could be described as 45,XY,der(5)t(5;9),-9,del(11)(q14),del(14)(q12)[4]/46,XY,der(5)t(5;18),del(4)(p13~14),-9,del(11)(q14),del(14)(q12),der(18)t(18;5;9)[3]/46,XY[14]. MCB using chromosome specific probes for #5, #9 and #18, respectively, revealed the presence of three different malignant clones. All three clones had one normal #5. Clone 1 had a derivative chromosome 5 der(5)t(9;5;9), clone 2 one derivative of chromosome 5 der(5)t(18;5) and one of chromosome 18 der(18)t(18;5;9), and clone 3 had a chromosome 5 with deletion of the subtelomeric region in 5q – this result was conformed by subtelomeric probe for 5qter. Clone 1, 2 and 3 were present in 17%, 6% and 6%, respectively. The chromosomal changes detected in the present case will compared with previous reports from the literature.

Supported by Deutsche Krebshilfe (70-3125-Li1), INTAS (AISbl 03-51-4060), IZKF / TMWFK (TP 3.7 / B307-04004), DFG (436 ARM 17/2/04) and UICC (ICR/05/030).

P007

Identification of putative telomerase suppressor gene loci on chromosome 4 involved in cervical carcinogenesis

Backsch C.(1), Frank I.(2), Rudolph B.(1), Motzke K.(1), Dürst M.(1)
1) Klinik für Frauenheilkunde und Geburtshilfe der Friedrich-Schiller-Universität, 'Gynäkologische Molekularbiologie', Jena, Germany
2) Institut für Virologie des Universitätsklinikums, Essen, Germany

The importance of telomerase activation in human papillomavirus (HPV) - mediated carcinogenesis is underlined by the observation that hTERT mRNA expression is increased and telomerase activity is detectable in the majority of cervical carcinomas and in a subset of high grade cervical intraepithelial neoplasias (CINs). Previously we have provided evidence for the localization of a telomerase suppressor gene on chromosome 4. The aim of our study is to determine a region on chromosome 4 which is associated with telomerase suppression.
Microcell-mediated chromosome transfer was performed to introduce a single copy of the entire human chromosome 4 and different derivative chromosome 4 into HPK II cells. Cell lysates were prepared directly from individual hybrid cell colonies (100-300 cells). Telomerase activity was determined using a telomerase PCR ELISA kit. Genomic DNA from all donor cell lines was used for microsatellite PCR. A total of 51 polymorphic markers located on chromosome 4 were analysed.
Strong suppression of telomerase activity was only found in a subset of HPK II hybrids in which chromosome 4 or three of eight der(4) chromosomes had been introduced. By microsatellite analysis we could not define a deleted region on chromosome 4 which correlates exclusively with telomerase suppression. However, all hybrids retained 4q13, 4q25, 4q26 and 4q31.1-q31.2. Functional inactivation of telomerase associated genes in these regions by methylation or microdeletion may be a possibility.
In ongoing studies, we will compare the expression profiles of the genes in these particular regions in telomerase positive and negative precancers and cervical carcinomas. By this approach, we expect to identify a small number of genes that then can be assessed in functional studies.

P008

Application of fluorescence in-situ hybridisation (24-color-fish) for testing mutagenicity of ethylmethanesulphonate in preneoplastic human colon cells using the oecd guideline 473

Knoll N.(1), Weise A.(2), Liehr T.(2), Pool-Zobel B.L.(1), Glei M.(2), Marian B.(3), Claussen U.(2)
1) Department of Nutritional Toxicology, Institute for Nutritional Sciences, Jena, Germany
2) Institut für Humangenetik und Anthropologie, Jena, Germany
3) Institute for Cancer Research, University of Vienna, Vienna, Austria

Genetic toxicology assays include well established test systems like the Comet Assay and modifications thereof with FISH. This method can be used to investigate the genotoxic potentials of compounds in mammalian cells and can detect global and gene-specific DNA damage, respectively. Latest developments are to assess numeric and structural chromosomal aberrations using the 24-color-FISH technique in human colon adenoma cells (LT97) treated with food contaminants. For this a new protocol, based on the OECD guideline for Testing of Chemicals 473, was adapted to the LT97 adenoma cells. Ethylmethanesulphonate (EMS), a monofunctional alkylating agent with carcinogenic and mutagenic potential, was used as positive control without metabolic activation. EMS induces micronuclei, sister chromatid exchanges and specific DNA modifications (alkylation) resulting in random point mutations leading to base transi-

tions. In this study we determined the mutagen-induced chromosomal changes in detail to confirm its eligibility in the modified mutagenicity test and compared the specific clastogenic effects to other compounds that are of interest in toxicology. For this, LT97 cells were exposed to EMS for 6 hours and maintained in plain culture medium for an additional cell cycle. Metaphase spreads were prepared and hybridised using combinational labelling for unambiguous identification of all 24 chromosomes. Evaluation of 50 metaphases revealed that about 80% were aberrant. Alterations like deletions, translocations, isochromosomes and marker chromosomes were observed. Altogether, EMS induced 2.9 breaks per aberrant cell particularly addressed to specific chromosomes. So we point out that, although EMS is known to induce random point mutations on the level of DNA bases, it causes numerous structural chromosomal aberrations in preneoplastic human colon cells proving its suitability as control in the 24-color-FISH technique to detect structural chromosomal damage. Thanks to BLE for support.

P009

ArrayCGH as a useful tool to identify distinct mechanisms of clonal development in relapsed childhood acute lymphoblastic leukemia

Kirschner-Schwabe R.(1), Matthäi A.(2), Steinhoff C.(3), Eckert C.(1), Schröck E.(2), Vingron M.(3), Henze G.(1), Seeger K.(1), Hagemeier C.(4)

1) Klinik für Pädiatrie m. S. Onkologie/Hämatologie, Universitätsmedizin Charité, Berlin, Germany

2) Institut für Klinische Genetik, TU Dresden, Dresden, Germany

3) MPI für Molekulare Genetik, Abt. Vingron, Berlin, Germany

4) Klinik für Allgemeine Pädiatrie, Universitätsmedizin Charité, Berlin, Germany

Acute lymphoblastic leukemia (ALL) is the most common childhood tumor from which approximately 25% of patients suffer a relapse. Relapse has been assumed to represent a re-emergence the initial leukemic clone, which escaped or survived from initial therapy. This is supported by studies of immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements highly specific for a distinct lymphoid cell and its progeny. In most cases, initial and relapse leukemic clones share common Ig and TCR markers. However, particularly in TEL-AML1 positive leukemia, Ig and TCR markers can be distinct between disease stages. Typically, these relapses occur late after cessation of treatment and exhibit a good therapy response comparable to the initial leukemia. These notions led to the hypothesis that a late relapse derives from a putative persisting pre-leukemic clone by a second independent transformation event. To challenge this hypothesis we systematically analyzed by array-CGH 10 patients with late relapse of ALL including 4 TEL-AML1 positive patients, one MLL-ENL positive case and 5 patients lacking characteristic leukemic fusion genes. To back trace potential clonal events we included samples from initial diagnosis, first relapse and, for 9 cases, from second relapse. Analysis of one TEL-AML1 positive patient presenting with distinct Ig and TCR markers revealed a higher number of aberrations in the initial versus relapsed stage of the disease. Importantly, aberrations were distinct except for a 12q deletion, supporting the existence of a pre-leukemic clone as a common ancestor for distinct reactivating events. In contrast, aberrations in the MLL-ENL positive patient were additive over the course of relapses, indicating a linear progression of a recurrent initial leukemic clone as an alternative mechanism of relapse. ArrayCGH should prove useful in further characterizing clonal events in leukemic disease leading to a better understanding of the pathogenesis of relapsed leukemias.

P010

Alterations of tumor suppressor genes on chromosome 9 in pleomorphic xanthoastrocytomas as revealed by array-based comparative genomic hybridization (CGH) and candidate gene analysis

Hoischen A.(1), Zipper P.(2), Kaulich K.(2), Blaschke B.(2), Radlwimmer B.(3), Becker A.J.(4), Wiestler O.D.(3), Lichter P.(3), Reifenberger G.(2), Weber R.G.(1)

1) Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn, Germany

2) Department of Neuropathology, Heinrich-Heine-University, Duesseldorf, Germany

3) German Cancer Research Center, Heidelberg, Germany

4) Department of Neuropathology, Rheinische Friedrich-Wilhelms-University, Bonn, Germany

Pleomorphic xanthoastrocytomas (PXAs) are rare astrocytic neoplasms that mainly affect children and young adults. They correspond histologically to WHO grade II. PXAs usually show circumscribed growth and favourable prognosis despite exhibiting a high degree of cellular pleomorphism.

By chromosomal CGH analysis, we previously showed that chromosome 9 loss is the hallmark imbalance present in 25 of 50 (50%) tumors. To identify the tumor-suppressor-genes located on chromosome 9 associated with PXA-tumorigenesis, we now performed high resolution array-based CGH and molecular genetic studies. Ten PXAs with available high molecular weight DNA were investigated by array-based CGH on a genome wide 8k-array. An array-CGH pattern consistent with a loss of one chromosome 9 and a homozygous deletion involving the *CDKN2A/p14^{ARF}* and *CDKN2B* loci at 9p21.3 was detected in five tumors. These data were confirmed by interphase fluorescence in situ hybridization (FISH) on tumor sections. Two PXAs were found to have no chromosome 9 loss but a deletion of a clone containing *CDKN2A/p14^{ARF}* and *CDKN2B* by array-CGH. In one of these cases, homozygous deletion of this clone was shown by interphase FISH on tissue sections. Altogether, a homozygous deletion involving the CDKN2A/p14^{ARF} and CDKN2B loci was detected in at least 6 of 10 PXAs. Mutational analysis of two candidate tumor suppressor genes on 9q, *PTCH* (9q22.3) and *TSC1* (9q34), revealed no mutations in PXAs with 9q loss. However, PXAs consistently showed low expression of *TSC1* mRNA but no evidence of *TSC1* promoter methylation. Taken together, our study identified homozygous deletions involving the *CDKN2A/p14^{ARF}* and *CDKN2B* loci in a substantial proportion of PXAs and suggests a possible role of low *TSC1* expression in these tumors.

P011

Comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analyses of pediatric and adult germ cell tumors

Zahn S.(1), Sievers S.(2), Alemazkour K.(1), Göbel U.(1), Schneider D.T.(1)

1) Clinic of Pediatric Oncology, Hematology and Immunology, Heinrich-Heine-University, Duesseldorf, Germany

2) Max Planck Institute of Molecular Physiology, Dortmund, Germany

Germ cell tumors (GCTs) constitute a heterogeneous group of tumors that significantly varies with regard to clinical presentation and histology. In childhood, the majority of GCTs develop at extragonadal sites, and show teratoma and yolk sac tumor (YST) histology, whereas gonadal tumors predominate after the onset of puberty and are divided into seminomas and nonseminomas. Cystic ovarian teratomas and spermatocytic seminomas are considered distinct entities. In order to define recurrent chromosomal imbalances and to correlate the genomic patterns with clinical presentation and histopathologic differentiation, we examined 65 GCTs with CGH (20 teratomas, 19 YSTs, 7 seminomas, 19 non-seminomas). Since loss of distal 1p represents one of the most frequent aberrations in embryonal tumors of childhood, CGH analysis was supplemented with LOH analysis using 23 microsatellite markers at 1p13-p36.

Virtually all teratomas show a balanced karyotype on CGH analysis. In malignant GCTs, CGH profiles distinguish different biologic subgroups. Childhood YSTs are characterized by imbalances of chromosome 1 (-1p, +1q), loss of 6q and gain of 20q. In contrast, gain of 12p constitutes the most frequent imbalance found in adolescence GCTs. LOH analysis detects allelic loss at 1p with a commonly deleted region from 1p35-1pter in most childhood YSTs (89%), and less frequently in adolescence GCTs (13%).

Genetic analysis of pediatric GCTs allows for the differentiation of distinct biologic subgroups that correlate with general epidemiological patterns. In accordance with our previous CGH studies, we observed a high frequency of LOH at distal 1p in malignant childhood GCTs. Thus, imbalances at this chromosome are related to true allelic loss. These changes resemble those found in other embryonal tumors of childhood, in which loss at 1p portends a poor prognosis. Therefore, a prospective study is warranted in order to evaluate the potential prognostic impact of LOH 1p in pediatric GCTs.

P012

Immunohistochemical and genetic analysis of localized extranodal and nodal dendritic cell tumours and implications for therapy

Muradyan A.(1), Gruber-Moesenbacher U.(2), Leslie K.(3), Halbwedl I.(4), Langner C.(4), Beham-Schmid C.(4), Popper H.H.(4), Ullmann R.(1)

1) Max Planck Institute for Molecular Genetics, Human Molecular Genetics, Berlin, Germany

2) Institute of Pathology, Feldkirch, Austria

3) Institute of Pathology, Mayo Clinics, Scottsdale, United States of America

4) Institute of Pathology, Graz, Austria

Dendritic cell tumors of the antigen presenting system can develop as interdigitating and follicular variants. Given their extremely rare occur-

rence, diagnosis as well as prediction of biological behaviour still poses major problems. Eight non-metastatic dendritic cell tumors, two follicular (FDCT) and six interdigitating (IDCT) variants, among them the first case reported in the lung, were analysed by histology and immunohistochemistry using a panel of specific markers. Six of these patients, together with one recurrence, were additionally investigated by chromosomal- and array comparative genomic hybridisation (CGH) using a 36k whole genome tiling path Bacterial Artificial Chromosome DNA array. Both chromosomal and array CGH revealed a heterogeneous pattern of chromosomal aberrations, with partial loss of 8p in 4/6 and 11q in 3/6 as well as a partial gain of 17q in 3/6 as the only overlap. Particularly those very small high copy amplifications, which escaped detection by chromosomal CGH, highlighted possible candidate genes for improved diagnosis and defect-targeted therapies. For example, her2/neu was found to be amplified at the DNA level and over-expressed at the protein level, making these patients likely candidates for successful therapy by Herceptin. This study demonstrates the potential of high-resolution array CGH to contribute to the advancement of individualized therapies for rare tumors where established therapeutic schemes do not yet exist.

P013

Endostatin phenylalanines 31 and 34 define a receptor binding site

Stahl S.(1), Gätzner S.(1), Müller T.D.(2), Felbor U.(1)
1) Department of Human Genetics, Würzburg, Germany
2) Department of Physiological Chemistry II, Würzburg, Germany

Endostatin has achieved much attention as a naturally occurring inhibitor of angiogenesis and tumour growth. Endostatin is derived from collagen XVIII's C-terminal domain and deleted or truncated in most patients suffering from Knobloch syndrome blindness. A delayed regression of the hyaloid vascular system which supplies the embryonal lense and vitreous appears to play a major role in the pathogenesis of the congenital myopia.
To evaluate the functional significance of two surface-exposed hydrophobic phenylalanines at positions 31 and 34 of endostatin, two arginines essential for endostatin's heparin binding, R27 and R139, and two human sequence alterations within endostatin, A48T and D104N, we applied the alkaline phosphatase fusion protein method. Replacement of F31 and F34 with alanines led to complete loss of characteristic in situ binding while heparin binding remained intact. In contrast, the non-heparin binding alkaline phosphatase-tagged human endostatin lacking R27 and R139 bound to specific tissue structures such as the tunica vasculosa lentis vessels. The two Knobloch syndrome-associated endostatin sequence variants did not result in altered in situ binding to murine embryonal tissues, human endothelial cells, heparin and immobilized laminin. However, expression of the endostatin mutant A48T was significantly reduced. This observation might be explained by a lower folding efficiency due to the structural constraints of A48 residing in the hydrophobic core.
Our data suggest that residues F31 and F34 form a putative receptor binding site acting independently from heparan sulfate binding and that the A48T mutation destabilizes the endostatin molecule.

P014

First in vivo analysis of the 12q13-15 amplicon in a glioblastoma and its recurrencies

Fischer U.(1), Deutscher S.(1), Meese E.(1)
1) Humangenetik, Homburg, Germany

DNA amplification occurs in multidrug resistant cells and in tumor cells but not in normal human cells. Numerous reports describe gene amplifications in human glioma by cytogenetic and molecular genetic means. However, the mechanisms underlying the development of amplicons are still unknown. Most studies on the development and alterations of an amplification unit were done predominantly with cell systems under selective pressure of drugs driving the amplification of genes that were responsible for a specific drug resistance. A shortening of an initially large amplification unit to a smaller amplification unit including the target drug resistance confering gene was observed under in vitro conditions. However, most of the amplification units contain no known drug resistance confering genes and the mechanisms driving the amplification of these "target genes" remains unclear. Here we have for the first time investigated the development of an amplification unit under in vivo conditions. Our results revealed no shortening of the amplification unit to a smaller region containing a single or only a few genes in a glioblastoma and its recurrencies. We detected a large amplification unit that was stable in size over many years. These results strongly indicate that the driving mechanisms under in vivo conditions select for a variety of genes with many of them contributing to an advantage for the cells. In addition our results indicate that the amplification prozess is dynamic and the amplification level for specific genes within this amplification unit can vary to adopt to the permanantly changing conditions in the tumor environment.

P015

EZH2 expression in human prostate cancer

Bachmann N.(1), Hoegel J.(1), Haeusler J.(1), Perner S.(2), Kuefer R.(3), Vogel W.(1), Maier C.(1)
1) University of Ulm, Department of Human Genetics, Ulm, Germany
2) University of Ulm, Department of Pathology, Ulm, Germany
3) University of Ulm, Department of Urology, Ulm, Germany

EZH2 (enhancer of zeste homolog 2), a polycomb group transcriptional repressor, is considered as a potential candidate gene for the development of aggressive prostate cancer for two reasons. First, because of its location at 7q35. Genetic alterations in this region have been implicated in the prostatic tumor progression. Second, because of its increased expression in metastatic prostate cancers.
In our previous work we have genotyped eleven variants within the EZH2 gene in genomic samples of prostate cancer probands and controls. We have examined the variants and the resulting haplotypes for association with prostate cancer. Our results suggested that genetic variations of the EZH2 gene are not responsible for the linkage of 7q to aggressive prostate cancer. We suppose that the "wild-type" per se is the most aggressive allele of EZH2.
It was shown mainly by gene expression profiling and immunohistochemical analyses that EZH2 is overexpressed in prostate cancer, particularly in metastases. We examined, therefore, the EZH2 expression in primary prostate tumors and included CAV-1, an adjacent tumor suppressor gene. We quantified the mRNA of EZH2 and CAV-1 in 35 matched samples of prostate tumor tissue and tumor-free tissue via Real-time-PCR after reverse transcription. We detected an elevated expression level of EZH2 and a decreased expression level of Caveolin-1 in almost all tumors.
Furthermore, we analyzed the methylation status of the EZH2 and CAV-1 promoters via bisulfite sequencing in order to assess if this epigenetic mechanism is responsible for the altered gene expression. We showed that down-regulation of CAV-1 is mediated by promoter hypermethylation in these carcinoma specimens. We also observed changes in the methylation profile of EZH2.
In addition, we are currently looking for amplifications/deletions by determining the genomic copy number of both genes via Real-time-PCR to get more insights in the regulatory mechanisms of the altered gene expression.

P016

Partial uniparental disomy is recurrent in mantle cell lymphoma and affects chromosomal regions supposed to harbor tumor supressor genes

Nieländer I.(1), Martin-Subero J.I.(1), Wagner F.(2), Martinez-Climent J.A.(3), Siebert R.(1)
1) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
2) German Resource Center for Genome Research (RZPD), Berlin, Germany
3) Center for Applied Medical Research CIMA, University of Navarra, Pamplona, Spain

Recent studies have highlighted partial uniparental disomy (pUPD) as a frequent mechanism of tumor suppressor gene inactivation in acute leukemias and solid tumors. By loss of (part of) one parental chromosome and gain of the homologue from the other parent, pUPD results in loss of heterozygosity (LOH) without chromosomal deletion. As the gene dosage is not altered, pUPD cannot be detected by conventional cytogenetics, FISH or arrayCGH. So far, partial UPD has not been extensively studied in lymphomas. Thus, we have performed genome-wide analyses of single nucleotide polymorphisms (SNPs) using a 100K SNP-array (GeneChip® Human Mapping 100K Set, Affymetrix; Santa Clara, CA) in five t(11;14)-positive mantle cell lymphoma (MCL) cell lines, which have been extensively characterized by CGH to BAC/PAC arrays. Copy number and LOH analyses were performed using the Chromosome Copy Number Analysis Tool (Affymetrix) applying a 0.5Mb genome smoothing filter. Partial UPD was defined as a region spanning at least 50 SNPs with homozygous allele calls and a –log10 (p-value) for LOH above 15 in the absence of a deletion. A median number of 8 (range= 5 - 20) pUPDs were identified in the samples indicating that pUPD is a common phenomenon in MCL cell lines. Most interestingly,

the regions of pUPD detected in the MCL cell lines affected regions of recurrent chromosomal deletions described in recently published array-CGH studies of MCL like 1p, 8p, 9q, 11q and 17p.
In summary, our findings show pUDP to be recurrent in mantle cell lymphoma and to affect regions commonly targeted by deletions. Thus, pUPD has to be considered as an alternative mechanism of tumor suppressor gene inactivation in lymphomas.
Acknowledgements: This study was supported by the Lymphoma Research Foundation (NY).

P017

Identification of novel germline MUTYH mutations in patients with familial adenomatous polyposis
Laner A.(1), Grabowski M.(2), Holinski-Feder E.(1)
1) MGZ - Medizinisch Genetisches Zentrum, München, Germany
2) Ludwig-Maximilians-Universität München, Abteilung Medizinische Genetik, München, Germany
Familial adenomatous polyposis (FAP) is a premalignant disease characterized by numerous adenomatous polyps of the colon and rectum. One or more of these polyps progress through dysplasia to malignancy, leading to cancer at any age from late childhood through the seventh decade. Germinal mutations in the base excision repair (BER) gene MUTYH have been recently described in association with predisposition to FAP. In contrast to the classical dominant condition of FAP due to germinal mutations in the tumor suppressor gene APC (adenomatous polyposis coli), the MUTYH polyposis is an autosomal recessive disease. Here we report screening for germinal mutations in the MUTYH gene by direct sequencing and MLPA deletion screening of exons 2 and 16 in 144 individuals with multiple colorectal adenomas or classical FAP, in whom no mutation in the APC gene had been identified. In total 20 biallelic and 4 monoallelic germline MUTYH mutations have been detected in this group. Furthermore, we report the identification of six novel MUTYH mutations (c. 601G>A, p.V201M; c.605G>A, p.G202E; c.842C>T, p.P281L; c.1034C>T, p.P345L; c.1092delA fs E364>394X and c.1172C>T, p.P391L) which, to our knowledge, have not been previously described in the literature. All missense mutations mentioned above correspond to conserved amino acids located in predicted functional domains (V201 and G202 in the Helix-hairpin-Helix motiv; P281 in the Endo III Fe-S binding domain; P345 in a PCK-phosphorylation site and P391 in the NUDIX hydrolase domain) of the MUTYH gene. Interestingly, the two patients with the mutations c.692G>A p.G382D / c1103delC fs 379>405X and c.692G>A p.G382D / c.1034C>T p.P345L were initially referred to HNPCC testing as they both had several adenomas at the age of 56 / 47 years with a possible family history for colorectal cancer. We now routinely screen all patients with a clear FAP but without a detectable APC mutation for mutations in the MUTYH gene by complete sequencing.

P018

For-client letters in routine genetic counselling for suspected hereditary cancer
Schäfer D.(1), Kettner M.(2), König R.(1)
1) Institut für Humangenetik, Frankfurt am Main, Germany
2) Private Universität Witten/Herdecke, Studium fundamentale, Witten/Herdecke, Germany
In genetic counselling, for-client letters are supposed to meet several important claims. In particular, they should record the actual state of knowledge and its individualized interpretation, facilitate clients' deliberations beyond the temporal and other constraints of the counselling session and help clients to arrive at valid decisions concerning health-related problems with a genetic component. However, genetic counselling concerning hereditary cancer gets more and more cognitively complex, fragmented and scattered over time.
Therefore, supporting the dialogical phase by communication media like for-client letters becomes increasingly important. In a former study (70 families with breast/ovarian cancer, HBOC, 30 with cancer of the colon, CC, randomized, double blind) we had compared a standard letter (SL) with letters (EL), which had been enriched systematically, based on a tape-recording of the counselling session. We could prove that clients, who received EL, had a significantly better and temporarily sustained knowledge and comprehension of relevant facts.
However, in routine genetic counselling a simple and timesaving procedure for employing EL is mandatory. Therefore, we simplified the process of writing EL and tested its usefulness in another 90 families with suspected hereditary cancer (60 HBOC and 20 CC). An established EL was modified according to notes made by the counsellor during the counselling session, avoiding any tape recording (simplified EL = SEL). SL and SEL were given to the clients and compared in the same way as in our former study. Our preliminary analysis revealed that clients receiving a SEL had a significantly better knowledge and comprehension of relevant facts. This effect was comparable to the effect of EL in our former study. We conclude that it is feasible to write communicatively enriched for-client letters in routine genetic counselling for suspected hereditary cancer.

Supported in part by BMBFFKZ01KU9904

P019

Assessment of differentiation and progression of benign and malignant hepatic tumors using array based comparative genomic hybridization
Skawran B.(1), Steinemann D.(1), Becker T.(2), Tauscher M.(1), Weigmann A.(1), Wingen L.(1), Tauscher S.(1), Hinrichsen T.(1), Hertz S.(1), Flemming P.(3), Flik J.(4), Wiese B.(5), Kreipe H.(3), Lichter P.(6), Schlegelberger B.(1), Wilkens L.(1)
1) Institute of Cell and Molecular Pathology, Hannover, Germany
2) Department of Visceral and Transplantation Surgery, Hannover, Germany
3) Institute of Pathology, Hannover, Germany
4) Institute of Virology, Hannover, Germany
5) Institute of Biometry, Hannover, Germany
6) Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany
With a total of more than 250.000 deaths per year worldwide, hepatocellular carcinoma (HCC) is one of the most significant tumors affecting the human population today. Array-CGH was performed on 24 cases of HCC (hepatocellular carcinoma) and 10 cases of HCA (hepatocellular adenoma). Copy number changes on the genomic scale were found by array-CGH in all cases. In HCC, gains of chromosomal regions 1q (91,6%), and 8q (58,3%), and losses of 8p (54%) were found most frequently. Based on these array-CGH results, hierarchical cluster analysis branched all HCA from HCC. However, in two adenomas with a known history of glykogenosis type I and adenomatosis hepatis gains of 1q were found, too. The critically gained region was narrowed down to bands 1q22-23.1. Whereas no significant differences in the mean number of chromosomal aberrations were seen between adenomas and well differentiated carcinomas (2.7 vs 4.6), a significant increase accompanied the dedifferentiation of HCC (14.1 in HCC-G2 and 16.3 in HCC-G3; $p<0.02$). Dedifferentiation of HCC was also closely correlated to losses of 4q and 13q ($p<0.001$/$p<0.005$, respectively). Thus losses of 4q and 13q seem to occur later during tumor progression. To get more insight into the development of HCC we performed gene expression profiling in parallel (Stanford Functional Genomics Facility, Stanford, CA, USA). Remarkably, when looking at gene sets regulated in a concerted manner we identified the genes mapping to cytoband 1q22 as discriminating between HCA and HCC. In conclusion, gains of the region 1q22 seem to be important for the initiation of HCC development, whereas the increased chromosomal instability during dedifferentiation of HCC leads to an accumulation of additional chromosomal aberrations.

P020

Association of CDKN2A alterations and MC1R variants with B-RAF and N-RAS mutations in melanoma
Thirumaran R.(1), Ugurel S.(1), Sandra Bloethner S.(1), Gast A.(1), Mueller-Berghaus J.(1), Hemminki K.(1), Schadendorf D.(1), Kumar R.(1)
1) German Cancer Research Center, Heidelberg, Germany
Aberrations in B-RAF, N-RAS and CDKN2A genes constitute major genetic alterations detected in cutaneous malignant melanoma. We determined mutations and homozygous deletions in the CDKN2A gene and mutations in the B-RAF and N-RAS genes in 148 melanoma cell lines from 120 individuals. In addition we also determined polymorphisms in the entire MC1R gene. Our results showed 83 (56%) cell lines from 61 (51%) individuals carried mutations in the B-RAF. Another 31 (21%) cell lines from 27 (23%) individuals carried N-RAS mutations. Homozygous deletions at the CDKN2A locus was found in 54 (37%) cell lines from 42 (35%) individuals and mutations in the gene were detected in 27 (18%) cell lines from 21 (18%) individuals. One or more single nucleotide polymorphisms were detected in MC1R gene with 81 (70%) individuals with variant allele frequencies (for 9 polymorphisms) ranged between 0.01 and 0.12. Our results showed that mutations in B-

RAF/ N-RAS genes were in a direct association with the CDKN2A mutations and homozygous deletion (OR 6.7 95%CI 2.6-17.2; P<0.0001); and in an inverse association with total MC1R polymorphisms (OR 0.11 95%CI 0.03-0.50; P 0.001). In a Kaplan-Meier survival model cases with any MC1R polymorphism showed better survival (median 44 versus 26 months) in a non-significant association (Log rank test P value 0.15).

P021

Translocations affecting the BCL3 locus in 19q13 are present in a wide spectrum of human lymphomas

Martin-Subero J.I.(1), Wlodarska I.(2), Bastard C.(3), Callet-Bauchu E.(4), Harder L.(1), Klapper W.(5), Siebert R.(1)
1) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
2) Center for Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium
3) Laboratoire de genetique oncologique, centre Henri-Becquerel, Rouen, France
4) Service d'Hématologie Biologique, Centre Hospitalier Lyon Sud, Lyon, France
5) Institute of Hematopathology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

The translocation t(14;19)(q32;q13) is a recurrent chromosomal aberration in B-cell chronic lymphatic leukemia (B-CLL). This translocation leads to deregulation of the BCL3 oncogene in 19q13 through its juxtaposition to the immunoglobulin heavy chain (IGH) locus in 14q32. We have studied B-cell and T-cell lymphomas with breakpoints in 19q13 as well as classical Hodgkin lymphomas for rearrangements in the BCL3 locus by FISH using a novel self-developed BCL3 break-apart probe.

Among 50 B-cell lymphomas with a t(14;19)(q32;q13) or variants, we detected 43 cases with IGH/BCL3 fusion, two with IGL/BCL3, and one with IGK/BCL3. Interestingly, these cases not only included B-CLL, but also other B-cell lymphomas like marginal zone lymphomas and diffuse large B-cell lymphomas. Moreover, we studied three peripheral T-cell lymphomas with cytogenetically-proven t(14;19)(q11;q13). In all three cases, a chromosomal break affecting the BCL3 locus and colocalization to TCRAD in 14q11 was confirmed. These findings suggest TCRAD-driven BCL3 activation as possible novel oncogenic mechanism in T-cell neoplasms. Finally, two out of 20 classical Hodgkin lymphomas showed signal patterns indicating BCL3 breakpoints. In one of them the partner gene was identified as IGH whereas the partner remains unknown in the other.

Our results show that the spectrum of lymphatic neoplasias with BCL3 rearrangements goes far beyond B-CLL. To our knowledge, this is the first time that BCL3 breaks are described in T-cell lymphomas and classical Hodgkin lymphomas. The relevance of this finding is strengthened by recent reports pointing to an important role of the BCL3 oncoprotein in these two tumor entities.

This work was supported from grants of the Deutsche Krebshilfe (70-3173-Tr3) and the Fund for Scientific Research of Flanders (FWO-Vlaanderen; Grant no. G.0338.01).

P022

Comprehensive cytogenetic characterization of an esthesioneuroblastoma

Holland H.(1), Koschny R.(2), Krupp W.(3), Meixensberger J.(3), Ahnert P.(1)
1) Biotechnical-Biomedical Centre (BBZ), Faculty of Medicine, University of Leipzig, Leipzig, Germany
2) Div. of Apoptosis Regulation, German Cancer Research Center (DKFZ), Heidelberg; Dept. of Internal Medicine, University of Heidelberg, Heidelberg, Germany
3) Clinic of Neurosurgery, University of Leipzig, Leipzig, Germany

Esthesioneuroblastoma is a malignant neuroectodermal tumour originating from olfactory epithelial cells in the nasal vault. Due to the rarity of this tumour entity cytogenetic data are very limited. Therefore, we performed comprehensive cytogenetic analysis using classical karyotyping by standard trypsin-Giemsa staining (GTG-banding), multicolour fluorescence in situ hybridization (M-FISH) and interphase-FISH (I-FISH) of an esthesioneuroblastoma obtained from the Clinic of Neurosurgery at the University of Leipzig.

Primary esthesioneuroblastoma cells were isolated from surgical specimens. For the karyotyping study we analysed 20 metaphases of the primary cell culture using GTG-banding and 15 metaphases using M-FISH. For each I-FISH probe [LSI N-MYC (2p24.1)/CEP X; LSI C-MYC (8q24.12-q24.13)/CEP 8] 200 interphase cells were analysed.

Using GTG-banding we found 12 structural and 14 numerical aberrations among 20 analysed metaphases. Most of the 12 identified structural aberrations were localized on chromosome arms 1p, 1q, 2p, 3q, 6q, and 17q. Numerical changes were most frequently affected on chromosomes 5, 8, 17, and 22. M-FISH analysis revealed numerical aberrations on chromosomes 4, 5, 10, 12, 16, 17, 18, 19, 20, and 21. In contrast to common neuroblastoma, esthesioneuroblastoma do not overexpress N-MYC. Accordingly, we could not detect an amplification of the N-MYC locus 2p24.1 or an overexpression of the N-MYC protein, using I-FISH and Western Blot, respectively. I-FISH analysis also did not show an amplification of the C-MYC locus 8q24.12-q24.13 but demonstrated a trisomy 8 in 10 of 200 cells.

More cytogenetic investigations of esthesioneuroblastoma are necessary to define tumour- specific chromosomal aberrations.

P023

Molecular cytogenetic and gene expression profiling refines Burkitt lymphoma and identifies novel prognostic groups within mature aggressive B-cell lymphoma

Siebert R.(1), Löffler M.(2), Trümper L.(3), Stein H.(4), on behalf of the Deutsche Krebshilfe Verbundprojekt "Molekulare Mechanismen bei Malignen Lymphomen"
1) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
2) Institut für Medizinische Informatik, Statistik und Epidemiologie, Leipzig, Germany
3) Klinikum Georg-August Universität, Göttingen, Germany
4) Institut für Pathologie at Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

The Burkitt translocation t(8;14)(q24;q32) and its variants are the hallmark of Burkitt lymphoma (BL). Nevertheless, these translocations leading to fusion of the MYC locus to one of the immunoglobulin (IG) loci are not specific for BL. They also occur e.g. in diffuse large B-cell lymphomas (DLBCL), which can be difficult to distinguish from BL using current criteria. To define BL and to identify clinically relevant subgroups of lymphomas we performed gene expression profiling employing Affymetrix GeneChips (U133A) in 220 mature aggressive B-cell lymphomas. Chromosomal abnormalities were detected by interphase FISH and array-CGH. In the cohort, presence of a MYC break was associated with an "avalanche" of differentially expressed genes (5000 genes at FDR 0.004). Using a novel method called "core group extension" we generated a molecular signature of BL which comprises 74 probe sets (58 genes). This signature classified 44 cases as molecular Burkitt lymphomas (mBL) including eleven cases (25%) with the morphology of DLBCL. 38/43 (88%) mBL contained an IG-MYC fusion mostly in the context of a low number of chromosomal imbalances (median complexity score: 2). With a single exception, IGH-BCL2 fusions and BCL6 breakpoints were absent in mBL. The vast majority of the 176 cases without Burkitt lymphoma signature were DLBCL. A MYC break frequently (40%) involving non-IG-partners was detectable in 20% of these 176 cases and was associated with a high number of chromosomal imbalances (median complexity score: 8.5), presence of IGH-BCL2 fusions or BCL6 breaks (48.6%) and adverse prognosis. Classification of breakpoints affecting MYC, BCL2 and BCL6 as well as determination of the number of chromosomal imbalances allowed the definition of three cytogenetic groups (MYC-simple, MYC-complex and MYC-negative). The MYC-simple status was associated with the mBL signature whereas the MYC-complex status was a strong adverse prognostic factor independent from clinical risk factors.

P024

The hematopoetic key regulator Ikaros is a candidate for interaction with the leukemogenic CALM/AF10 fusion protein

Greif P.(1), Tizazu B.(1), Bohlander S.(1)
1) GSF-Clinical Cooperative Group "Leukemia" and Dept. of Med. III, München, Germany

The focus of our research group is the study of the t(10;11)(p13;q14) translocation that leads to the fusion of the CALM and AF10 genes. This translocation is found in acute lymphoblastic leukemia (ALL), acute myeloid Leukemia (AML) and also in malignant lymphomas. In some patients the t(10;11) is the sole cytogenetic abnormality, which indicates that the CALM/AF10 fusion is a causal event during leukemogenesis. Previous studies of our group have shown that the expression of CALM/AF10 in primary murine bone marrow cells triggers the development of an aggressive leukemia in a murine bone marrow transplantation model. However, the mechanism of CALM/AF10 dependent leukemogenesis, remains unkown. Recently, we could show that

AF10 interacts with the transcription factor Ikaros (ZNFN1A1) in yeast-two-hybrid assays. Interestingly, Ikaros is a key regulator of hematopoesis, required for normal differentiation and proliferation of B- and T-lymphocytes. In various forms of leukaemia an aberrant expression pattern of Ikaros has been found. In a murine model, the expression of a dominant negative isoform of Ikaros causes leukemias and lymphomas. Therefore, we are currently investigating the interaction between AF10 and Ikaros and the significance of this interaction in leukemogenesis. To this end, GST-pull downs and in vivo co-localization experiments are carried out. In particular, we want to determine, whether Ikaros interacts with the octapeptide / leucine zipper domain of AF10, which is required for both MLL/AF10 and CALM/AF10-mediated malignant transformation. In this context, it would be of special interest, if CALM/AF10 causes leukemia in the absence of Ikaros. This question might be answered by expressing the CALM/AF10 fusion gene in bone marrow cells of Ikaros -/- mice. These studies may provide new insights into the mechanism of CALM/AF10 induced leukemia and thereby facilitate the development of new therapies.

P025

Identification of epigenetic modifications in t(11;14)-positive mantle cell lymphoma cell lines using microarray-analysis after 5-aza-2'-deoxycytidine treatment

Ripperger T.(1), von Neuhoff N.(1), Emura M.(1), Lehmann U.(2), Tauscher M.(1), Skawran B.(1), Rudolph C.(1), Schlegelberger B.(1), Steinemann D.(1)

1) Institute of Cell and Molecular Pathology, Hannover, Germany

2) Institute of Pathology, Hannover, Germany

Mantle cell lymphoma (MCL) is defined as a mature B-cell NHL, which is clinically characterized by an aggressive course with a median survival of only 3 years. The genetic hallmark of the disease is the translocation t(11;14)(q13;q32) that causes an overexpression of Cyclin D1. However, experimental evidence has indicated that additional alterations are required to induce lymphomagenesis. In order to identify potential tumor suppressor genes probably involved in the occurrence of MCL, microarray analysis was performed on a MCL cell line Granta-519 after treating it with the demethylating agent 5-aza-2'-deoxycytidine (5-aza-CdR).

Demethylation of promoter CpG islands leads to the re-expression of epigenetically silenced genes. Microarray analysis (Stanford Functional Genomics Facility, Stanford, CA, USA) displayed more than 1.000 upregulated genes, 27 of them >3-fold. The findings were validated by quantitative real-time PCR for 13 candidate genes (CCNA1, CXCL10, CXCL9, RGS1, TFPI2, ATM, ING1, PARG1, RUNX3, DAPK1, JUNB, BNIP3L, PUMA) located in chromosomal regions of recurrent loss or involved in the regulation of proliferation, apoptosis, or DNA repair. Afterwards, gene expression and promoter methylation status of selected genes were studied by real-time PCR and methylation-specific PCR or combined bisulfite restriction analysis in five t(11;14)-positive cell lines (Granta-519, Hbl-2, Jvm-2, JeKo-1, NCEB-1) and compared with CD19+ normal B cells.

In conclusion, a reduced expression of ING1, RUNX3 and BNIP3L was displayed in at least 4 of 5 cell lines. For the first time, methylation of the promoter region of ATM was demonstrated in all studied cell lines. In addition, PARG1 was found to be methylated, at least partially, in all studied cell lines. So, this data indicate that epigenetic silencing may be involved in the inactivation of ATM and PARG1 in MCL. Further investigations are under way to prove this hypothesis in primary tumor samples.

P026

Effect of common B-RAF and N-RAS mutations on global gene expression in melanoma cell lines

Bloethner S.(1), Chen B.(1), Hemminki K.(2), Mueller-Berghaus J.(3), Ugurel S.(3), Schadendorf D.(3), Kumar R.(2)

1) German Cancer Research Center, Division of Molecular Genetic Epidemiology, Heidelberg, Germany

2) Department of Biosciences, Karolinska Institute, Huddinge, Sweden and German Cancer Research Center, Division of Molecular Genetic Epidemiology, Heidelberg, Germany

3) German Cancer Research Center, Skin Cancer Unit, Heidelberg, Germany

Melanoma is a potentially lethal skin cancer. The MAPK signaling pathway plays an important role in melanocytic neoplasia and activation of this pathway occurs through oncogenic mutations of BRAF or NRAS. BRAF is mutated in a majority of melanomas. Over 90% of the mutations in BRAF affect a valine-to-lysine change in codon 600, which results in an increased basal activity. Mutations in NRAS complement those in BRAF and occur in about 30% of melanomas. The consequences of these mutants on global gene expression are not understood. We studied global gene expression in 3 melanoma cell lines with the most common V600E mutation in the BRAF gene, 4 cell lines with a common Q61R mutation in the NRAS gene and 3 cell lines with no mutations using human HG-U133A 2.0 microarrays. Data analysis revealed several significantly upregulated and down-regulated genes specifically in cell lines with BRAF and NRAS mutations compared to cell lines without mutations, as well as genes with overlapping up- and down-regulation. Expression data of 9 selected genes were validated with quantitative RT-PCR. Expression of genes, that encode members or regulators of the MAPK pathways or are involved in metastasis or invasion, was affected in cell lines with mutations in BRAF and NRAS. Up-regulated genes in cell lines with mutations included dual-specificity phosphatase 6 (DUSP6), sprouty homolog 2 (SPRY2), and v-akt murine thymoma viral oncogene homolog 3 (AKT3).Interleukin 18 (IL18) and inhibitor of DNA binding 2 (ID2) were down-regulated. Our results provide a novel insight into the effect of mutations in the BRAF and NRAS genes on global gene expression in melanoma.

P027

Characterization of chromosomal rearrangements in human malignant glioma cells using 24-color-FISH, comparative genomic hybridization (CGH) and array-based CGH with the aim to identify novel tumor relevant genes

Trost D.(1), Hoischen A.(1), Wick W.(2), Weber R.G.(1)

1) Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn, Germany

2) Department of General Neurology and Hertie Institute of Clinical Brain Research, University Tübingen, Tübingen, Germany

Malignant gliomas, the most frequent primary tumors of the brain, have a particularly bad prognosis. This poor clinical course is paralleled by a high incidence of chromosomal imbalances in malignant glioma cells causing the activation of oncogenes and the inactivation of tumor-suppressor-genes. In order to identify recurrent translocations for further molecular breakpoint characterization and genomic imbalances involved in tumor development, we applied 24-color-FISH, chromosomal CGH and array-based CGH together with banding techniques to nine cell lines derived from malignant gliomas, including three newly established cell lines. In eight of nine cell lines, complex structural and numerical alterations were identified by cytogenetic analyses. 24-color-FISH identified recurrent unbalanced translocations: t(1;19), t(1;20), t(3;16), t(3;19), t(4;6), t(5;15), t(6;8), t(6;19), t(6;20), t(8;14), t(11;15) and t(12;16) as well as balanced rearrangements: t(X;4), t(11;19) and t(2;20). In one of the newly established cell lines, clonal evolution was found with cells containing a near diploid karyotype and others showing a near tetraploid karyotype and sharing some of the structural rearrangements of the diploid clone. Only one rearrangement, a der(9)t(7;9)(p22;p22), was present in all clones of this tumor. This translocation is thus likely to represent an early event in the development of this glioma. In addition to the imbalances revealed by chromosomal CGH in the nine malignant glioma cell lines, array-based CGH identified losses of CDKN2A/p14ARF and CDKN2B (in 6/9) and PTEN (in 1/9), as well as amplification of CDK4 and SAS (in 2/9) and EGFR (in 1/9). The translocations identified here will be characterized further by cytogenetic techniques. This will allow the selection of pertinent chromosomal translocations for molecular analysis and possibly the identification of novel genes rearranged in malignant glioma cells that are relevant for tumorigenesis.

Supported by NGFN2

P028

Gain of the chromosomal region 18q21 represents an independent negative prognostic factor and is associated with the activated B-cell gene expression subtype in diffuse large B-cell lymphoma

Dierlamm J.(1), Murga Penas E.M.(1), Schwänen C.(1), Wessendorf S.(1), Berger H.(1), Bentink S.(1), Hinz K.(1), Becher C.(1), Stürzenhofecker B.(1), Hummel M.(1), Bokemeyer C.(1), Stein H.(1), Spang R.(1), Löffler M.(1), Trümper L.(1), Siebert R.(1), On behalf of the network project 'Molecular Mechanisms of Malignant Lymphomas' (1)

1) Verbundzentrale: Klinikum der Georg-August-Universität Göttingen, Göttingen, Germany

Diffuse large B-cell lymphoma (DLBCL) represents a heterogeneous disease entity. In contrast to other lymphoma subtypes, reliable single genetic markers indicating the patients´ prognosis are lacking. In the present study, 138 DLBCL were analyzed by interphase fluorescence in situ hybridization (FISH) with *MALT1*-specific probes, comparative genomic hybridization microarray (array-CGH), and gene expression profiling. A gain of the *MALT1* gene ranging from 3 to 6 copies (46 cases) to gene amplification (3 cases) was detected by FISH in 49 cases (36%). This might also include changes in ploidy, which was not corrected for. In more than 50% of the cases with a *MALT1* gain, a concomitant gain of *BCL2*, which lies about 5Mb telomeric to *MALT1*, was detected by array-CGH. By gene expression profiling, the group with a *MALT1* gain showed a significantly higher expression of genes located on chromosome 18q21 than the group without *MALT1* gains. Moreover, a significant clustering of cases with *MALT1* gain in the activated B-cell gene expression (ABC) subtype was observed (p=0,0006). The groups with and without *MALT1* gain did not significantly differ with respect to sex ratio, performance status, B-symptoms, clinical stage, bulky disease, extranodal involvement, bone marrow infiltration, and lactate dehydrogenase level. However, time to treatment failure (p=0,019) was significantly different in both groups. In the multivariate analysis the *MALT1* status remained an independent negative prognostic factor. Our data indicate that gain of the region 18q21 including *MALT1* caused by changes in ploidy or partial chromosomal gains represents an independent prognostic factor in DLBCL and adds important information to the known clinical prognosis parameters.

P029

Mutations causing FAP in Polish population

Plawski A.(1), Podralska M.(1), Skrzypczak M.(2), Lipinski D.(1), Slomski R.(1)

1) Institute of Human Genetics, Poznan, Poland

2) Institute of Human Genetics, Leipzig, Germany

Familial adenomatous polyposis (FAP) is an autosomal dominant predisposition to initiate numerous polyps in the colon and rectum which develop to the carcinoma if left untreated. FAP is caused by inherited or germ line mutations in the APC gene. Early recognition of the mutation carriers is very important for the medical treatment of persons from the high-risk group. The DNA bank for Polish FAP patients at the Institute of Human Genetics at Poznan was established in 1997. FAP diagnoses were performed in cooperating health centers. 620 DNA samples from persons belonging to 240 FAP families were collected. 280 patients were diagnosed with FAP, 215 persons belong to risk group and 67 persons excluded from the risk group. The entire APC gene coding sequence was screened for mutations in 220 families. The APC gene mutations were identified in 105 Polish FAP. Thirty one of them have not been described before. Seven mutations types recurred two ore more times. The recurrent mutations were detected in 52% of diagnosed families. 90 persons without mutations in the APC gene were further examined for occurrence of MYH gene mutations. Two of the most frequent mutations of the MYH gene (Y165C and G382D) found in this gene occurred in a heterozygotic system in 13% of patients. In the investigations of the MYH gene, no other mutations in coding sequence were recorded. The results indicate that in our group of patients with the diagnosed FAP but without mutation in the APC gene, the proportion of the mutation in the MYH gene has minor impact on preconditioning the disease.

P030

Leupaxin is expressed in mammary carcinomas and represents a novel coactivator of the estrogen receptor alpha

Kaulfuß S.(1), Büchner A.(1), Herr A.-M.(1), Hemmerlein B.(2), Thelen P.(3), Kickstein M.(1), Burfeind P.(1)

1) Institute of Human Genetics, Göttingen, Germany

2) Department of Pathology, Göttingen, Germany

3) Department of Urology, Göttingen, Germany

Leupaxin is a cytoskeleton adaptor protein belonging to the paxillin- protein family of LIM-domain containing proteins preferentially expressed in cells of hematopoietic origin. In a recent study we already showed that leupaxin is involved in the invasiveness of prostate carcinoma cells and that it interacts and activates the androgen receptor.

Here we demonstrate that leupaxin is also expressed in several mammary carcinoma cell lines. Immunocytochemical studies at the subcellular level revealed that leupaxin is mainly localized in focal adhesion sites and that leupaxin shuttles between the cytoplasm and the nucleus in mammary carcinoma cells. Furthermore, by using immunohistochemistry we could show that leupaxin is expressed in 13% of human mammary carcinoma specimens analyzed.

To investigate the function of leupaxin in mammary carcinoma cells the expression of leupaxin was down-regulated in invasive MDA-MB-231 cells using the RNAi technique. The leupaxin knock-down resulted in a 72% reduction of invasiveness of these cells, whereas the proliferation of MDA-MB-231 cells was not affected. Moreover, interaction of leupaxin with the estrogen receptor alpha was demonstrated and in addition, leupaxin was shown to transactivate the estrogen receptor alpha in a ligand-dependent manner.

In conclusion, the results of our present studies indicate that leupaxin could serve as a potential candidate involved in the progression of mammary carcinomas.

P031

Activation of cyclin D2 by chromosomal translocations in cyclin D1-negative mantle cell lymphomas

Gesk S.(1), Nagel I.(1), Klapper W.(2), Martin-Subero J.I.(1), Fu K.(3), Weisenburger D.D.(3), Harder L.(1), Siebert R.(1)

1) Institute of Human Genetics, University Hospital Schleswig Holstein, Campus Kiel, Kiel, Germany

2) Department of Pathology, University of Hospital Schleswig Holstein, Campus Kiel, Kiel, Germany

3) Nebraska Medical Center, Department of Pathology & Microbiology, Omaha, United States of America

Mantle cell lymphoma (MCL) is a lymphoproliferative disorder with distinct pathological and clinical features. Genetically, MCL is characterized by the translocation t(11;14)(q13;q32), which leads to deregulation of the gene encoding cyclin D1 in 11q13 through juxtaposition to the immunoglobulin heavy chain (IGH) locus in 14q32. By gene expression profiling, we have recently shown that rare MCLs lacking the t(11;14) and cyclin D1 expression exist. Otherwise, these Cyclin D1-negative MCLs show morphological, immunhistochemical and clinical features very similar to typical cyclin D1-positive MCL (Fu et al., Blood 2005).

Here, we present two cases of cyclin D1-negative MCL with typical growth pattern and immunophenotype (CD5+, CD20+, CD10- and CD23-). In both cases the translocation t(11;14) was ruled out by FISH. We then investigate the presence of chromosomal rearrangements affecting the gene loci encoding cyclin D2, D3, E1 and E2. Both MCL displayed breakpoints involving the CCND2 locus in 12p13 and the IGK locus in 2p12. In one of these cases cytogenetic analysis revealed a translocation t(2;12)(p12;p13). Furthermore, by immunohistochemistry the cyclin D2 protein was shown to be expressed in both cases.

These results indicate that cyclin D1-negative MCLs can arise through translocations fusing the CCND2 and IG loci. Additionally, these data suggest that cyclin D2 activation can substitutive cyclin D1 to transit the cell cycle, especially during the G1 to S phase in these as yet incurable tumors.

This work was supported from a grant of the Deutsche Krebshilfe (70-3173-Tr3).

P032

Novel WT1 germline mutation in a fetal rhabdomyomatous bilateral Wilms Tumor with an additional mutation in CTNNB1

Uschkereit C.(1), de Torres Gomez-Palette C.(2), Schumacher V.(1), Mora J.(2), Royer-Pokora B.(1)

1) Universität Düsseldorf, Institut für Humangenetik, Düsseldorf, Germany

2) Hospital Sant Joan de Deu, Laboratory of Developmental Tumors, Barcelona, Spain

We describe a female patient with a novel germline WT1 mutation (p.Ser50 stop) who developed a bilateral WT at the age of 12 months. The tumor was initially treated with empirical chemotherapy, but showed no clinicoradiological response and was subsequently completely removed. The histology was predominantly fetal rhabdomyomatous with some areas of blastemal

clusters. Immunohistological staining with a CTNNB1 antibody revealed a cytoplasmic and membraneous staining as well as a few focal areas with nuclear positivity in muscle cells. Blastemal cells showed only a strong membranous and cytoplasmic staining. The tumor presented loss of the wild type WT1 allele (LOH) and an additional tumor specific heterozygous mutation in CTNNB1 (p.Ser45Pro). Future molecular analysis of microdissected muscle, blastemal, malignant tubuli and normal kidney cell types from the tumor will reveal the timing of genetic events during tumor development.
The patient was afterwards not treated with chemotherapy and did well for one year. At the age of two years she developed a bilateral renal relapse and both tumors were removed. Pathology findings about the relapses were complex and different from those af the initial tumors. There was a large classical triphasic WT on the left side that was totally resected. The tumor of the right kidney was entirely different and appeared as a small and round WT of the rhabdomyomatous type that could be resected as a tumor ball. This tumor was arising from a thin abnormal layer of tissue that was also partially removed. Analysis revealed that this abnormal tissue from where the rhabdomyomatous WT developed was histologically a triphasic WT. Further molecular studies and histological examinations of the relapses will help to understand the complex development of triphasic and monotonous WTs from a cell that is haploinsufficient for WT1.

P033

Gene expression profiles of T-cell prolymphocytic leukemia with inv(14)(q11q32) reflect patterns of chromosomal imbalances

Bug S.(1), Dürig J.(2), Klein-Hitpass L.(3), Harder L.(1), Baudis M.(4), Dührsen U.(2), Siebert R.(1)
1) Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
2) Department of Hematology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
3) Institute of Cell Biology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
4) Division of Pediatric Hematology/Oncology, University of Florida, Gainesville, Gainesville, United States of America

About 80% of cases of T-cell prolymphocytic leukemia (T-PLL) carry an inv(14)(q11q32) or t(14;14)(q11;q32) as characteristic chromosomal aberration. Thus, the oncogenes TCL1A and TCL1B in 14q32.1 get under the influence of enhancer elements of the T-cell receptor alpha/beta locus in 14q11. Besides the inv(14) or t(14;14), T-PLLs display a highly characteristic pattern of recurrent genomic alterations.
Here we performed Affymetrix 50K SNP-Chip analyses in 12 cytogenetically well characterized T-PLL with inv(14). Recurrent gains were detected in 6p and 8q, losses in 6q, 8p, 10p, 11q, 18p and 22q. Partial uniparental disomies (UPDs) were rare. Copy number counts in the designated candidate regions were confirmed by Interphase-FISH studies with a panel of more than 50 probes. The pattern of chromosomal aberrations was consistent with previous studies (Soulier et al., GCC, 2001). Moreover, we could show that the chromosomal breakpoints leading e.g. to the recurrent iso(8q) are not strictly conserved.
Parallel gene expression profiling (Affymetrix U133A) was performed on CD3-positive T-cells of 5 inv(14)-positive T-PLL and of 8 healthy donors. Hypergeometric distribution analysis identified a significant clustering of overexpressed genes in the chromosomal arms of 6p, 7q and 8q and significant accumulation of underexpressed genes in 6q, 8p, 10p, 11q and 18p. Comparison to the genomic data set revealed a strong correlation of chromosomal imbalances and gene expression levels. Our results therefore suggest that the pattern of chromosomal imbalances dominates the gene expression profile in T-PLL.

P034

Detection of TORC1-MAML2 fusion transcripts in paraffin-embedded mucoepidermoid carcinoma

Fehr A.(1), Röser K.(2), Belge G.(1), Schumacher U.(3), Löning T.(2), Bullerdiek J.(1)
1) University of Bremen, Center for human genetics, Bremen, Germany
2) University Medical Centre Hamburg-Eppendorf, Institute for Oral pathology, Hamburg, Germany
3) University Medical Centre Hamburg-Eppendorf, Institute for anatomy II, Hamburg, Germany

Mucoepidermoid carcinoma (MEC) is the most frequent type of cancer in the major and minor salivary glands and the second most frequent lung tumour of bronchial gland origin. The diagnosis and clinical management of these tumours is complicated due to their varying biological behaviour and relative rareness. MECs are often characterized by a t(11;19) translocation. Previous studies of MECs of the salivary and bronchial glands have identified the fusion gene, TORC1 (a.k.a. MECT1)-MAML2, resulting from this translocation. RT-PCR can help to identify MECs by detection of the TORC1-MAML2 fusion transcript in tissues. We have developed a method to detect the TORC1-MAML2 fusion transcript in up to 10 year old formalin-fixed paraffin-embedded (FFPE) tissues from human MECs and control tissues derived from MECs xenotransplanted to SCID-mice by RT-PCR. We analysed a total number of 20 human MECs and seven control tissues derived from MECs xenotransplanted to SCID-mice for the presence of the TORC1-MAML2 fusion transcript. RT-PCR using total RNA from FFPE tissue sections were performed. For PCR positive control we used NCI-H292. Previous reports described in approximated 70% of deep-frozen MECs with t(11;19) translocation a TORC1-MAML2 fusion transcript. Our results correspond roughly to these data, 66% of our tested human MECs and all control tissues from SCID-mice contained such a fusion gene. We could demonstrate that it is possible to detect the TORC1-MAML2 fusion transcript in up to 10 years old paraffin-embedded tissues by RT-PCR. The TORC1-MAML2 fusion gene is a potential usefulness molecular marker for the diagnose of MECs by RT-PCR. This allows further studies on paraffin-embedded salivary gland archival material, which could be of interest for large retrospective studies.

P035

Allele loss and deletion on 16q24 define the location of a candidate tumor suppressor that is targeted in a subgroup of retinoblastomas

Lohmann D.(1), Rieder H.(2), Ullmann R.(3), Schneider S.(4), Kappler M.(5), Zeschnigk M.(1), Gratias S.(1)
1) Humangenetik, Essen, Germany
2) Institut für Humangeentik und Anthropologie, Düsseldorf, Germany
3) Max Planck Institute for Molecular Genetics, Berlin, Germany
4) Institut für klinische Genetik, Marburg, Germany
5) BGFA, Epidemiologie, Bochum, Germany

Mutations that inactivate both alleles of the RB1 gene are a prerequisite for the initiation of retinoblastoma. Additional genomic alterations are frequent in this eye tumor including gains of 1q and 6p and losses on 16q. It is plausible that the identification of the functional changes caused by these alterations will help to identify targets for molecular therapy. To identify a minimal deleted region (MDR) on in retinoblastoma 16q we analyzed 22 STR-loci in tumor and blood DNA from 58 patients. RNA was available from 12 tumors and was used for microarray expression analysis (HG-U133A) and quantitatve RT-realtime-PCR. A subset of tumors was also investigated by conventional and matrix CGH. The mutational status at the RB1 locus was known in all patients. Detailed phenotypic data (clinical manifestation and histomorphology) were obtained.
40/58 (69%) tumors showed no LOH at any informative 16q marker. LOH was detected in 18/58 (31%) tumors including 5 that also showed allelic imbalance (AI) for some markers. Three tumors with LOH and AI showed complex genomic changes in mCGH that were not deteted in conventional CGH. Two tumors showed LOH only at markers near the telomere of 16. Because normal CGH and mCGH, LOH is due to isodisomy in some regions. Results in all tumors with chromosome 16 alterations are in line with a single MDR spanning about 6.5 Mb in 16q24.1 to 16q24.3. The centromeric boundary defined by D16S422 (Mb 81.5). On the telomeric side all tumors with LOH showed alterations at D16S3026 (Mb 88), the most distal STR known on 16q. The parental origin of allele loss was paternal and maternal in nine and six tumors, respectively. Clinical presentation and histomorphology of tumors with alterations in the MDR on 16q was distinct.
Specifically, 12 of 13 tumors with alterations MDR showed diffuse intraocular seeding. This genotype-phenotype correlation suggests that a member of the cadherin gene cluster on 16q is involved.

P036

Identification and molecular cytogenetic characterization of a supernumerary neocentromeric derivative chromosome 3 in bone marrow cells of a Fanconi anemia patient

Marchal J.(1), Gerlach A.(1), Ebell W.(2), Neitzel H.(1), Tönnies H.(1)
1) Charité, Universitätsmedizin Berlin, Institute of Human Genetics, Chromosome Diagnostics and Molecular Cytogenetics, Berlin, Germany

2) Charité, Universitätsmedizin Berlin, Department of General Pediatrics, Bone Marrow Transplant Unit, Berlin, Germany

Fanconi Anemia (FA) is a congenital disorder characterized by chromosome instability, progressive bone marrow failure, susceptibility to myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML). Bone marrow (BM) cells of FA patients frequently develop clonal chromosome aberrations, which can represent an adverse risk factor in FA, as reported for monosomy of 7 and/or tri- and tetrasomies of 3q. We present the cytogenetic characterization of clonal aberrations present in bone marrow cells of a patient with FA and AML. Conventional cytogenetic analyses of bone marrow cells revealed a complex karyotype in all analyzed cells, with trisomies for chromosomes 8, 10, 13, a derivative chromosome 1, defined as 1qter->1q21::1p36->1qter, and a supernumerary marker chromosome. For the characterization of the marker chromosome several molecular cytogenetic studies were performed. GTG-banding, FISH, and CGH analyses demonstrated that the marker consisted of a inverted duplication of the distal part of chromosome 3 (3qter->3q26.2::3q26.2->3qter). Hybridization with an all-centromere probe produced no signal on the derivative chromosome 3, demonstrating the absence of alpha-satellite repeated sequences despite a prominent visible constriction in chromosome band 3q26.3. Subsequently, the marker chromosome was shown to contain a functional neocentromere, as two signals were observed when performing immunofluorescence with CENP-C antibodies.

In conclusion, we found in this patient a mainline BM population with an aberrant karyotype: 50,XY,+8,+10,+13, der(1)(1qter->1q21::1p36->1qter),+inv dup (3)(qter->q26.2::q26.2->q26.3->neo->q26.3->qter). The presence of a neocentromeric chromosome with a 3q content is particularly remarkable and important due to the adverse risk demonstrated for 3q gains in FA. Thus, at the time of our analyses the FA patient was also affected by AML, probably as a consequence of the extension of this particular clonal aberration among the BM population.

P037

Family characteristics of individuals with multiple juvenile malignancies

Kohlschmidt N.(1), Schneider-Rätzke B.(1), Spix C.(2), Galetzka D.(1), Haaf T.(1)
1) Institute of Human Genetics, Mainz University School of Medicine, Mainz, Germany
2) Institute of Medical Biostatistics, Epidemiology, and Informatics, Deutsches Kinderkrebsregister, Mainz, Germany

Causes of malignant diseases may be environmental and genetic with varying shares, depending on factors like age, lifestyle habits, environmental pollution, family history, and chance. Genetic causes are probably more important for childhood than for adult cancer. Those with two malignancies may be carriers of especially unfavourable genetic information regarding tumor development, detection, and deletion. Whilst single responsible genes are only known for limited types of cancers, the application of array chips, testing the expression of several hundred genes simultaneously, promises to reveal additional genetic causes.

We recruited individuals with at least two malignancies in childhood or early adulthood in cooperation with the German Childhood Cancer Registry. Participants agreed in blood sampling and skin biopsies for laboratory tests.

All those enrolled were asked for information on their families, especially on cancer and examined for syndromic features. Pedigree analysis shows that in some subjects' families, cancer is unusually common, indicating a genetic risk, whilst in other subjects' families; cancer is not more frequent than in the general population. We present preliminary data on the hitherto examined subjects, matched controls with only one malignancy in childhood, and matched cases with no childhood cancer. Pedigree data will not allow deriving probability figures appropriate for prospective individual risk management.

P038

NF1 haploinsufficiency and increased noise

Kemkemer R.(1), Gruler H.(2), Kaufmann D.(3)
1) MPI for Metal Research, Stuttgart, Germany
2) Department of Biophysics, University of Ulm, Ulm, Germany
3) Department of Human Genetics, University of Ulm, Ulm, Germany

One consequence of neurofibromin haploinsufficiency is an altered morphology of NF1 cells in vitro. We demonstrated by biophysical investigations and stochastic calculations that these differences in cultured NF1 melanocytes are the consequence of an altered signal-to-noise ratio or an increase in inaccuracy in dendrite formation. It is shown in mathematical simulations that increased noise in determination of the amount of a gene product is a general effect in a monoallelic system in contrast to a biallelic system. This increased noise can result in an additional problem: an increased occurrence of normally very rare stochastic events due to reduction of the gene product under a threshold. Here, we investigated if a defined physical signal reduces the increased noise in cultured NF1 melanocytes. As physical signals we tested artificial variations of the topography of the cell culture tissue surface: grooves with heights of 25 to 200 nanometer in a distance of 2 to 10 micrometer. Analysis of the number of dendrites in NF1 melanocytes cultured on such grooves showed that they behave like control melanocytes. This extracellular signal normalizes the signal-to-noise ratio in NF1 cells. The melanoyctes orientate on the grooves. Interestingly, the orientation can be described by this simple equation: Orientation ~ (height (of grooves) x separation)^2. The comparative analysis of the orientation behaviour demonstrated, that control melanocytes show a better orientation than NF1 melanocytes. NF1 melanocytes show a reduced sensitivity for extracellular topographic signals in vitro. Similar results were gained in experiments with cultured fibroblasts: the control fibroblasts show a better orientation than NF1 fibroblasts.

P039

Early treatment sensitivity in childhood acute lymphoblastic leukemia is associated with gain of chromosome 21

Steinemann D.(1), Cario G.(2), Stanulla M.(3), Tauscher M.(1), Weigmann A.(1), Hinrichsen T.(1), Michaelsen G.(1), Viehmann S.(4), Lichter P.(5), Schrappe M.(2), Schlegelberger B.(1)
1) Institute of Cell and Molecular Pathology, Hannover, Germany
2) Department of Pediatrics, Kiel, Germany
3) Pediatric Hematology/Oncology, Hannover, Germany
4) Pediatric Hematology/Oncology, Giessen, Germany
5) Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany

ALL is the most common malignancy in childhood. In vivo response to initial therapy, as assessed by determination of minimal residual disease at 5 and 12 weeks of treatment, has evolved as the strongest prognostic factor in pediatric ALL patients treated according to the BFM regime. In this study, ten patients with a poor treatment response (MRD load > 10-3 at week 12, MRD-high risk, HR), five treatment-sensitive patients (no measurable MRD at weeks 5 and 12, MRD-standard risk, SR) and three patient with an intermediate treatment sensitivity (any MRD positivity at week 5, MRD load < 10-3 at week 12, MRD intermediate risk, IR) were investigated by means of high-resolution array-based comparative genomic hybridization. To ensure homogeneity with regard to prognostic factors, the following inclusion criteria were used: B cell precursor or common ALL, DNA index of 1.0, no BCR/ABL, no MLL/AF4, no TEL/AML1 rearrangements. A gain for all chromosome 21-related BAC clones was observed in all five SR as well as in two out of three IR risk patients. None of the ten HR patients showed a gain of any region of chromosome 21. This result could be confirmed by means of fluorescence in situ hybridization using the LSI AML1/ETO DC probe set. Interestingly, the one MRD-IR patient demonstrating no gain of chromosome 21 relapsed 29 months after diagnosis. Recurrent genomic alterations in the group of HR patients were loss of chromosomal region 2p11.22 (9/10), a gain of 8q24.13 (7/10) and loss of 14q32 (8/10). To exclude that these alterations may indicate genomic polymorphisms we additionally profiled the DNA prepared from the patients blood at remission stage. No genomic alterations were observed in this material. Besides the basic level of chromosome 21 gain, higher levels of gain or amplification observed for individual regions were found in 4 SR/IR patients. The further characterization of these amplified sequences may lead to the identification of therapy-related target genes.

P040

Unusual immunohistochemical results in the tumour of a patient with Lynch-Syndrome and a pathogenic MSH6 germline mutation

Rahner N.(1), Friedrichs N.(2), Sengteller M.(1), Abou Jamra R.(1), Büttner R.(2), Mangold E.(1), Pagenstecher C.(1)
1) Universitätsklinikum Bonn, Institut für Humangenetik, Bonn, Germany
2) Universitätsklinikum Bonn, Institut für Pathologie, Bonn, Germany

Lynch syndrome (HNPCC) is a cancer susceptibility syndrome caused by germline mutations in DNA mismatch repair genes, predominantly MLH1 and MSH2. In most cases the results of immunohistochemical staining in tumour tissues point to a pathogenic germline mutation in one of the MMR genes. Nevertheless the identification of a pathogenic germline mutation is not possible in all cases. One reason could be a methylation of the MLH1 promotor causing false positive results in immunohistochemistry staining.
Here we report on an interesting case of a 35yrs old woman from a family fulfilling the Amsterdam criteria with high genomic instability (MSI-H) and an unexpected loss of expression of the MMR protein complex MLH1/PMS2 as well as an isolated loss of MSH6 in her tumour tissue. Mutation analyses of MLH1, MSH2 and MSH6 genes from genomic DNA isolated from peripheral blood lymphocytes revealed the pathogenic germline mutation MSH6,c.3367G>T;p.Glu1123X but no mutation in MLH1 and MSH2. To find out the reason for the loss of expression of the MLH1/PMS2 complex we performed mutation analyses on all exons of MLH1 from genomic DNA isolated from the patient's tumour tissue. We identified the splice mutation MLH1,c.307-2A>G in tumour but not in peripheral blood DNA. The second hit may be MLH1 promotor methylation or an yet unidentified mutation.
This case indicates that the results of immunohistochemical staining in tumour tissues do not definitely point to a germline mutation in the according MMR gene and that somatic mutations, too, can cause false positive results. Knowing that protein expression of MSH6 and PMS2 is not performed in all laboratories, this case shows its necessity especially in young patients and conspicuous families.
We therefore suggest immunohistochemical staining in tumour tissue for all MMR proteins for a higher mutation detection rate in families with the Lynch syndrome.

The study was supported by the Deutsche Krebshilfe.

P041

The CALM/AF10 interacting protein CATS interacts with the anti-apoptotic protein HAX1

Fröhlich-Archangelo L.(1), Fontanari Krause L.(1), Krause A.(1), Kremmer E.(2), Buske C.(1), Bohlander S.K.(1)
1) Department of Medicine III and GSF, Clinical Cooperative Group Leukemia, University of Munich, Munich, Germany
2) Institute of Immunology, GSF, Munich, Germany

The CATS gene was first identified as a CALM interacting protein (CALM interacting protein expressed in thymus and spleen). The CATS interaction region of CALM is contained in the leukemogenic fusion protein CALM/AF10, which is found in acute myeloid leukemia, malignant lymphoma and acute lymphoblastic leukemia. High expression of CATS in proliferating cells and in tumor cells as well as its nucleolar localization suggest a role of CATS in the control of cell proliferation. RT PCR analysis showed CATS expression in patient cells and cell lines carrying the CALM/AF10 rearrangement. Further expression analysis showed CATS expression in different cell subpopulation from the murine thymus and bone marrow but very low expression in B220+ cells.
Interestingly, there was high CATS expression in CALM/AF10 expressing B220+ leukemic cells from a CALM/AF10 murine bone marrow transplant model. Immunofluorescence showed that endogenous CATS protein is localized to the nucleus and nucleoli. We used Leptomycin B to inhibit the nuclear export in 293T cells transfected with YFP-CATS, YFP-CALM and YFP-CALM/AF10. CATS localization was not affected by LMB treatment. However both CALM and CALM/AF10, which are normally cytoplasmic proteins, strongly accumulated in the nucleus, where they can interact with CATS. CATS was used as a bait to screen a Hela cDNA library in a yeast two hybrid screen. Among different interacting partners of CATS, HAX1 was identified. HAX1 has been found in anti-apoptotic signaling counteracting the pro-apoptotic effects of BAX and was identified as a protein interacting partner of HS1 (hematopoietic lyn substrate 1), which is part of the B-cell and T-cell receptor signaling cascade. We confirmed the CATS-HAX1 interaction in the yeast system and by co-immunoprecipitation. This interaction suggests that CATS plays a role in the regulation of apoptosis and suggests a link between the leukemogenic fusion protein CALM/AF10 and T- and B-cell receptor signaling.

P042

Securin and chromosomal stability

Pfleghaar K.(1), Heubes S.(2), Cox J.(3), Stemmann O.(2), Speicher M.(1)
1) Institut für Humangenetik der TU, München, Germany
2) Max Planck Institut für Biochemie, Martinsried, Germany
3) Genedata GmbH, Martinsried, Germany

Aneuploidy is one of the most common features in aggressive human tumors. Chromosomal instability may facilitate tumorigenesis through amplification of oncogenes or loss of tumour suppressor genes. Therefore, exploring the underlying molecular mechanisms of chromosomal instability remains an important issue. A previous study suggested that securin, an anaphase-regulating gene product in chromatid segregation, is required for chromosomal stability. After securin knockout in the chromosomally stable HCT116 colorectal cancer cell line, HCT116 cells showed gross rates of chromosome losses. When we reinvestigated the HCT116 knockout cell line, we found that after few passages, hSecurin -/-cells regained chromosomal stability and executed anaphases normally (Pfleghaar et al. 2005, PLoS Biol 3:e416). This was unexpected, as the securin loss resulted in a peristing reduction of the sister-separating protease separase and inefficient cleavage of the cohesion subunit Scc1. These biochemical defects were initially discussed as a possible reason for the CIN phenotype, which occurred after securin knockout. In order to find genes involved in a potential back-up mechanism, which could have stabilized the genome, we compared the gene expression of stable knockout and wildtype cells with the Affymetrix HG U133 chip. Three genes with important roles in mitosis, namely PLK2, RCC1 and SMC6, showed significantly different expression profiles in the two cell lines. This suggests that these genes may be involved in mechanisms to compensate for the securin loss. The exact role of these three genes in stabilizing cells in the absence of securin remains to be elucidated.

P043

High resolution oligonucleotide array CGH in Myelodysplastic syndrome

Evers C.(1), Drechsler M.(1), Hildebrandt B.(1), Beier M.(1), Servan K.(2), Royer H.-D.(2), Royer-Pokora B.(1)
1) Institut für Humangenetik und Anthropologie, Universität Düsseldorf, Düsseldorf, Germany
2) Caesar Forschungszentrum, Bonn, Germany

Myelodysplastic syndrom (MDS) is a clonal bone marrow disorder characterized by abnormalities of haematopoiesis and an increased risk of transformation into acute myeloid leukemia (AML). The most frequent cytogenetic abnormality of this disease is an interstitial deletion of the long arm of chromosome 5 (del5q) occuring in 30% of all patients with chromosomal abnormalities. An isolated del5q is associated with a good prognosis. The additon of other karyotype aberrations to a del5q indicates a significantly worse overall survival. Due to the limited resolution of classical cytogenetics only deletions larger than 2-5 MB are detectable and the exact breakpoints remain unclear.
New molecular techniques like array-based comparative genomic hybridisation (array-CGH) allow an exact localisation of the chromosomal breakpoints as well as the identification of small submicroscopic deletions and amplifications.
Oligonucleotide CGH-arrays (Agilent) with an average spatial resolution of 75kb are a new addition to the diverse techniques for genomic studies. To determine the exact breakpoints and to identify submicroscopic genomic imbalances we performed a genomewide high resolution array-CGH study with patients with an isolated del5q in at least two metaphases and at least 70% aberrant cells as determined by FISH studies. The characterised breakpoints differ among these patients and one patient showed additional submicroscopic chromosomal aberrations. The results demonstrate that the cytogenetic breaktpoints are highly variabel and that array-CGH allows the identification and characterisation of the exact deletion endpoints. Furthermore additional hidden chromosomal abnormalities that are not detectable with classical cytogenetics but may have an progostic and therapeutic impact can be identified.

P044

Unusual somatic exon 10 mutation in the WT1 gene in a Wilms tumor from a 9 month old child

Royer-Pokora B.(1), Küff M.(1), Quante A.(1), König J.(2), Jürgens H.(2), Uschkereit C.(1)
1) Universität Düsseldorf, Institut für Humangenetik und Anthropologie, Düsselorf, Germany
2) Universitätsklinikum Münster, Klinik und Poliklinik für Kinderheilkunde, Pädiatrische Hämatologie/Onkologie, Münster, Germany

The patient presented with a WT at the age of 9 month. A cell culture was established from the normal kidney and the tumor, which showed epithelial differentiation. The histology of the tumor was stromal-predominant. A mutation analysis

of the blood DNA did not reveal a mutation and polymorphism in exon 1 was found. In the tumor a 1 bp insert at c1293 in exon 10 was found, leading to a frameshift and elongation of the protein. The mutation is described as 431fsX475. The amino acid change disrupts the fourth zinc finger (ZF) and most likely alters the DNA binding properties. Mutations in the last exon (10) have only rarely been described, possibly because the protein retains most of its normal function. However the mutation described here demonstrates that an intact ZF4 is important for its tumor suppressor activity. The tumor showed loss of the wild type allele and in addition had a somatic mutation in CTNNB1, which supports previous data describing a correlation of WT1 and CTNNB1 mutations. RT-PCR analysis of the tumor cells in culture demonstrated that WT1 and Vimentin are expressed which shows that the cells are derived from kidney and correspond to an early (more mesenchymal) stage of differentiation. Currently we are performing experiments to verify the tumor origin of the cells in culture by demonstrating the presence of a WT and CTNNB1 mutation.
The availability of a WT1 mutant tumor cell culture system is important for unravelling the effect of the mutant WT1 protein with an altered ZF4 in comparision to the wild type protein.

P045

Differential contribution of NBS1 gene alterations to breast cancer susceptibility

Bogdanova N.(1), Festchenko S.(2), Sohn C.(3), Dörk T.(1)
1) Hannover Medical School, Gynaecology Research Unit, Hannover, Germany
2) Mother and Child Hospital, Minsk, Belarus
3) University of Heidelberg, Department of Gynaecology, Heidelberg, Germany

The NBS1 gene encodes a protein, nibrin/p95, that functions as a sensor and transducer in DNA double strand break repair. NBS1 gene mutations in the homozygous state cause Nijmegen Breakage Syndrome, a radiation sensitivity disorder. Heterozygous carriers of the major NBS1 gene mutation, 657del5, have been suggested to be at an increased risk for breast cancer. Two conserved NBS1 missense variants, R215W and I171V, have also been associated with various cancer types. We assessed the frequency of the 657del5, R215W and I171V mutations in a large series of breast cancer patients and control individuals from the Republic of Belarus, a population of predominantly Slavic descent. The 657del5 mutation was detected in 5 out of 1143 breast cancer patients but was not observed in any out of 611 controls (p=0.09). Four of the five patients carrying the 657del5 allele had premenopausal disease. Two patients reported a first- or second-degree family history of breast cancer. The frequency of the R215W substitution was not different between breast cancer cases and population controls (OR=0.6, 95%CI 0.2-2.5), and the I171V substitution was less frequent in the cases than in the control group (OR=0.4, 95%CI=0.1-0.9). In summary, while the two investigated missense variants did not appear to predispose towards breast cancer, our data provide some support for the proposed role of frameshift mutation NBS1*657del5 as a rare breast cancer susceptibility allele in Slavic populations.

P046

Functional polymorphisms of MDM2 and TP53 may modulate the age at onset of breast cancer

Reincke S.A.(1), Bremer M.(2), Karstens J.H.(2), Sohn C.(3), Dammann O.(4), Dörk T.(1)
1) Hannover Medical School, Gynaecology Research Unit, Hannover, Germany
2) Hannover Medical School, Department of Radiation Oncology, Hannover, Germany
3) University of Heidelberg, Department of Gynaecology, Heidelberg, Germany
4) Hannover Medical School, Perinatal Epidemiology Unit, Hannover, Germany

The Mdm2 protein regulates the intracellular fate of the tumour suppressor protein p53. A functional polymorphism in the MDM2 promoter, SNP309, has been reported to determine Mdm2 expression and to modulate cancer penetrance in patients with Li-Fraumeni syndrome, a multiple-cancer syndrome that is often caused by p53 germline mutations. We therefore investigated whether the two common polymorphisms in MDM2 (SNP309) and in TP53 (Arg72Pro) affect the age at disease onset in breast cancer patients outside of Li-Fraumeni families. A hospital-based group of 1047 German breast cancer patients and 500 population controls were genotyped for both variants. The rare allele frequencies of MDM2*SNP309 were 0.36 among patients and 0.39 in controls (OR 0.86; 95%CI 0.74-1.01) and the TP53*Pro allele frequencies were 0.27 in the patient group and 0.29 among controls (OR 0.88; 95%CI 0.75-1.04), indicating that neither of both variants is associated with increased breast cancer susceptibility. Median ages at breast cancer diagnosis were 56, 57 and 57 years for MDM2 rare homozygotes, heterozygotes and common homozygotes, respectively, and the same result was obtained for TP53 genotypes Pro/Pro, Arg/Pro and Arg/Arg (ptrend= 0.1). Median age at diagnosis was 51 years for patients who were combined homozygotes for the two rare genotypes (n=10), and was 54 years for those patients who were homozygous for the p53 Pro allele and carried at least one high-producer allele of MDM2 (n=40, p=0.03). Our data suggest that MDM2 and TP53 polymorphisms do not exert gross effects on breast cancer susceptibility. Additional studies are required to confirm whether their interaction might modulate the age at onset of breast cancer.

P047

Breast cancer in patients carrying a CHEK2 mutation: a follow-up analysis

Meyer A.(1), Dörk T.(1), Sohn C.(2), Karstens J.H.(1), Bremer M.(1)
1) Hannover Medical School, Department of Radiation Oncology, Hannover, Germany
2) University of Heidelberg, Department of Gynaecology, Heidelberg, Germany

Cell-cycle checkpoint kinase 2 (CHEK2) is a central mediator of cellular responses to DNA damage including cell cycle arrest and apoptosis. Germline mutations of the CHEK2 gene confer an increased risk for breast cancer, but data about outcome and prognosis for such patients after standard multimodality treatment are scarce at present. We investigated the outcome of radiotherapy following breast-conserving surgery in a hospital-based group of German breast cancer patients with non-metastasized early-stage breast cancer (T1-2). 25 patients were heterozygous for one of three CHEK2 gene mutations (I157T, n=13; 1100delC, n=10; IVS2+1G>A, n=2). The comparison group consisted of 125 early-stage breast cancer patients (non-carriers) without a CHEK2 gene mutation. Median follow-up was 87 months. Actuarial metastasis-free and overall survival at 7 years were 64 % vs. 84 % (p = 0,045) and 69 % vs. 87 % (p = 0,097), respectively. In a multivariate step-wise Cox regression analysis, the presence of a CHEK2 mutation remained a significant discriminator for metastasis-free survival (p = 0,048). The results indicate that the presence of a CHEK2 mutation is associated with an adverse outcome. If confirmed in larger studies these data may serve as a basis for the development of future surveillance and treatment strategies taking into account the individual´s mutational status.

P048

Fluorescence in situ hybridization (FISH) on paraffin-embedded tissue sections for detection of chromosomal aberrations in thyroid tumors

Drieschner N.(1), Belge G.(1), Focken T.(1), Löschke S.(2), Bullerdiek J.(1)
1) Universität Bremen, Zentrum für Humangenetik, Bremen, Germany
2) Forschungszentrum Borstel, Klinische und experimentelle Pathologie, Borstel, Germany

Benign thyroid lesions are cytogenetically well described epithelial tumors. Translocations including the chromosomal regions 19q13.4 or 2p21 and trisomies of chromosome 7 are the most common chromosomal aberrations in benign thyroid tumors. These aberrations account for nearly 60% of all cytogenetic subgroups in benign thyroid tumors. Nevertheless these aberrations were mainly investigated either by cytogenetic or molecular-cytogenetic methods. Fluorescence-in situ-hybridization (FISH) opens up the possibility to detect these aberrations in both metaphase preparations as well as in interphase nuclei of thyroid tissues. In comparison to cytogenetic investigations FISH on paraffin-embedded tissues has the advantage to assign these aberrations to specific cell types within the tissue section. For the application of FISH on paraffin tissue sections of the thyroid the protocol has to be optimized. In detail pretreatment as well as digestion of the thyroid tissue prior to hybridization has to be improved. Here we present first results with the optimized FISH protocol for detection of chromosomal aberrations including trisomy of chromosome 7 and translocations of chromosome 2p21 and 19q13.4. The results were compared with data obtained by cytogenetic investigations of the corresponding thyroid tumors.

P049

Non genetics health professionals´ confidence in caring for individuals and families with genetic conditions: Preliminary data from the European GenEd study.

Nippert I.(1), Julian-Reynier C.(2), Harris H.J.(3), Ten Kate L.P.(4), Kristoffersson U.(5), Schmidtke J.(6), Benjamin C.(7), Challen K.(7), Harris R.(7)

1) Institut für Humangenetik/Frauengesundheitsforschung, Universitätsklinikum Münster, Münster, Germany
2) INSERM, Marseille, France
3) Brooklands Beacon Medical Practice, Manchester, United Kingdom
4) University Hospital, Amsterdam, Netherlands
5) University Hospital, Lund, Sweden
6) MHH, Hannover, Germany
7) St Mary's Hospital, Manchester, United Kingdom

Introduction: Non-genetics health professionals will increasingly be faced with individuals and families who have a need for genetics information and services. It is of increasing importance that non-genetic health professionals are ready to understand the role of genetics in health care and to provide adequate information and services.

Methodology: In order to assess the preparedness to care for individuals and families with a need for genetic services, 12 genetic service items were listed in a questionnaire that was distributed in 5 European countries and more than 4.500 health professionals responded. Service items ranged from taking a family history to obtaining informed consent before taking blood for DNA-tests. Respondents were asked to report their confidence in their ability to carry out these services.

Results: The majority of the respondents was confident or highly confident in identifying specialist genetic services in their area of practice (59%) or in taking a family history (55.9%), however, less than a third of the respondents were confident or highly confident in providing 8 out of 12 services. The lowest confidence rates were reported for counselling a woman for a predictive testing for Huntington´s disease (9.5%) and for identifying patient support groups for rare genetic disorders (19.7%). Professionals with continuing medical education in genetics were significantly more confident in providing 10 out of 12 services than professionals with no education in genetics. Significant differences for overall confidence scores were found between each speciality ranging from general practitioners (lowest) to obstetricians to paediatricians (highest).

Conclusion: The study results indicate that although there are significant differences in self-reported confidence between specialities, the confidence rates are low for most genetic service items included in this survey.

P050

A contiguous TBX5/ TBX3 deletion results in a surprisingly mild limb phenotype of radial and ulnar malformations

Borozdin W.(1), Bravo-Ferrer Acosta A.M.(1), Seemanova E.(2), Unger S.(1), Kohlhase J.(1)

1) Institut für Humangenetik und Anthropologie, Universität Freiburg, Freiburg, Germany
2) Institute for Biology and Medical Genetics, 2nd Medical Faculty, Charles University, Prague, Czech Republic

Heterozygous mutations in the gene TBX5, encoding a transcriptional activator, cause Holt-Oram syndrome (HOS), an autosomal dominant disorder characterized by radial ray upper limb malformations in combination with congenital heart defects and/ or cardiac conduction anomalies. Mutations in the closely linked gene TBX3 encoding a transcriptional repressor cause ulnar-mammary syndrome (UMS). HOS and UMS are thought to result from haploinsufficiency of TBX5 and TBX3, respectfully. While performing deletion screening for TBX5 by quantitative Real Time PCR we detected a heterozygous deletion in a family diagnosed with HOS. Further mapping showed that the 2.2 Mb deletion encompassed both TBX5 and TBX3. Clinical re-examination revealed mild limb features of ulnar-mammary syndrome, i.e. clinodactyly of the fifth digits and hypoplastic hypothenar eminences in the affected mother and both affected daughters. The radial involvement was also rather mild, with only one daughter showing unilateral radial aplasia and the two other affected family members having small but mostly functional hands. Other features of HOS in this family included cardiac septal and conduction defects, whereas UMS features were mammary hypoplasia, axillary gland and hair hypoplasia, and subglottic stenosis. We conclude that a heterozygous deletion for both TBX5 and TBX3 results in a rather mild limb phenotype.

P051

Prenatal diagnosis of harlequin ichthyosis using fetal skin biopsy and future facilitated possibilities after identification of underlying ABCA12 mutation

Hausser I.(1), Akiyama M.(2), Theile U.(3), Shimizu H.(2), Wolff G.(4), Rauskolb R.(5)

1) Universitäts-Hautklinik, EM-Labor, Heidelberg, Germany
2) Department of Dermatology, Hokkaido University Graduate School, Sapporo, Japan
3) Institute of Human Genetics,Mainz University, Mainz, Germany
4) Institute of Human Genetics, University Freiburg, Freiburg, Germany
5) Gynecological Department, Albert-Schweitzer-Hospital Northeim, Northeim, Germany

Harlequin ichthyosis is one of the most severe heritable keratinization disorders with armour-like hyperkeratoses, deep fissures and contractures, a grossly abnormal facial appearance, and usually perinatal death. Patients surviving under retinoid therapy suffer from a severe ichthyosiform erythroderma. Diagnosis is based on the specific morphological abnormality, that is a complete lack of normal keratinosomes/lamellar bodies and an accumulation of defective forms of these organelles, pivotal for terminal differentiation and epidermal barrier function, in the uppermost living cells as well as within horny scales. The condition is transmitted as an autosomal recessive trait and prenatal diagnosis is strongly sought by families with an affected child. With keratinization starting around hair follicle openings during mid-pregnancy, the ultrastructural diagnostic marker can be identified in fetal skin samples. We report prenatal diagnoses performed in week 20-23 EGA in eight pregnancies of five unrelated at-risk families. The diagnosis of harlequin ichhyosis could be excluded in five cases and confirmed in three cases. Recently, mutations within the gene coding for the lipid transporter ABCA12 have been found to underlie harlequin ichthyosis. An ABACA12 mutation was identified in the index case of one of the families. This will allow DNA-based early prenatal diagnosis in this family.

P052

Proximal trisomy 14 in a child with West syndrome

Koehler U.(1), Blanck C.(2), Ostendorf A.(1), Schönfeld B.(1), Holinski-Feder E.(1)

1) Medizinisch Genetisches Zentrum, München, Germany
2) Kinderkrankenhaus St.Marien, Landshut, Germany

We report on a cytogenetic investigation of a 1/2 year old girl with West syndrome.

Prenatal genetic counselling was implemented due to a polyarthritis and medication (methotrexate, cortisone, Arava, Vioxx) of the childs father. During pregnancy bleeding at 15th gestational week occurred and a hospitalisation was necessary because of an abortus imminens. At 39th gestational week the mother gave birth to a girl by caesarean section. Birth weight was 3390g, length 51cm and head circumference 31cm. Dysmorphic features were not observed.

At the age of 6 weeks an increasing restlessness occurred. MRT of the cranium showed moderate coarsening of the gyrus (fronto-parieto-temporale) with expanded liquor cavity and in other respects properly devoloped cerebral structures. The EEG was pathologic with hypersynchronous activity, tonic seizures and signs of hypsarrhythmy.

Cytogenetic investigation (GTG-banding and Multiplex-FISH) revealed an aneuploid karyotype with a duplication of the proximal part of the long arm of chromosome 14 resulting in a partial trisomy 14. Karyotype: 46,XX,der(14)dup(14)(q11.2q13) de novo. Karyotyping of the parents revealed normal results.

Despite the fact that the proximal region of chromosome 14 comprises many genes involved in brain development no correlation of this chromosomal imbalance and West syndrome or other forms of epilepsy has been shown to date.

P053

A rare case of a singleton pregnancy with an androgenetic, monospermic complete hydaditiform mole and co-twin fetus

Koehler U.(1), Neitzel B.(1), Holinski-Feder E.(1)

1) Medizinisch Genetisches Zentrum, München, Germany

We report a rare case of a complete hydaditiform mole (CM) monospermic in origin coexistent with a co-twin fetus. Hydaditiform moles originate from a genetically abnormal conception. Com-

plete hydaditiform mole are mostly androgenetic in origin. Mostly all cases of molar change coexistent with a co-twin fetus reported so far represent dizogotic twin pregnancies in which one fertilisation results in a CM and the other in a normal co-twin fetus.
The present study is a rare case in which a co-twin fetus coexisted with a complete hydaditiform mole which both originates from the same sperm cell.
Cytogenetic investigation of both the mole and the fetal cells revealed female karyotypes. Fetal cells were analysed after the pregnancy was terminated because of the potentiality of a trophoblast disease. However, molecular genetic testing with polymorphic markers showed that all the paternal alleles were identical in the mole, the placental and the fetal cells. No maternal alleles were detected in the molar cells. In addition, we found a biparental distribution of alleles in the placental and fetal cells.
To best of our knowledge this is the second case of an androgenetic and monospermic origin of both complete hydaditiform mole and co-twin fetus. The results displayed a singleton pregnancy with molar change in which one single sperm cell fertilised one oocyte. After fertilisation, an asynchronous mitotis of the male pronucleus could have given rise to a cell containing two identical male pronuclei and one female pronucleus. This resulted in two blastomeres, one of these comprised only a single male pronucleus which caused the complete androgenetic mole after a second failure of cytokinesis (e.g. endoreduplication).The other blastomere contained a male and a female pronucleus and gave rise to the co-twin fetus.

P054

Analysis of the role of hydroxysteroid dehydrogenase type 10 in embryonic development and neurodegeneration

Grziwa B.(1), Steinbeisser H.(1), Sass O.(2), Johannes Z.(1)
1) Humangenetik Heidelberg, Heidelberg, Germany
2) Uniklinik Freiburg, Freiburg, Germany

Hydroxysteroid dehydrogenase type 10 (HSD10) became an intriguing research topic when it was found to bind to the peptide Aβ, the central pathogenetic factor in Alzheimer`s Disease and to mediate its neurotoxic effects. HSD10, the product of the X-chromosomal HADH2 gene, is a soluble protein mainly residing in the mitochondria matrix. Its functions are only partly characterised. We previously showed that HSD10 is mutated in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, a disorder of isoleucine metabolism. Affected patients show an unusual neurodegenerative disease course, which we investigated further. All patients identified so far show missense mutations in the HADH2 gene. Although patients with the same mutation show a characteristic clinical pattern there is no strict correlation between enzyme activity and clinical symptoms. It is thus possible that neurodegeneration in MHBD deficiency and Alzheimer disease is mediated by an as yet unidentified function of HSD10. The importance of the protein beyond its role in isoleucine breakdown was also supported by the observation that HSD10 mutants (Scully) in Drosophila are embryonic lethal. For further characterization of HSD10 function in early embryonic development, we chose Xenopus as an animal model and identified HSD10 orthologues in Xenopus laevis and Xenopus tropicalis. By a developmental RT-PCR and whole mount in situ hybridization we monitored the expression of HSD10 in Xenopus embryos. The contribution of HSD10 to early embryogenesis will be analyzed in gain-of-function (GOF) and loss-of-function (LOF) experiments.

P055

Heterogeneity of geroderma osteodysplastica hereditaria and wrinkly skin syndrome in 22 patients from Oman and exclusion candidate loci.

Rajab A.(1), Kornak U.(2), Meyer B.(3), Hoffmann K.(2), Jaeken J.(4), Nürnberg P.(3), Mundlos S.(2)
1) Genetic Unit, DGHA, Ministry of Health, Muscat, Sultanate of Oman, Muscat, Oman
2) Institut für Medizinische Genetik, Charité Universitätsmedizin, Berlin, Germany
3) Cologne Center for Genomics (CCG), Universität zu Köln, Köln, Germany
4) Center for Metabolic Disease, University Hospital Gasthuisberg, Leuven, Belgium

We report 22 Omani patients from eight consanguineous families with Geroderma Osteodysplastica Hereditaria (GO MIM 231070), Cutis Laxa with Growth and Developmental Delay (CLGDD, MIM 219200) and Wrinkly Skin Syndrome (WSS, MIM 278250). Evaluation of the clinical and radiological features of WSS and CLGDD patients showed no significant differences. Although there are numerous identical features in WSS/CLGDD and GO patients we were able to delineate characteristic differences in early childhood which may facilitate the differential diagnosis. The WSS phenotype seen in our patients was uniform and evolved during early childhood. Most dramatic features were observed in the first 2-3 years of life with excessive skin folds, poorly growing sparse hair, hypodontia, caries and broken dental crowns, herniae, diverticulae, infantile emphysema, malposition of the feet, delayed bone age, osteopenia, hip dislocations and persistence of anterior fontanel beyond 3 years of age. A serum sialotransferrin type 2 pattern was found in three WSS cases but not in one investigated GO patient. Isoelectrofocusing of serum apolipoprotein CIII (marker for O-glycosylation) in these three patients was normal. This suggests that WSS is due to or secondary to a (still unexplained) N-protein glycosylation defect probably at the level of processing (CDG-IIx). Our GO cases display a wide interfamiliar heterogeneity and had unusual features of bleeding from gums and hematuria. Adult WSS and GO patients may be underdiagnosed and may present with early onset osteoporosis, tendency to fractures and vertebral collapse. We excluded four known loci for cutis laxa and WSS on 2q31, 5q23-q31, 7q11 and 14q32 by microsatellite screening.

P056

Three more cases with small supernumerary marker chromosome (sSMC) derived from chromosome 2 further confirm the suggested genotype/ phenotype correlation

Ziegler M.(1), Mrasek K.(1), Hickmann G.(2), Mazauric M.(2), Kozlowski P.(2), Fuster C.(3), Santos Verdaguer M.(3), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Praenatal-Medizin und Genetik, Düsseldorf, Germany
3) Univ Autònoma de Barcelona - Unitat de Biologia - Facultat de Medicina, Bellaterra - Barcelona, Spain

sSMC are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are equal in size or smaller than a chromosome 20 of the same metaphase spread [Liehr et al., 2004, Cytogenet Genome Res]. Thus, sSMC are characterizable only by molecular cytogenetics methods, i.e. fluorescence in situ hybridization (FISH). We present here the characterization of three more cases with a de novo sSMC(2) by means of (sub)centromere-specific multicolor-FISH = (sub)cenM-FISH. The obtained results support the recently suggested genotype-phenotype correlation for sSMC derived from chromosome 2 [Mrasek et al., 2005, J Histochem Cytochem; Liehr et al., 2006, Cytogenet Genome Res]: i.e. gains of the heterochromatin of chromosome 2 centromere and/or of the euchromatic region 2q11.2 are not associated with clinical abnormalities, while gains of centromere-near euchromatin in 2p and gains of regions larger than 2p11.2 lead to clinical symptoms. Case 1 was a prenatal case with a heterochromatic dic(2)(:p11.1→q11.1::q11.1→p11.1:) were a normal child was born. Cases 2 and 3 were postnatal. Case 2 is a 10 year old boy without major malformations and good gross motor skills but severe growth retardation and severe speech development delay; a min(2)(:p11.2→q11.1:) was characterized, confirming an adverse prognosis for gain of centromere-near material of the short arm of chromosome 2. Case 3 is another child with multiple malformations and a sSMC characterized as min(2)(:p11.1→q12.1:). This case confirmed a negative effect of partial trisomies larger than 2q11.2. In summary, the previously suggested genotype-phenotype correlation for sSMC derived from chromosome 2 is supported and confirmed by these three additional cases.

Supported in parts by SAF2003-03894, Dr. Robert Pfleger Stiftung and the Evangelische Studienwerk e.V. Villigst. The support of the family of case 2 is gratefully acknowledged.

P057

Narrowing the candidate region of Albright hereditary osteodystrophy-like syndrome by quantitative real-time PCR in 10 patients with terminal deletions of chromosome 2q

Auber B.(1), Zuther K.(1), Herold S.(1), Boehm D.(1)
1) Institute of Human Genetics, Goettingen, Germany

There have been a number of reports concerning patients presenting with an Albright Hereditary Osteodystrophy (AHO)-like syndrome, concomitant with a deletion of chromosome 2q. The

AHO-like syndrome has certain features, like short stature, mental retardation, short hands and feet due to an abnormal shortening of one or more metacarpals or –tarsals, especially the IVth and Vth. We analysed 10 patients with terminal deletions of various sizes of chromosome 2q with a real time-PCR based aproach. The smallest deletion found in a patient with AHO-like syndrom has a size of 3.4 Mb. Another patient suffering from only mild mental retardation and obesity, but not from a typical AHO-like syndrome has a deletion of 2.9 Mb. Our data indicate that the „AHO-like syndrome critical region" thus has a size of about 0.5 Mb and contains one gene, histone deacetylase 4 (HDAC4). No disease due to a mutation in HDAC4 is known up to now, but HDAC4-null mice display premature ossification of developing bones due to early onset chondrocyte hypertrophy. The deletion of HDAC4 might trigger the bony malformations in AHO-like syndrome patients.

P058

Towards measurement of clinical validity and utility of genetic testing in Europe

Javaher P.(1), Schmidtke J.(1)
1) Medizinische Hochschule Hannover, Institut fuer Humangenetik, Hannover, Germany

In recent years a great deal of attention has been paid at the national and international level to develop policies in the field of genetic service provision. Eurogentest aims at addressing the challenges through the creation of an European Network of Excellence (NoE) in genetic testing by involving leading experts from across Europe and developing the necessary infrastructure, tools, resources, guidelines and procedures that will structure, harmonise and improve the overall quality of all European genetic services. Towards achievement of these objectives, we examined access to and uptake of as well as funding policies and costs of genetic testing in 6 European countries with the aim of finding criteria to measure clinical validity and utility of genetic testing.

A survey questionnaire was created and responses were received from presidents of human genetics societies and other experts representing following countries with populations ranging from 5 to 80 million: Finland, Sweden, Portugal, UK, France and Germany.

The comparison between these countries indicates differences and similarities, such as a similar increase of DNA-based testing in Germany and the UK from 1999 to 2002 despite considerable differences in system regulation. In Sweden, DNA diagnostic and PND cytogenetic testing raised from 1996 to 2003, with PND testing increasing by a factor of 1.5. Whereas approximately 21,000 DNA-based tests per year are performed in Finland with a population of 5 m, only about 12,000 are performed in Portugal with a population of 10 m and at a relatively high price level in comparison with other countries.

There is as yet no consensus within the scientific community and among national and/or international health care providers how to measure clinical validity and clinical utility of genetic testing. Therefore further investigations are strongly needed and standards are to be developed to give general guidance.

P059

Knowledge about genetic information among participants of a pilot study on hemochromatosis screening

Stuhrmann M.(1), Nippert I.(2), Hoy L.(3), Schmidtke J.(1)
1) Medizinische Hochschule, Humangenetik, Hannover, Germany
2) Universität Münster, Humangenetik, Münster, Germany
3) Medizinische Hochschule, Biometrie, Hannover, Germany

Objectives: Genetic information is an important part of genetic screening programmes. We assessed the knowledge outcome among participants of a pilot study on genotype-based screening for hereditary hemochromatosis and evaluated the impact of genetic counselling, age, educational background and gender.

Methods: Questionnaires were sent to all participants for whom a homozygous (67) or heterozygous (485) result was obtained. Four questions were assessing knowledge of the following clinical and genetic aspects of hemochromatosis: prevention, penetrance, inheritance and impact of heterozygosity. Post test genetic counselling had been offered to all homo- and heterozygotes.

Results: The overall uptake rate of counselling was low (19.9%). The highest (72.5%) and lowest rates (31.7%) of correct responses were given for the questions regarding prevention and penetrance, respectively. Younger participants gave significantly ($p<0.001$) more correct answers on prevention, penetrance and heterozygosity than older participants; and counselled individuals more often gave correct answers on prevention and heterozygosity than those without counselling (87.7% vs 70%, $p<0.05$; 63.1% vs 34.0%, $p<0.001$). Significantly more correct answers to these questions were given by older individuals who underwent counselling than by those without (85.4 vs 62.8%, $p<0.01$; 56.1 vs 24.2%, $p<0.001$). Higher educational status significantly influenced the correctness of responses regarding inheritance and heterozygosity (55.8 vs 43.1%, $p<0.05$; 48.7 vs 34.2%, $p<0.01$). Gender was not associated with correctness of answers.

Conclusion: Understanding the results of a genetic screening program is highly depending on age and – less pronounced - on the educational background of the participants. Genetic counselling is a crucial component of the information process and can significantly raise the level of understanding in older individuals participating in genetic screening.

P060

Isolated macrocephaly requires mutational screening of the PTEN gene including exclusion of mosaicism

Wieczorek D.(1), Gillessen-Kaesbach G.(1), Aretz S.(2), Friedl W.(2)
1) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany
2) Institut für Humangenetik, Universitätsklinikum Bonn, Bonn, Germany

The tumor suppressor gene PTEN encodes a lipid phosphatase that mediates cell cycle arrest and apoptosis. Somatic PTEN inactivation is found in sporadic neoplasia; overlapping germline mutational spectra are reported in patients with Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS) and Proteus-like syndrome (PSL). To summarise these conditions a unifying molecular-based diagnosis, PTEN Harmartoma Tumor Syndromes (PHTS), was suggested. The classical phenotypes of CS, BRRS, PS and PSL are well characterised, but little is known on the early-childhood and the mosaic phenotypes. Here we present one sporadic, 4-year-old patient with macrocephaly, motor developmental delay and lipoma, developing during the first year of life, who carries a de novo missense mutation c.389G>A in exon 5 (Arg130Gln) of the PTEN gene. In addition, we report on a 14-month-old boy with macrocephaly and a hemangioma at one leg with the missense mutation c.737C>T (Pro246Leu) in exon 7 of the PTEN gene. The father of the second patient presents with macrocephaly and multiple lipomas, however, the mutation identified in his son was not detected in DNA from a venous blood sample, but was found in DNA from buccal swabs pointing to somatic mosaicism. Initially, we considered the father's lipomas to be part of the clinical spectrum of PHTS. However, the paternal grandfather, who also display lipomas, does not carry the mutation. Therefore, lipomas are a coincidental finding in this family.

We conclude that macrocephaly might be the only clinical feature in patients of young age carrying a PTEN mutation and in older patients with somatic mosaicism of PTEN.

P061

Six patients with infantile hepatocerebral syndrome and mitochondrial DNA depletion caused by mutations of the deoxyguanosine kinase (DGUOK) gene

Freisinger P.(1), Fütterer N.(1), Lankes E.(1), Santer R.(2), Horvath R.(3)
1) Kinderklinik TU München, Stoffwechselzentrum, München, Germany
2) Universitätsklinikum Eppendorf, Kinderklinik, Hamburg, Germany
3) Städt. Klinikum GmbH, Institut für mitochondriale Genetik, München, Germany

Pathogenic mutations in the DGUOK gene were identified in 6 patients with the hepatocerebral from of mtDNA depletion. We report on clinical, neuroradiological, histological and genetic findings in these cases. All patients showed severe hepatopathy, however the involvement of other organs (skeletal muscle, brain) was very variable. Five new unpublished mutations (one of them occurring twice) and two previously described mutations were identified. In two patients three different mutations were detected all affecting the start methionine, suggesting a mutational hot spot. From our collective of 12 cases with infantile hepatoencephalopathies with mtDNA depletion sequencing of DGUOK revealed pathogenic mutations in 6 patients suggesting that this gene defect is a relatively frequent cause of the hepatic form of mitochondrial DNA depletion syndrome. One of our patients underwent liver transplantation and unexpectedly, additionally to a diffuse hepatopathy, a hepatocellular carcinoma was detected implying a possible link between mtDNA depletion and tumorigenesis.

P062

Idiopathic Adulthood Ductopenia in a family of six affected and five healthy siblings

Gotthardt D.(1), Runz H.(2), Imparato S.(1), Flechtenmacher C.(3), Schirmacher P.(3), Zschocke J.(2), Sauer P.(1)
1) University Hospital Heidelberg, Internal Medicine IV, Heidelberg, Germany
2) Department of Human Genetics, Heidelberg, Germany
3) Department of Pathology, Heidelberg, Germany

Idiopathic Adulthood Ductopenia (IAD) is a rare liver disease defined as a paucity of the intrahepatic bile ducts lacking other causes for this phenomenon. The natural history ranges from asymptomatic to progressive liver disease, eventually leading to liver cirrhosis, liver failure and death. Its relationship to the non-syndromic paucity of intrahepatic bile ducts in infancy is under debate. So far only a few dozen cases have been described in the literature, most of them were sporadic and only a minority presented as familial cases. Here we present a Transsilvanian family of eleven siblings, ten female and one male. The parents showed no signs of liver disease and normal laboratory parameters.
Consanguinity could not be excluded. The son and one daughter died during childhood at the age of 7 and 5 resp due to liver failure. Four of the remaining nine children showed abnormal liver parameters; one of these four died of liver cirrhosis at the age of 44, one was referred to liver transplantation and two have elevated GGT, AP, ALAT, ASAT, but show no signs of impaired synthesis and bilirubin clearance. Histological examination showed paucity of intrahepatic bile ducts, resembling most closely a ductal plate malformation. All examinations revealed no hint to a liver disease of known etiology. The family consented to a genetic analysis of this disease and a linkage analysis has been initiated. Here we present the clinical and genetic data acquired so far.

P063

Unusual keratinisation disorder in a 7-year-old boy: A variant of the Olmsted syndrome?

Herdemann S.(1), Hausser I.(1), Wortmann G.(2), Hartschuh W.(1), Enk A.(1), Jappe U.(1)
1) Universität Heidelberg, Hautklinik, Heidelberg, Germany
2) Deutsches Wollinstitut DWI, Aachen, Germany

A 7year-old boy with nonconsanguineous healthy parents and siblings presented with painful focal palmoplantar hyperkeratoses, persisting and slightly growing since early infancy that forced him to crawl on his knees instead of walking upright. He suffered from hair- and naildystrophy, periorificial, periauricular as well as perianal skin lesions and behavioural disturbance. Light microscopy of the hair shafts revealed trichorrhexis nodosa and, under polarized light, a “tiger tail pattern” suspicious of trichothiodystrophy. Amino acid analysis revealed decreased cystein and increased lysine contents. However, the overall chemical composition was not characteristic for trichothiodystrophy. Histopathology and electron microscopy of a specimen from the plantar area revealed massive hyperkeratosis, acanthosis, and papillomatosis as well as quantitatively disturbed keratinisation. Local treatment with salicylic acid 10% in petrolatum and daily mechanical reduction of the hyperkeratoses improved the symptoms. However, these measures only induce intermittent relief as long as the patient and the family are sufficiently compliant. Though presenting overlapping clinical symptoms as well as histopathological features with trichothiodystrophy, our diagnosis is Olmsted syndrome. Olmsted syndrome is a rare keratinisation disorder which was first described in 1927. It is characterized by the combination of bilateral palmoplantar transgredient keratoderma and periorificial keratotic plaques with possible involvement of other ectodermal derivatives including follicular keratoses, linear keratotic streaks, thin nails or onychodystrophy, leukokeratosis of the oral mucosa, diffuse alopecia and hair shaft defects. The disease mostly occurred spontaneously although possible autosomal and X-linked recessive transmissions have been suggested. Pathogenesis as well as the relation to the ectodermal dysplasia syndrome complex and trichothiodystrophy is still not fully understood.

P064

Long term prognosis of early onset autosomal dominant polycystic kidney disease prenatally diagnosed by ultrasound

Mavany M.(1), Rudnik-Schöneborn S.(1), Bergmann C.(1), Zerres K.(1)
1) Institut für Humangenetik, RWTH Aachen, Aachen, Germany

Although the age of clinical onset of autosomal dominant polycystic kidney disease (ADPKD) is typically in the third to fifth decade of life, about 2 % of gene carriers present with severe early manifestation in childhood or even prenatally. In 1982 the first case of prenatally diagnosed autosomal dominant polycystic kidney disease was published (Zerres et al., Lancet 1982; 2:988). The diagnosis was made in the 33rd gestational week in a 27-year-old woman, when distinctly enlarged fetal kidneys with predominantly centrally located cystic structures were seen on sonography. In the context of the family history ADPKD was suspected. The pregnant woman´s 53-year-old mother was on hemodialysis since three years. The diagnosis of ADPKD in the young mother, who had no knowledge about her risk so far, was made post partum. At that time no information of in utero onset ADPKD was available. Genetic counselling of the couple covered all thinkable consequences, including necropsy in case of neonatal death. To our surprise the clinical course was much more benign than was considered by the fetal manifestation. Hemodialysis started only recently at the age of 24 years. ADPKD was also diagnosed prenatally in the younger sister, being on hemodialysis from the age of 19 years, which underlines the observation of comparable clinical severity in affected siblings. All affected family members showed the same PKD1-mutation (E2771K). Meanwhile much more data about the clinical course of in utero diagnosed ADPKD is available. In comparison to prenatally diagnosed autosomal rezessive PKD the outcome is often more favourable, although lethal manifestations can occur. We observed two severely affected children of a father with typical ADPKD. Both sibs showed anhydramnions and a Potter phenotype leading to death after birth. A review including own and published data of around 100 cases with in utero onset ADPKD will be given.

P065

Two complementary recombinant chromosomes 5 in a healthy woman

Bartsch O.(1), Ali Ergun M.(2), Balci S.(3), Kan D.(2), Eggermann T.(4), Kotzot D.(5)
1) Institut für Humangenetik, Universitätsklinikum, Mainz, Germany
2) Department of Medical Genetics, Gazi University, Ankara, Turkey
3) Department of Clinical Genetics, Hacettepe University Children’s Hospital, Ankara, Turkey
4) Institut für Humangenetik, Universitätsklinikum, Aachen, Germany
5) Abteilung für Klinische Genetik, Medizinische Universität, Innsbruck, Austria

We report a healthy woman with two abortions who is a carrier for a rare heterozygous double recombinant of an inv(5) chromosome, karyotype 46,XX,rec(5)dup(5p)inv(5)(p13q22),rec(5)dup(5q)inv(5)(p13q22). Her father had a 46,XY,inv(5)(p13q22) karyotype; his consanguineous wife had died. Molecular investigation of 11 highly polymorphic markers spanning chromosome 5 revealed biparental inheritance for two markers (D5S406, D5S681) on 5p15.3 and 5q13.1, and an allele constellation not compatible with paternal heterodisomy for marker D5S623 on 5q11.2. Eight markers were not informative. Three mechanisms of formation have to be discussed: First, fertilization of a normal oocyte by a sperm carrying the two recombinant chromosomes 5, followed by postzygotic recombination between the normal maternal homologue and the rec(5)dup(5p), and by loss of the mitotically recombined maternal homologue, leading to segmental paternal heterodisomy 5q13?qter (trisomic rescue). Second, postzygotic recombination in a 46,XX,inv(5)(p13q22) zygote resulting in the 46,XX,rec(5)dup(5p)inv(5)(p13q22),rec(5)dup(5q)inv(5)(p13q22) karyotype, followed by absence of the original cell line in lymphocytes. Third and most likely, both parents were inv(5) carriers and complementary recombinations in maternal and paternal meiosis resulted in a zygote with two recombinant chromosomes 5. Our patient denied any further studies but later reported the birth of a phenotypically normal child. This is the first report known to us of complementation by two non-homologous recombinant chromosomes in a phenotypically normal woman, and the first example of a child born to a carrier of complementary recombinant chromosomes.

P066

Primary ciliary dyskinesia in a large family maps to chromosome 7p15.3 harbouring DNAH 11

Schwabe G.(1), Niggemann B.(2), Wahn U.(2), Mundlos S.(3), Hoffmann K.(3)
1) Klinik f. Allgemeine Pädiatrie, Charité, Campus Virchow, Berlin, Germany
2) Klinik für Pädiatrie m. S. Pneumologie und Immunologie, Charité, Campus Virchow, Berlin, Germany
3) Institut für Medizinische Genetik, Charité, Campus Virchow, Berlin, Germany

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder characterized by ciliary dysfunction with marked genetic heterogeneity. Patients with PCD exhibit bronchiectasis and upper airway infections, 50 per cent of the patients have situs inversus (Kartagener syndrome). We

here describe a family with six affected and five unaffected children from not consanguineous parents. One affected son exhibits a situs inversus totalis, all other members have a situs solitus. Pulmonary symptoms are clinically heterogeneous ranging from recurrent pneumonia to merely increased incidence of sinusitis. Ciliary beat frequency (CBF) was analysed by light microscopy from nasal or bronchial brush specimen. The CBF ranged from 0 to 11 Hz and was reduced in affected individuals, corresponding to the susceptibility and frequency of pulmonary infections. A linkage analysis was performed for five genetic loci harbouring genes associated with PCD. The family analysed showed linkage to chromosome 7p15.3 harbouring DNAH11, encoding axonemal heavy chain dynein type 11. To date only one DNAH11 nonsense mutation has been described in a patient with PCD and uniparental disomy of chromosome 7. DNAH11 mutation analysis in the described kindred is currently under way.

P067

Evidence for polygenic inheritance of premature ovarian failure

Ledig S.(1), Jakubiczka S.(1), Häusler G.(2), Wieacker P.(1)
1) Institut für Humangenetik, Molekulargenetik, Magdeburg, Germany
2) Universitätsklinik für Kinder- und Jugendheilkunde, Klin. Abteilung für Allgemeine Pädiatrie, Wien, Austria

Ovarian dysgenesis in women with XX karyotype can be caused by homozygous or compound heterozygous mutations in the FSHR gene which have been described almost exclusively in the Finnish population. Furthermore, it has been shown that BMP-15, a X-linked member of the TGF-β super family, is another important factor for folliculogenesis. BMP-15 is secreted by oocytes and promotes granulosa cell (GC) proliferation independently of FSH. Recently, a heterozygous mutation in the BMP-15 gene affecting the pro domain of the corresponding protein has been found in two italian sisters with hypergonadotropic ovarian failure.
Here, we report on a 25 years old woman with premature ovarian failure due to an arrest of folliculogenesis. Mutational analysis of FSHR gene revealed a previously described heterozygous mutation in exon 6 (Ile160Thr) which impairs cell surface expression. Since one heterozygous mutation in the FSHR gene is not sufficient for developing hypergonadotropic ovarian failure, screening of further candidate genes has been performed in this patient. A heterozygous substitution (Ala180Thr) was identified which destroys a β-sheet structure in the pro region of BMP15. While the FSHR mutation has been inherited from the patient's father, the unaffected mother is the carrier of the BMP15 missense mutation.
We propose that double heterozygosity in the FSHR and BMP15 genes are causative for the phenotype of our patient. Amino acid substitution of BMP15 decreases GC proliferation in the developing follicles. This effect may be amplified by reduced expression of FSHR in GC due to mutation Ile160Thr. However, the patient's sister, who exhibits normal ovarian function, is also heterozygous for the same mutations in the FSHR and BMP15 gene. The discrepancy of the phenotypes of the two sisters may be explained by different patterns of X inactivation. Furthermore, gene defects in other genes effective in folliculogenesis can not be excluded.

P068

De novo EFNB1 gene deletions in two patients with CFNS

Jakubiczka S.(1), Weidner C.(1), McDonald-McGinn D.(2), Zackai E.H.(2), Kreß W.(3), Collmann H.(4), Wieacker P.(1), Wieland I.(1)
1) Department of Human Genetics, University Hospital, Magdeburg, Germany
2) Clinical Genetics Center, Children's Hospital of Philadelphia, Philadelphia, United States of America
3) Department of Human Genetics, University Hospital, Würzburg, Germany
4) Children's Hospital, University Hospital, Würzburg, Germany

Craniofrontonasal syndrome (CFNS; MIM304110) is characterised by hypertelorism, strabismus, coronal synostosis, craniofacial asymmetry including orbital asymmetry, bifid nasal tip, grooved nails, abnormalities of the thoracal skeleton, as well as wiry and curly hair. Females are more severely affected than males, who commonly show only mild manifestations such as hypertelorism. Recently, we have demonstrated that mutations of EFNB1 in Xq12 cause CFNS. Here, we report on the detection of deletions encompassing the whole EFNB1 gene in two sporadic CFNS patients. Both girls exhibit typical signs of CFNS. Sequence analysis of the coding part of EFNB1 did not reveal any alteration in both patients. For further elucidation, quantitative Southern-blot analysis was performed and in both patients half of the normal female doses of EFNB1 was detected, indicating that gene deletion is the cause of CFNS. Estimation of the deletion intervals was carried out by combined microsatellite and quantitative Southern-blot analysis. In patient 1 the deletion comprises a minimum of 1,3 Mbp and a maximum of 2,6 Mbp of DNA, while the deletion in patient 2 encompasses approximately 40 - 60 kbp. OPHN1 involved in X-linked mental retardation 60 (MRX60; MIM 300486) and neighbouring EFNB1, is included in the deletion interval of patient1 suggesting a contiguous gene syndrome. Interestingly, both deletions have occurred on the paternal genetic background. So far, we have detected 12 point mutations and one previously reported deletion of exons 2 - 5 of EFNB1 in familiar CFNS. 31 small sequence changes and the two de novo deletions reported here, have been observed in sporadic CFNS patients. Thus, we detected EFNB1 deletions in 6,8 % of our CFNS patients. Our data indicate that deletions are not an uncommon event in the aetiology of this X-linked disease.

P069

Unusual molecular finding in two sisters with juvenile-onset vitelliforme macular dystrophy type 2 (= VMD2)

Welling B.(1), Stoehr H.(2), Weber B.H.F.(2), Horst J.(1)
1) Institut für Humangenetik, Westfälische-Wilhelms-Universität Münster, Münster, Germany
2) Institut für Humangenetik, Universität Regensburg, Regensburg, Germany

We report on two sisters with juvenile-onset vitelliforme macular dystrophy type 2 (= VMD2, also known as Best disease) - a central retinopathy primarily characterised by an impaired function of the retinal pigment epithelium - who presented at the age of eleven respective six years for genetic counselling at our institute. The girls were accompanied by their 35 year old father and their younger, four year old brother. The father, the 32 year old mother and the little brother reported no vision problems. The mother wears glasses only for reading. Extensive eye examination in both girls were performed at the Eye Clinic Münster and DNA testing was done in the VMD2-gene. The DNA analysis for the older girl revealed homozygousity for the mutation 140 G>A in codon 47 of exon 2 of the VMD2-gene. The consequence is an amino acid exchange from arginine to histidine. The same homozygous mutation was also found in the younger sister. To our knowledge only one patient has been described in the literature exhibiting this mutation, although with heterozygousity for the alteration. This patient was suffering from an adult form of Best disease. Because of the girls´ uncommon analyses results further clinical examinations and observations will be of interest. An assumed consanguinity of the parents was confirmed. Genetic analyses revealed heterozygousity for the above mentioned mutation in both parents.

P070

Hereditary hyperferritinaemia-cataract syndrome: An important differential diagnosis of hereditary hemochromatosis?

Gabriel H.(1), Gencik A.(1)
1) Zentrum fuer Medizinische Genetik, Osnabrueck, Germany

The hyperferritinaemia-cataract syndrome (HHCS) is an autosomal-dominant disorder characterized by high serum ferritin levels with normal iron saturation and early onset bilateral cataracts.
HHCS is caused by various mutations within the iron responsive element (IRE) of the L-ferritin gene leading to increased translation of L-ferritin mRNA.
The mutation A40G was found in two unrelated families. This mutation was first identified in a french family and is known as the "paris" mutation.
Our first patient was a 37 old women originally from Russia. She has an elevated ferritin level and bilateral cataract. The same mutation was detected in a 44 year old german man and his 7 year old daughter. In a 34 year old german woman with a serum ferritin level of 1330 µg/l the mutation G32A "Pavia 1" was found. As a result of a heterozygous Cys282Tyr mutation in the HFE gene the patient was initially misdiagnosed with hereditary hemochromatosis. The treatment of the suspected hemochromatosis by therapeutic phlebotomy resulted in a severe anaemia. Genetic counseling revealed a positive family history of cataracts. In conclusion, physicians should be aware that an isolated hyperferritinaemia is not a clear indication of iron overload , even if there is a mutation in the HFE gene. All cases of hyperferritinaemia should be checked for a family history of cataracts.

P071

Changing phenotype in a girl with diploid/triploid mosaicism (mixoploidy): a 14 year follow-up

Rittinger O.(1), Fauth C.(2), Kotzot D.(2), Kronberger G.(1)
1) Paracelsus Medizinische Privatuniversität Salzburg, Universitätsklinik für Kinder-und Jugendheilkunde, Salzburg, Austria
2) Medizinische Universität Innsbruck, Department für Medizinische Genetik, Molekulare und Klinische Pharmakologie, Innsbruck, Austria

Objectives: Diploid-triploid mixoploidy is a rare syndrome with a subtle but clinically recognizable phenotype, characterized by mental retardation, intrauterine and postnatal asymmetric growth failure, hypotonia, truncal obesity, syndactyly, camptodactyly and pigmentary dysplasia of the skin. Early craniofacial features are down-slanting palpebral fissures and a broad forehead. Mixoploidy may be underdiagnosed because in about 70% the triploid cell line is only seen in fibroblasts. Lymphocytes, however, show hardly more than 5% triploid cells. We report on a mildly retarded 14 y old girl diagnosed only recently due to a highly characteristic symmetric camptodactyly as reported in patients with Camera-Marugo-Cohen syndrome, some of them later on diagnosed with mixoploidy. Documentation of the changing phenotype was possible since early infancy. During the first years of life the girl presented with primordial growth retardation and a relatively large forehead, a phenotype reminiscent of Silver Russell Syndrome. Parallel to the onset of myoclonus epilepsy, sustained weight gain was observed. At school age, limitations of joint mobility and camptodactyly became more obvious. Recently she underwent surgery due to severe rotated thoracolumbal scoliosis. Lab. By cytogenetic and FISH analysis a triploid cell line was shown to be present in skin fibroblasts, urothelial cells and buccal smear cells. Microsatellite analysis demonstrated the additional haploid genome to be of maternal origin.

Conclusion: Diagnosis in mixoploidy is mainly due to the recognition of a typical phenotype in early infancy, changing to a syndromic obesity state with minor dysmorphic signs like camptodactyly. The examination of easily accessible tissues like urine or buccal smear cells may confirm the diagnosis without any invasive procedures. Moreover, clinical overlap with the phenotype of mat UPD 7 might indicate an additional epigenetic background.

P072

Identification of a novel 782InsC mutation in the WISP3 gene in a patient with progressive pseudorheumatoid dysplasia (PPD)

Gabriel H.(1), Zabel B.(2), Spranger J.(2), Gencik A.(1)
1) Zentrum fuer Medizinische Genetik, Osnabrueck, Germany
2) Johannes-Gutenberg Universität Mainz, Universitäts-Kinderklinik Mainz, Arbeitsgemeinschaft für Klinische Genetik, Zytogenetik & Molekulargenetik, Mainz, Germany

Progressive pseudorheumatoid dysplasia (PPD) is a rare autosomal rezessive disorder characterized by generalized platyspondyly, restricted joint mobility, osseous swelling of the interphalangeal and other joints and osteoporosis.
PPD is caused by mutations in the WISP3 gene, a member of the CCN gene family which encodes secreted proteins with function in cell growth and differentiation. So far only 11 different mutations were found mainly in patients from the Middle-East.
We hereby report the molecular study of the WISP3 gene in a consanguineous family originally from turkey.
The proband was a 15 year old boy presented with a 10-year history of polyarthritis. He had a limited range of motion, swelling of multiple joints and stiffness.
At the age of 8 years Duchenne muscular dystrophy was suspected. Muscle histology revealed no characteristic features of muscular dystrophy. Mutation analysis of the dystrophin gene was not performed. Futher examinations lead to the diagnosis of an “unclassified form of spondylo-metaphyseal dysplasia”. At the age of 12 years additional radiological tests were performed leading to the suspection of a progressive pseudorheumatoid dysplasia (PPD). Sequencing of the WISP3 gene revealed a homozygous insertion of a cytosine at position 782 resulting in a frameshift and premature stop codon at nucleotides 789-791. Sequence analysis of the parents revealed that both parents where heterozygous carriers of the 782InsC mutation.

P073

Clinical and genetic data of two sporadic spinal neurofibromatosis patients with malignant peripheral nerve sheath tumours

Wimmer K.(1), Fauth C.(2), Machherndl-Spandl S.(3), Beiglböck H.(1), Messiaen L.(4), Fonatsch C.(1)
1) Medizinische Universität Wien, Abteilung Humangenetik, Wien, Austria
2) Medizinische Universität Innsbruck, Department für Medizinische Genetik,, Innsbruck, Austria
3) Krankenhaus Elisabethinen, Linz, Austria
4) University of Alabama at Birmingham, Department of Genetics, Birmingham, Al, United States of America

Spinal neurofibromatosis (SNF), an alternate form of NF1, is characterized by a severe neurological phenotype with multiple spinal neurofibromas concomitant with a strikingly mild dermal involvement frequently limited to café au lait spots (CLS). SNF is clearly linked to the NF1 gene, but the genetic mechanisms leading to this peculiar phenotypic expression are largely unknown. We present two unrelated male patients with a SNF phenotype. Both patients were not suspected to suffer from NF1 until they presented with neurological deficits and NF1-related malignancies in their forties. Patient A initially presented with progressive peroneus paresis. A few months later a rapidly growing malignant peripheral nerve sheath tumour (MPNST) in the right thigh was diagnosed. MRI examination revealed multiple symmetrical spinal neurofibromas as well as an asymptomatic pheochromocytoma. Dermal features in this patient were restricted to 7 CLS. Patient B had neither CLS nor any other dermal sign of NF1. At the age of 42 he presented with incomplete paraparesis. Multiple spinal neurofibromas including an MPNST at the level L4 were detected by MRI. A splice mutation leading to the skip of NF1 exon 43 was identified in patient A. In patient B two NF1 alterations were identified, i.e. a Cys to Arg change in exon 18 and loss of one aminoacid in the second last NF1 exon. According to their nature and location these three novel NF1 alterations appear unusually mild compared with classic NF1 mutations, supporting the notion that the mild dermal phenotype of SNF may be related to a possible residual function of the mutated gene product. Further, uncovering the origin and location -in cis or in trans- of the two alterations found in patient B has the potential to shed further light on the genetic mechanisms leading to the phenotype of SNF and will be discussed.

P074

Longterm follow-up of three patients with Desbuquois syndrome

Albrecht B.(1), Schaper J.(2), Zerres K.(3), Gillessen-Kaesbach G.(1)
1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany
2) Institut für Diagnostische Radiologie, Kinderradiologie, Universitätsklinikum, Düsseldorf, Germany
3) Institut für Humangenetik, RWTH, Aachen, Germany

Desbuquois syndrome (MIM 251450) is a rare skeletal dysplasia with short stature, joint dislocations and premature ossification of carpal and tarsal bones. Two types of Desbuquois syndrome can be distinguished. Type 1 is characterized by additional hand malformations with dislocation of the fingers and the existence of an additional ossification centre of the second metacarpal bone. Type 2 shows no hand malformations. While the localisation of the gene responsible for type 1 Desbuquois syndrome is known (17q25.3), no linkage for Desbuquois syndrome type 2 was found. We report on three patients with Desbuquois syndrome type 2. Two patients were followed for more than 10 years. Both developed severe joint dislocations, which necessitated surgical intervention. The third patient showed genua vara already at the age of 2 years. All patients have severe short stature becoming more pronounced with age. The facial gestalt is similar in all three patients with a flat midface, a small nose and large protruding eyes. The joints are hyper extensible. All three patients have mild to moderate mental retardation. X-rays of all patients showed accelerated carpal bone maturation, short long bones, irregular ossification of the vertebral bodies, and a characteristic appearance of the femoral neck, described as “Swedish key appearance”. We will demonstrate the deterioration of the disease, and the therapeutic interventions. By homozygosity mapping, Faivre et al. (2003) obtained a maximum lod score of 4.61 at theta = 0.0 for locus D17S1806 supporting genetic homogeneity of type 1 Desbuquois syndrome. In type 2 Desbuquois syndrome Faivre et al. (2004) found heterozygosity for 9 microsatellite DNA markers from the 17q25.3 region, thus excluding the region as the locus in Desbuquois type 2 and demonstrating genetic heterogeneity. Molecular investigations in our three patients are ongoing (Valerie Cormier-Daire, Paris, Hôpital Necker-Enfants Malades).

P075

Lacrimoauriculodentodigital syndrome in two patients: Delineation of phenotype

Naumchik I.(1), Rumyantseva N.(1), Mironova S.(1), Kulak V.(1)
1) Republican Medical Center 'Mother&Child', Minsk, Belarus

Lacrimoauriculodentodigital syndrome (LADD; OMIM 149730) is a rare autosomal dominant disorder first described by Levy (1967) and Hollister (1973) and presenting with lacrimal malformations, cupped ears, hearing loss, dental anomalies, absent salivary glands, radial ray defects and genitourinary abnormalities. We report on the two unrelated girls with typical features of LADD and some additional anomalies. MM – the first child of young, healthy nonconsanguineous couple, her younger sister is in good health. At 6 years proposita showed mild mental and moderate growth retardation, microcephaly, triangular face, obstruction of nasal lacrimal ducts, simple cupped pinnas, normal hearing, peg-shaped lateral incisors, dry skin, hypotrichosis, clinodactyly of V fingers, normal thumbs, syndactyly of the I-II toes of right foot, broad left hallux, systolic murmur and US-detected cyst of right kidney.
KE – the only child of young healthy, unrelated parents. At 23 months she presented mild mental and moderate growth retardation, microcephaly, triangular face, obstruction of nasal lacrimal ducts, simple cupped ears without hearing loss, hypotrichosis, long fingers with V clinodactyly, partial II-III syndactyly of the toes and broad hallux, atrial and ventricular septal defects were surgical corrected at 12 months.
Chromosome analyses and metabolic screening results were normal in both girls. The clinical findings in our two patients with LADD included well-known abnormalities of lacrimal ducts, ears and teeth, absence some of typical features such as radial ray defects and hearing loss, abnormality of toes and kidneys documented only in some patients, and previously undescribed anomalies: microcephaly, growth and mental retardation, heart defect and hypotrichosis. Our findings in two patients will be discussed in two hypotheses: is there extended phenotype of LADD or there is a new MCA/MR-syndrome.

P076

Disruption of ARHGEF9 is associated with cognitive deficits: identification of a new candidate for X-linked mental retardation

Kalscheuer V.(1), Mentzel C.(1), Kübart S.(1), Tzschach A.(1), Gecz J.(2), van Bokhoven H.(3), Moraine C.(4), Fryns J.-P.(5), Chelly J.(6), Selicorno A.(7), Dalpra L.(8), Ropers H.-H.(1)
1) Max Planck Institute for Molecular Genetics, Human Molecular Genetics, Berlin, Germany
2) Women's and Children's Hospital, University of Adelaide, Genetic Medicine and Pediatrics, Adelaide, Australia
3) University Medical Centre, Human Genetics, Nijmegen, Netherlands
4) Services de Génétique -INSERM U316, CHU Bretonneau, Tours, France
5) Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium
6) Institut Cochin de Génétique Moleculaire, CNRS/INSERM, Paris, France
7) Clinica Pediatrica Università di Milano, Ambulatorio di Genetica Clinica, Milano, Italy
8) University of Milano-Bicocca, Experimental, Environmental Medicine and Medical Biotechnologies, Monza, Italy

Disease-associated balanced chromosome rearrangements have been instrumental in the identification of numerous genes for cognitive deficits. Here we report on the characterization of a de novo balanced t(X;9)(q11.3;q11.21) translocation in a girl with non-syndromic mental retardation. Fluorescence in situ hybridisation with BAC clones selected from both breakpoint regions indicated that on the X chromosome, the Cdc42 guanine nucleotide exchange factor (GEF) 9 (ARHGEF9) gene is truncated. Subsequent expression studies showed that full-length ARHGEF9 transcripts are absent in the patient cell line, suggesting that loss of functional ARHGEF9 is responsible for the clinical phenotype. To date, screening of a panel of unrelated patients with X-linked mental retardation did not result in additional mutations. However, disruption of ARHGEF9 has been previously associated with non-syndromic mental retardation in a female patient with inv(X)(q11.3;q26.3) (Marco et al., ASHG 2005), and a missense mutation in this gene has been reported in a patient with hyperekplexia and epilepsy (Harvey et al., J Neurosis, 2004). ARHGEF9 encodes collybistin (hPEM-2), a brain-specific GEF, which may regulate actin cytoskeleton dynamics and cell signalling. Together, these data suggest that ARHGEF9 is a novel X-chromosomal RhoGEF which is required for normal functioning of the human brain.

P077

„Agnathia-otocephaly": Report of two new unrelated cases and overview

Rumyantseva N.(1), Polityko A.(1), Mironova S.(1)
1) Republican Medical Center "Mother&Child", Minsk, Belarus

„Agnathia–otocephaly"– clinically recognizable developmental field defect due to incomplete development of mandibular process of first branchial arch (Bixler, 1985). We presented clinical, morphological findings of 2 affected infants with rare lethal "agnathia –otocephaly" complex.
Case 1: G2, gestation diabetes; P1 at term; male infant (BW=2500; BL=51cm; OFC=32cm), died at age 2.5 months.
Case 2: G2; P1 at 31 weeks, premature female (BW=1720; BL=43cm; OFC=32cm), died at age 1 month. Common data: sporadic cases; young nonconsanguineous parents; exposure to teratogenic factors during gestations was denied. Polyhydramnios. Asphyxia, tracheostomia. Infants showed neurological signs (dystonia, decreasing reflexis, strabismus), mental/growth retardation, otocephalic appearence-ventrally placed, but not fused dysmorphic ears, hypoplasia of zygomatic bones, down-ward slanting palpebral fissures, severe hypoplasia of mandibular arch, agnathia, microstomia, aglossia, esophagostenosis. External appearance of the eyes, limbs, chest, abdomen, genitalia were normal. Autopsy demonstrated no specific brain malformation, no defects noted in central nervous system, cardiovascular, genitourinary or skeletal systems. Analysis of phenotypes presented variability of clinical signs. Patient 1 was more severe affected: cleft palate, stenoses of external auditory canal, abnormal lung lobation. GTG-banded karyotypes were normal. Based on clinical and morphological findings (luck of holoprosencephaly-"marker" of "agnathia-holoprosencephaly" condition) diagnosis of "otocephaly" was documented. Interrelated associations "agnathia-otocephaly" and "agnathia-holoprosencephaly" are heterogeneous disorders; genetic, environmental or maternal factors may lead to such condition. We compared clinical picture of our patients with published data for further delineation of phenotypical spectrum.

P078

Unbalanced translocation 46,XY,der(9)t(3;9)(p25.3;p23) in two brothers with mental retardation and mild features of 9p-deletion syndrome but no sex reversal

Meins M.(1), Hameister K.(2), Gerresheim F.(1), Hagedorn M.(1), Epplen J.T.(1)
1) Department of Human Genetics, Ruhr-University, Bochum, Germany
2) Fachklinik für Kinderneurologie und Sozialpädiatrie Königsborn, Unna, Germany

Introduction: Whereas distal trisomy 3p has been found in mental retardation without major dysmorphic signs, monosomy 9p is described with characteristic dysmorphic features defined as 9p- syndrome. Moreover, sex reversal or ambiguous genitalia are a recurrent feature of 9p- in XY individuals.
Patients: The boys are first and second child of unrelated parents, the mother has a chronic eating disorder. Pregnancy and birth were reported normal in both. The older boy presented at 5 years with behavioural problems and psychomotor retardation. Retardation and muscular hypotonia were observed early in life. He has mild dysmorphisms like a long face, long philtrum and low set, dysplastic ears. Cranial MRI showed minor midline abnormalities. EEG was slightly abnormal, but he has no seizures. At 7 years he visits a school for mentally handicapped children. In the younger brother, feeding problems, retardation and muscular hypotonia were prominent in the first years. At 4 years he is mentally retarded, but has no dysmorphisms apart from strabismus and flat occiput. Examination of genitalia showed only bilateral testicular retraction. Growth parameters are normal in both.
Results: Cytogenetics revealed a karyotype 46,XY,der(9)t(3;9)(p25.3;p23) in both brothers. Mother is carrier of a balanced translocation. Fine mapping of the 9p translocation breakpoint in the older boy by quantitative PCR indicated a breakpoint in 9p23 between D9S168 and the TYRP1 gene. This confirms monosomy for both the 9p- critical region between D9S144 and D9S168 and for the sex reversal critical region between D9S1813 and D9S1810.
Conclusions: Our analyses confirmed an unbalanced karyotype 46,XY,der(9)t(3;9)(p25.3;p23) in both brothers. Thorough examination showed mental retardation with very mild features of 9p monosomy, and without signs of sex reversal. It is currently unclear what is the reason for the surprisingly mild phenotype associated with the identical translocation in both siblings.

P079

Molecular, molecular-cytogenetic, and clinical findings in a patient with ring chromosome 6

Höckner M.(1), Erdel M.(1), Utermann B.(1), Utermann G.(1), Kotzot D.(1)
1) Dep. of. Med. Genetik, Mol. and Clin. Pharmacology, Division of Clinical Genetics, Innsbruck, Austria

Ringchromosome 6 is a rare chromosome aberration characterized by microcephaly, growth retardation, mild to moderate mental retardation, and various dysmorphic signs such as, downslanting palpebral fissures, hypertelorism, epicanthis folds, small mouth, and low-set ears. The phenotype is highly variable depending mainly on the breakpoints but also on the percentage of ring chromosome, double rings, and monosomic cells. In general, the more proximal the breakpoints the more severe are the clinical findings.

The phenotype of the female patient presented here is characterized by only very mild developmental delay, short stature, and minor dysmorphic signs including synophrys, a broad philtrum, a small mouth, and short hands. Conventional karyotyping and FISH analysis with subtelomeric 6p and 6q probes and a probe with repetitive telomeric sequences resulted in a 46,XX,r(6)[16]/46,XX,dic r(6;6)[2]/47,XX,r(6),+r(6)[1]/45,XX,-6[2] karyotype with breakpoints distal to both subtelomeric probes indicating no loss of euchromatic material. Molecular investigations of the ring chromosome and its normal homolog revealed just paternal alleles on the ring chromosome suggesting either a formation in paternal meiosis or a mitotic origin from the paternal homolog.

In summary, ring chromosomes are not always associated with a severe phenotype. Breakpoints distal to the euchromatic material makes growth retardation and mild developmental delay most likely due to mosaicism with double rings and monosomic cells.

P080

Is the genetic background of the proinflammatory cytokine TNFa a predictor for the development of aggressive and/or chronic periodontitis?

Schulz S.(1), Machulla H.K.(2), Klapproth J.(1), Zimmermann U.(1), Schaller H.-G.(1), Altermann W.(3), Reichert S.(1)
1) Martin-Luther University Halle, Department of Operative Dentistry and Periodontology, Halle, Germany
2) privat, Halle, Germany
3) Martin-Luther University Halle, Institute of Medical Immunology, Halle, Germany

Periodontitis is a chronic inflammatory disorder of the periodontal supporting tissue of teeth. That's why several factors of the immune response and their genetic background have been proposed as potential markers for the development of this disease. However, existing data are very inconsistent. Therefore, the aim of the present study was to evaluate the importance of genomic variants of the potent proinflammatory cytokine TNFa for the incidence of chronic and aggressive periodontitis.

Methods: In the present study 66 periodontitis patients (chronic: n=34, mean age: 47+10.1y, 38.2% males; aggressive: n=32, mean age: 36.6 +7.3y, 40.6% males) and 32 control probands without periodontitis (mean age: 41.6+10.3y, 40.6% males) were included. We investigated genotype, allele and haplotype frequencies of the TNFa-SNP –308G>A and –238G>A by use of PCR-SSP (CTS).

Results: Hardy-Weinberg criteria were fulfilled for both SNPs in the patient groups. Investigating genotype and allele frequencies of both SNPs no significant disease specific differences could be detected in comparison with healthy controls. However, in the control group a distinct increase in the prevalence of the mutant genotypes (-308G>A: AG+AA; -238G>A: AG) and alleles (-308G>A: A, -238G>A: A), respectively could be described comparing with patients with chronic (-308G>A: 84.8%, -238G>A: 24.4%) and aggressive periodontitis (-308G>A: 39%, -238G>A: 133%). Compared with chronic (p=0.047), aggressive (p=0.024) and total patient group (p=0.015) in the control group a significant increase in carriers of the mutant haplotypes (haplotypes containing mutant A-allele vs. wild-type GG-GG) could be observed.

Conclusions: The significant higher prevalence of carriers of the mutant haplotypes in the control group could be an indication for an altered, possibly more effective immune response to periodontal pathogens since these SNPs were considered to trigger the TNFa production.

P081

Cardiovascular risk factors in patients with premature coronary heart disease, besides genetic disposition - enough room for primary prevention

Zerres K.(1), Kimmel M.(2), Crantz K.(2), Mevissen V.(2), Hoffmann R.(2), Ortlepp J.R.(2)
1) Institute for Human Genetics, University of Technology Aachen, Aachen, Germany
2) Medical Clinic I, University of Technology Aachen, Aachen, Germany

In 638 patients who suffer from coronary heart disease (CHD) before the age of 55 yrs the clinical diagnosis of myocardial infarction according to WHO criteria was made. Cardiovascular risk factors were defined as hypercholesterolemia (cholesterol level >200 mg/dl or medically treated), arterial hypertension (>140/90 mmHg or medically treated), diabetes mellitus (overnight fasting serum glucose > 126mg/dL on at least two occasions or medically treated), smoking (regular smoking habits), and obesity (a body mass index > 25 kg/m^2). The most present risk factors were smoking with a prevalence of 76%, followed by obesity with 71% and hypercholesterolemia with 68%, whereas hypertension (51%) and diabetes mellitus (15%) were less frequent. Most patients (98%) had at least one cardiovascular risk factor, whereas only 2% of patients with premature CHD at an early age had none of the five cardiovascular risk factors. Normal weight (BMI< 25.0 kg/m^2) and nonsmoking was only present in 92 patients (8%). Thus, the majority (92%) of patients with premature CHD were smokers and/or obese.

In addition we interviewed with a standardized questionnaire 104 patients younger than 55 yrs of age, who were referred for diagnostic left heart catherization about the general acceptance of genetic testing for CHD. The patients were asked if they would support genetic testing for potential genetic risk factors for CHD, if these tests could be used for a prevention program. 98% of all patients would support testing of genetic risk factors. Most of them think that the test should be done early in life (birth-6 yrs: 42%; 7-18 yrs: 20%; 18-30 yrs: 24%; >30 yrs: 9%). 76% stated that these results should never be given to any insurance.

Despite the high acceptance of genetic testing among patients with CHD, life style changes should be major goals of prevention programs in order to avoid risk factors with a major impact on CHD.

P082

Distinctive behavioural phenotype in a contiguous gene deletion syndrome at Xp22.3

Daumer-Haas C.(1), Hellebrand H.(2), Leiz S.(3), Meindl A.(2)
1) Pränatal-Medizin München, München, Germany
2) Frauenklinik und Poliklinik rechts der Isar, Gynäkologische Tumorgenetik, München, Germany
3) Kinderklinik Dritter Orden, München, Germany

We report a 6-year-old boy with ocular albinism, psychomotor retardation and behavioural abnormalities similar to attention deficit hyperactivity disorder (ADHD). He was born with a club foot on the right side and showed dry skin with scaling. Growth parameters were on the 3rd percentile. Psychomotor development was retarded (walking with 2.5 years). Facial dysmorphism was noted with blepharophimosis, narrow palpebral fissures, flat philtrum, thin upper lip and large deep set ears. His mother and maternal grandmother had severe hyperopia.

Because of the clinical symptoms ocular albinism, short stature, ichthyosis and ADHD a contiguous gene deletion syndrome with Xp deletion was suspected. The GTG-karyotype showed no structural abnormality of the X-chromosome and FISH analysis with the subtelomeric probe could exclude a terminal deletion. In contrast FISH-analysis with the Kallmann probe (LSI Kal) clearly demonstrated a deletion of this region. Consequently fine mapping studies were performed. The distal breakpoint was mapped between the markers DXS7100 and DXS7099 indicating that the SHOX and ARSE genes are not deleted in this patient. The proximal breakpoint was mapped between the markers KIAA1280 and DXS7103 indicating that the STS, KAL1 and OA1 genes are deleted in this patient. While the lack of these genes explain ichthyosis, Kallmann syndrome and ocular albinism found in the patient, some of the other genes located in the deleted region, such as neuroligin 4 or VCX might be responsible for the psychomotor retardation and the very distinctive behavioural abnormalities also diagnosed for this patient. The relevance of these genes might become aware by performing genotype-phenotype correlations for further patients exhibiting similar interstitial deletions.

P083

Atypical Rett syndrome in a boy with a sequence variant in exon 1 of the MECP2 gene

Strenge S.(1), Holinski-Feder E.(2), Merkenschlager A.(3), Kujat A.(1), Froster U.G.(1)
1) Institute of Human Genetics, Faculty of Medicine, University of Leipzig, Leipzig, Germany
2) Center of Medical Genetics, Munich, Germany
3) University Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany

We describe the clinical profile of a male patient with atypical Rett syndrome. Family history is unremarkable. The boy is the second child of nonconsanguineous parents. He was born in the 39th gestational week with a length of 49 cm (50th centile), a birth weight of 3020g (50th centile), and a head circumference of 33,5 cm (25th centile). Early development was normal except for hyperactivity. The boy did not show growth failure. Head size was in the normal range. He was able to speak 3-word-sentences. After normal development up to the age of 5 years there was a rapid deterioration of high brain functions. This deterioration progressed to dementia. The boy developed autistic-like behavior. He exhibited stereotypic hand movements. Thereafter, a period of apparent stability lasted for years.
Length at age 20 years is 171 cm (10th centile), and head circumference is 57 cm (75th centile). There is a severe mental retardation. The boy had never had seizures. He recovered some degree of speech with echolalia and is able to understand simple words.
The patient underwent comprehensive neurological, metabolic and genetic investigations. The analysis of G-banded chromosomes showed a normal male karyotype 46,XY. Studies of the methyl-CpG-binding protein 2 (MECP2) gene revealed the sequence variant c.46_48insGGA in exon 1 of the MECP2 gene, but there is no evidence that the mutation is pathogenic. The healthy mother is carrier of the mutation. A favourable skewed X-inactivation pattern can be an explanation, why there is no clinical expression in the mother.
We want to contribute this patient as a possibly further observation of a male patient with atypical Rett syndrome and a mutation of unknown significance in exon 1 of the MECP2 gene.

P084

Hypoglossia and situs inversus totalis

Koenig R.(1), Schneider W.(2), Klein C.(3), Schäfer D.(1)
1) Institut für Humangenetik, Frankfurt am Main, Germany
2) Kinderklinik, Frankfurt am Main, Germany
3) Klinik für Mund-,Kiefer- und Plastische Gesichtschirurgie, Frankfurt am Main, Germany

Hypoglossia is a rare congenital malformation, which occurs either as an isolated malformation or as part of several syndromes. We here describe a female infant who was born after an uneventful pregnancy as the second child of nonconsanguineous parents of Afghanian ancestry in the 42nd week of gestation. Birth weight was 3330 g, length 52 cm, head circumference 35 cm. At examination she presented with congenital hypoglossia and micrognathia. Due to glossoptosis and severe airway obstruction, she had to be intubated immediately. Feeding difficulties and swallowing dysfunction required nasogastric infusions. X-ray examination revealed dextrocardia. An abdominal ultrasound confirmed complete situs inversus. Ultrasound of the brain was normal. Karyotype was 46,XX.
A similar pattern of malformations has been described only seven times. All cases have been sporadic with normal karyotypes. Beside the main features hypoglossia, micrognathia and situs inversus the patients showed low set ears, choanal stenosis, pituitary hypoplasia, conductive hearing loss, PDA, vesicoureteric reflux, and asplenia. Two children died early, two showed developmental delay, and three developed well. There is overlap to the oromandibular-limb hypogenesis syndromes, a group of loosely defined entities. Apparently, their aetiology is heterogeneous. We discuss the possible underlying developmental relationships, in particular concerning the determination of the left-right axis.

P085

k-MED: Multimedia education in human genetics

Oeffner F.(1), Fuchs A.(1), Rauschendorf M.(1), Mützel T.(1), Fritz B.(1), Jürgen K.(1)
1) Philipps-Universität Marburg, Zentrum für Humangenetik, Marburg, Germany

k-MED (Knowledged Based Multimedia Medical Education) is a web based medical e-learning project funded by the German federal initiative for new media in education and the Hessian Ministry of Science and Arts. E-learning provides effective strategies for medical universities to meet the demands of the new German legislation for undergraduate medical education. The k-MED e-learning management system (ILIAS) supports online courses as well as online evaluation, online communication, and online exams (all in the same design, structure of web pages, access, navigation etc.).
Courses are held at the partner universities of k-MED: mainly at the medical schools of Marburg, Giessen, Frankfurt, and Hannover. Medical subjects include biochemistry, physiology, anatomy/histology, infectious diseases/immunology, pharmacology, dermatology, radiology/nuclear medicine, anaesthesia, and human genetics. Here, we present our teaching material currently organized in four e-learning modules: chromosomal aberrations, formal genetics, molecular diagnostics, and cytogenetics. These are basic courses intended to do the educational groundwork, which will enable academic teachers to concentrate on more sophisticated topics during their lectures.
The courses have been evaluated online by a large cohort of about 700 students at the Faculty of Medicine in Marburg. The group consists of scientists (human biology) and medical students during the basic study period or the clinical period, respectively. The results of the online-evaluation and their significance for the further development of k-MED are discussed.

P086

A patient with mosaic tetrasomy (15)(q24-qter) due to a supernumerary marker chromosome

Kuechler A.(1), Rauch A.(2), Doernbrack J.(3), Dost A.(3), Sitte-Zoellner P.(4), Trautmann U.(2), Claussen U.(1), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Institut für Humangenetik, Erlangen, Germany
3) Klinik für Kinder- und Jugendmedizin, Jena, Germany
4) Sozialpädiatrisches Zentrum Hochfranken, Hof, Germany

We report on a now 11 year old girl, second child of healthy unrelated parents. After an uneventful pregnancy, she was born by vacuum-assisted delivery in the 40th week of gestation with normal measurements (weight 2910 g (10th-25th centile), 52 cm (50th-75th centile), OFC 33.6 cm (25th centile)). After normal early motor development, a psychomotor delay became apparent soon. A developmental evaluation at the age of 2.5 years showed a delay of 1-1.5 years. The girl started to walk independently at 2 years and to speak first words at 2.5 years. Because of a suspected dysmorphism syndrome the girl was referred to the genetics' department during the first year of life. Chromosome analyses revealed a mosaic karyotype with a supernumerary marker chromosome in one and a normal karyotype in the other cell line. Further (molecular)cytogenetic studies could characterise the marker chromosome as deriving from the distal part of chromosome 15q consisting of an inverted duplication of (15)(q24-qter). Thus, the karyotype was defined as 47,XX,+der(15)(qter-q24::q24-qter)(11%)/46,XX(89%). At clinical reevaluation at 11 years, her height was 155 cm (75th-90th centile), weight 28.7 kg (3rd-10th centile), and OFC 54 cm (75th-90th centile). She had a friendly personality, her active speech comprised only single words but she had a good understanding. Clinical findings were body asymmetry, muscular hypotonia, and a severe progressive scoliosis. She had slender, mostly cold hands and feet but no long fingers/toes. She had mild facial dysmorphism (long nose and relatively short philtrum) and a high arched narrow palate. Echocardiography showed a mitral valve prolapse without insufficiency. Moreover, she suffered from chronic constipation and had reduced sensitivity to pain. Tetrasomy 15qter is a rare condition. To our knowledge, only 8 cases are published up to now. We review the literature and discuss the important clinical features.

P087

Trisomy 21 with XYY.

Garcia C.C.(1), Vera L.(2), Gonzalez D.(3), Fassrainer A.(4)
1) Catedrea de Fisiopatologia. UCV, Caracas, Venezuela
2) Hospital de Ninos JM de lo Rios, Caracas, Venezuela
3) Hospital de Ninos JM de los Rios, Caracas, Venezuela
4) Hospital de Ninos JM de Los Rios, Servicio de Genetica, Caracas, Venezuela

We report a 2 months old male baby from the North East coast of Venezuela, with a phenotype mostly resembling the typical physical characteristic of Down Syndrome including flat facial

profile, depressed nasal bridge, hypertelorism, large tonge, but with a discrete epicanthal fold. Other clinical features include cryptorquidism, partial AV defect and ostium secundum. Cytogenetic study performed on peripheral blood leucocytes culture, revealed the existence of double aneuploidy involving chromosome 21 and Y. A review of the literature points towards the existence of only 28 cases, thus the clinical information on this rear disorder is limited. In this work, we also discuss the possible origins and effects of this chromosomal abnormality.

P088

Mutation spectrum of type I glycogen storage disease in Hungary

Miltenberger-Miltenyi G.(1), Szonyi L.(2), Balogh L.(2), Utermann G.(1), Janecke A.R.(1)
1) Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck, Austria
2) 1st Department of Pediatrics, Budapest, Hungary

We performed mutation analysis in 12 Hungarian type I glycogen storage disease (GSD1) patients in order to determine the mutation spectrum. All patients were clinically classified as GSD1a. Nine patients carried biallelic G6PC mutations (p.Q27fsX35, p.D38V, p.W70X, p.K76N, p.W77R, p.R83C, p.E110Q, p.G222R), with E110Q reported only in Hungary. However, 3 patients displayed 2 common G6PT1 (SLC37A4) mutations (p.L348fsX400, p.C183R) which were originally described in association with GSD1non-a. Review of the literature and our data show that G6PT1 mutations are not associated with neutropenia and related clinical findings in approximately 10% of these cases. Homozygosity for the truncating G6PT1 mutation p.L348fsX400 can be observed with and without neutropenia indicating that one or more modifiers of G6PT1 action exist. Our data are suitable to provide DNA-based and thus non-invasive confirmation of diagnosis in Hungarian patients with this disorder.

P089

A 17 Mb interstitial duplication of chromosome 6p12.3-q12 associated with multiple malformations and features of cleidocranial dysplasia

Lehmann K.(1), Klopocki E.(1), Bommer C.(1), Tinschert S.(2), Ropers H.-H.(3), Mundlos S.(1), Ullmann R.(3)
1) Charité Universitätsmedizin Berlin, Institute of Medical Genetics, Berlin, Germany
2) Carl Gustav Carus Technical University, Institute of Clinical Genetics, Dresden, Germany
3) Max-Planck Institute for Molecular Genetics, Berlin, Germany

We report on a male newborn with craniofacial dysmorphism, skeletal abnormalities and organ malformations. The prenatal sonographic examinations showed polyhydramnion, an abnormal facies, a narrowed thorax with severe lung hypoplasia and additional gastrointestinal anomalies. The newborn died 4h after birth due to severe respiratory problems. The postnatal radiographical examination revealed thin and hypoplastic ribs, gracile and shortened clavicles, a hypoplastic mandible and hypocalcification of the cranial bones with streaky changes and wide skull sutures. Because some of these features resembled cleidocranial dysplasia, we performed a mutation analysis in the gene *CBFA1* but no mutation was found. Subsequently, we investigated patient's fibroblasts cytogenetically and the karyotype revealed a structure aberration on chromosome 6q. FISH analysis using an α-satellite probe detected 2 signals on chromosome 6. As shown by whole chromosome paint 6 (wcp6), the additional material derived from chromosome 6. Further characterization by high-resolution array-CGH on a 36k tiling path array detected a 17 Mb interstitial duplication with breakpoints in 6p12.3 and 6q12. FISH analysis confirmed a tandem duplication on chromosome 6. Investigation of the parents revealed the same chromosomal aberration in the father. Clinical examination of the father showed phenotypic similarities with the patient like shortened clavicles and narrowed upper thorax. However, the overall appearance was much milder than the one of the patient.

P090

Trisomy 4p16 and monosomy 8p23 due to an unbalanced translocation t(4;8) in two patients: molecular cytogenetic analysis with delineation of the phenotype

Neumann L.M.(1), Klopocki E.(2), Matthäi A.(3), Ropers H.-H.(4), Mundlos S.(2), Ullmann R.(4)
1) Charité Universitätsmedizin Berlin, Institute of Human Genetics, Berlin, Germany
2) Charité Universitätsmedizin Berlin, Institute of Medical Genetics, Berlin, Germany
3) Carl Gustav Carus Technical University, Institute of Clinical Genetics, Dresden, Germany
4) Max-Planck Institute for Molecular Genetics, Berlin, Germany

Unbalanced translocations t(4;8) resulting in a derivative chromosome 4 have been described as a cause of Wolf-Hirschhorn syndrome. Here we report two cases from two families with unbalanced de novo translocations t(4;8) leading to derivative chromosome 8. This translocation was not recognized in the initial conventional chromosome analysis. Microarray-based comparative genomic hybridization (array CGH) using a 1 Mb and a 36k BAC array identified a duplication of 9 Mb of chromosome 4p16 and a deletion of 7 Mb of chromosome 8p23. Translocation breakpoints in both cases are located within the olfactory receptor gene clusters on 4p16 and 8p23. Subsequently, partial trisomy 4p and partial monosomy 8p were confirmed by FISH and demonstrated to be de novo. Our patients show some phenotypic similarities (coarse face, accentuated supraorbital ridges, bushy eyebrows and full lower lips). They are both tall and obese with relatively large heads. Furthermore their grade of mental retardation is similar. We present a comparison of the phenotype of the two cases and a literature review. Wether a phenotypic pattern associated with trisomy 4p16.1-p16.3 and monosomy 8p23.1-p23.3 can be established has to remain open since the number of comparable cases is low.

P091

Partial trisomy 8q in a 43 year old male with moderate intellectual disability, epilepsy and large cell non-Hodgkin´s lymphoma

Helbig I.(1), Wirtenberger M.(2), Jauch A.(1), Hager H.-D.(1), Tariverdian G.(1), Hemminki K.(2), Burwinkel B.(2), Klaes R.(3)
1) Institut für Humangenetik, Heidelberg, Germany
2) DKFZ, Abteilung Molekulargenetische Epidemiologie, Heidelberg, Germany
3) Zentrum für Humangenetik Mannheim, Mannheim, Germany

Partial trisomies of 8q are rare genetic abnormalities resulting in a broad range of malformations. Whereas diagnosis of these syndromes is usually made in early childhood, we report on a patient diagnosed at the age of 43 years with a 47,XY,+der(22)t(8;22)(q24.13;q11.21) karyotype. To our knowledge, this is the oldest patient described with a partial trisomy 8. The patient presented with moderate intellectual disability, a past history of epilepsy and facial dysmorphologies. In addition to the characteristic features of this syndrome, the patient suffered from a Large-B-Cell-Non-Hodgkin-Lymphoma in adulthood. Detailed breakpoint mapping by single nucleotide polymorphism (SNP) arrays revealed that the derivative chromosome contains a full-length copy of the C-MYC oncogene. Given that trisomy 8q is one of the most frequent secondary chromosomal abnormality in haematological diseases, the possibility of a genetic predisposition for these disorders in patients with partial trisomy 8 is raised.

P092

Teasing out information from 2D pictures of dysmorphic faces: finding a model for integrated side and frontal views and age effects yields accuracy of > 90% in syndrome classification

Maus B.(1), Vollmar T.(1), Tasse C.(1), Gillessen-Kaesbach G.(1), Wurtz R.(2), Horsthemke B.(1), Wieczorek D.(1), Boehringer S.(1)
1) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany
2) Ruhr-Universität Bochum, Institut für Neuroinformatik, Bochum, Germany

Formalizing facial appearance through 2D and 3D image analysis is now the subject of research in several groups. In our ongoing inquiry, we try to understand a complex data set representing 2D pictures of faces from patients with 10 different syndromes (MPS III, CDL, FraX, PWS, WBS, 5p-, 22q-, Noonan S., Sotos S., SLO).
Here, we present the two key components of developing computer aided syndrome classification. The first step involves transforming the image data and choosing relevant subsets to build a model that can successfully discriminate features of syndromes. The second step involves clinical validation of the findings. In addition to formal tests of decisions derived from our algorithm (e.g. symmetry), the latter step also involves clinician intervention to verify the plausibility of computer decisions. The verification compares expected clinical findings with the features of the face that were highlighted by our computer-generated model for each syndrome. We show that careful model selection which involves both choosing appropriate subsets of the data and applying various mathematical trans-

formations can drastically improve classification accuracy. We demonstrate that accuracy exceeds 90% for the 10 syndromes on frontal views, in contrast to roughly 80% based on previous research for the same data set.
Interestingly, side views alone exceed 80% in accuracy, underlining the importance of this aspect in clinical diagnosis. Combining side and front views further improves classification results. Age is an important aspect influencing facial traits. In this data set no linear pattern can be found explaining variation with age. Nevertheless age can be dealt with by stratifying the data set.
In conclusion, 2D image analysis can be optimized to handle all available syndromes in our data set in a near optimal way. We therefore expect applicability to an even larger set of syndromes, with the potential for widespread use of computer aided facial analysis.

P093

Novel mutations in *KRAS* in two patients with cardiofaciocutaneous (CFC) syndrome
Schulz A.L.(1), Zenker M.(2), Koch M.C.(3), Barth H.(4), Morlot S.(5), Kutsche K.(1)
1) Institut für Humangenetik, Universitätsklinikum Eppendorf, Hamburg, Germany
2) Institut für Humangenetik, Universität Erlangen-Nürnberg, Erlangen, Germany
3) Zentrum für Humangenetik, Marburg/Lahn, Germany
4) Zentrum für Kinder- und Jugendmedizin, Klinik für Neonatologie und Neuropädiatrie, Marburg, Germany
5) Praxis für Humangenetik, Ärztliche Partnerschaft Wagner-Stibbe Hannover, Hannover, Germany
Recently, mutations in HRAS have been described to cause Costello syndrome, a rare congenital disorder characterized by postnatal failure to thrive, coarse face, macrocephaly, loose skin with deep palmar and plantar creases, heart abnormalities, and mental retardation. HRAS belongs to the family of RAS proteins including KRAS, NRAS, and ERAS, which act as molecular switches in the cell implicated in the activation of various signaling cascades. Costello syndrome, cardiofaciocutaneous (CFC) syndrome, and Noonan syndrome display some phenotypic similarities suggesting that autosomal dominant *de novo* mutations in other RAS genes may be responsible for these disorders. Indeed, Kratz and colleagues described germ line mutations in KRAS (p.V14I, p.T58I, and p.D153V) in five patients with Noonan syndrome as well as one alteration (p.P34R) in a patient with CFC syndrome. These data prompted us to analyse 12 patients with supposed Costello syndrome and no HRAS mutation as well as 9 patients with CFC syndrome for mutations in KRAS. Indeed, in one patient with CFC syndrome we identified the heterozygous c.64C→G (p.Q22E) mutation and in a patient with supposed Costello syndrome the c.468C→G (p.F156L) alteration was present. Both mutations occurred *de novo*. The two patients shared numerous features, such as coarse face, loose skin with deep palmar and plantar creases, pulmonary stenosis, and postnatal growth failure. In addition, the patient with the p.F156L mutation presented with hypertrophic cardiomyopathy and Dandy-Walker malformation with hydrocephalus. He died at the age of 13 months. The patient harboring the p.Q22E mutation also showed additional characteristics, such as sparse hair, hypotonia, and mild psychomotor retardation. In summary, mutations in KRAS give rise to a broad spectrum of clinical phenotypes comprising Noonan syndrome and mild or severe CFC syndrome that, in the latter case, can also resemble Costello syndrome.

P094

Gomez-Lopez-Hernandez syndrome – description of a new case and review of the literature
Schell-Apacik C.(1), Cohen M.(1), Vojta S.(1), Klopocki E.(2), Ullmann R.(3), Ertl-Wagner B.(4), Heinrich U.(5), von Voss H.(1)
1) Institut für Soziale Pädiatrie und Jugendmedizin der Ludwig-Maximilians-Universität, Kinderzentrum München, München, Germany
2) Institut für Medizinische Genetik, Charité Universitätsmedizin Berlin, Berlin, Germany
3) Max-Planck-Institut für Molekulare Genetik, Berlin, Germany
4) Institut für Klinische Radiologie der Ludwig-Maximilians-Universität München, München, Germany
5) Zentrum für Humangenetik und Laboratoriumsmedizin Dres. Klein & Rost, Martinsried, Germany
We report on a patient with synostosis of the lambdoid suture, partial scalp alopecia, corneal opacity, mental retardation, striking phenotypic features including brachyturricephaly, hypertelorism, midface hypoplasia, and low-set ears consistent with Gomez-Lopez-Hernandez syndrome. In early childhood the patient had behavioural problems with aggressive behaviour and periods of raging. He also had seizures which were adequately controlled by antiepileptic medication. Magnet resonance imaging (MRI) revealed rhombencephalosynapsis with a complete fusion of the cerebellar hemispheres and absence of the vermis, also consistent with Gomez-Lopez-Hernandez syndrome.
We performed conventional chromosome analysis and telomere FISH analysis with normal results. Currently, we are performing array-CGH (Comparative Genomic Hybridization) analysis to investigate the patient's DNA for submicroscopic rearrangements.
Gomez-Lopez-Hernandez syndrome is a very rare genetic disorder first described by Gomez M.R. in 1979 and Lopez-Hernandez in 1982. To our knowledge, there have been only 15 cases reported in literature so far. Laboratory diagnoses were inconspicuous, although array-CGH analysis had never been performed before.
We present the clinical findings of the patient at the ages of 3 10/12 and 15 8/12 years as well as the laboratory and MRI findings, comparing our results with the available literature data.

P095

Diagnosis of Shprintzen-Goldberg syndrome in a 24 years old woman
Liebrecht D.(1), Daumer-Haas C.(1)
1) Pränatal-Medizin München, München, Germany
The Shprintzen-Goldberg syndrome (SGS) is a rare disorder of unknown cause characterized by craniosynostosis, a marfanoid habitus and neurological anomalies. The diagnosis of SGS depends on clinical findings.
We report on a 24 years old female patient with known diagnosis of bronchial asthma, myopia, hearing loss and two events of pneumothorax. The patient showed a marfanoid habitus. She was born after an uneventful pregnancy with inguinal hernia on both sides. The childhood of the patient was complicated by mild developmental delay. Craniofacial dysmorphism was noted with dolichocephaly, hypertelorism, exophthalmos, ptosis, strabismus, anteverted nares and micrognathia. In addition to the craniofacial findings the patient had skeletal anomalies: scoliosis, pectus excavatum, arachnodactyly, long toes. She had a normal karyotype (46,XX). Analysis of FBN1 gene revealed no mutation. New medical history of our patient includes a diagnosis of mitral valve prolaps/ insufficience of mitral valve and further ophthalmological anomalies (hyperopia, astigmatism, cataracta congenita). CT scan showed moderate enlargement of external and lateral ventricles and an Arnold-Chiari-I-malformation.
First given extern diagnosis was an untypical form of Marfan syndrome. However especially cardial and ophthalmological findings of our patient were not typical for Marfan syndrome, but nearly all findings of our patient have been described in SGS.
Many of the features of SGS are shared with Marfan syndrome. In conclusion it seems to be important for patients with marfanoid habitus to consider other rare marfanoid syndromes.

P096

New perspectives in DGGE: Development of a capillary approach.
Cammaert R.(1), Jansen B.(2), Weichenhan D.(3), de Vos G.(1)
1) Ingeny International BV, Goes, Netherlands
2) Institute of Human Genetics, Heidelberg, Germany
3) Otto Meyerhof Zentrum, Heidelberg, Germany
As our knowledge of the human genome progresses, the demand for higher throughput in mutation screening emerges. The use of several methods as a reliable pre-screen, followed by sequencing proved it´s value in the past years. DGGE, established in 1978 by Fisher and Lehrman, has built a very good reputation as a reliable screening method for mutations in human disease genes. After careful set-up a nearly 100% mutation detection rate was accomplished in many laboratories. It was successfully used to screen for mutations in e.g. BRCA1, BRCA2, CFTR, RET, RB, DMD, PKU, MLH1, MSH2, MSH6. Here we present a capillary approach, which will speed up the procedure considerately. In a glass capillary (1 mm inside diameter, 100 mm length) we have cast a denaturing gradient poly acrylamide gel, for the separation of the fragments. The patterns produced are very similar to those produced on a normal DGGE. The top part of the capillary is left empty during preparation of the gel. After polymerization the top part can be used to inject a PCR-mix. We developed a device that will thermocycle before applying voltage for electrophoresis. The gels are stained with EtBr during the electrophoresis. A CCD camera system is used to make images of the gels. The design allows 96 capillaries to be run simultaneously. This allows

mutation detection in 96 fragments of an average length of 300 base pairs with minimum effort and in a shorter time. A normal DGGE experiment takes app. 3 hours for PCR and overnight for the DGGE, after which the gel can be stained. Capillary DGGE takes between 1 and 3 hours for the complete experiment (PCR, electrophoresis, staining and imaging).

P097

Further genetic heterogeneity and diagnostic pitfalls in Meckel-Gruber syndrome

Bergmann C.(1), Frank V.(1), Küpper F.(1), Seidel H.(2), du Bois G.(3), Grimm T.(4), Senderek J.(1), Zerres K.(1)
1) Department of Human Genetics, Aachen University of Technology, Aachen, Germany
2) Department of Human Genetics, Ludwig-Maximilians-University Munich, Munich, Germany
3) Institute of Chromosomal Diagnostics and Genetic Counselling, Böblingen, Germany
4) Department of Human Genetics, University of Würzburg, Würzburg, Germany

Meckel-Gruber syndrome is an autosomal recessive, usually lethal multisystemic disorder characterized by developmental anomalies of the central nervous system (typically occipital meningo-encephalocele), cystic kidneys, and postaxial polydactyly. In most cases, a ductal plate malformation of the liver can also be observed. The majority of patients manifests antenatally with perinatal demise. However, the phenotype may considerably vary even among affected siblings and a wide range of further associated features has been reported. So far, three different loci for Meckel-Gruber syndrome have been mapped: MKS1 on 17q22-q23, MKS2 on 11q13, and MKS3 on 8q24. Very recently, the MKS1 gene could be identified, whereas the genetic basis for MKS2 and MKS3 is still to be determined. We will give a short overview of critical differential diagnoses of this entity and report on two siblings diagnosed with Meckel-Gruber syndrome until school age when retinitis pigmentosa manifested and a diagnosis of Bardet-Biedl syndrome (BBS) was made. This is in accordance with a recent study on BBS mutations in patients diagnosed as having Meckel-Gruber syndrome and underlines that BBS may mimic Meckel-Gruber syndrome in cases without CNS malformation. We will also present linkage data obtained in six consanguineous Turkish (multiplex) kindreds in whom at least one affected sib of each family was pathoanatomically diagnosed with Meckel-Gruber syndrome comprising occipital meningo-encephalocele. Intriguingly, haplotyping revealed that not all families are linked to one of the known MKS loci. Thus, we hypothesize further locus heterogeneity in Meckel-Gruber syndrome. A genome-wide SNP analysis is currently underway and we would like to include further families in our ongoing studies.

P098

Fibrodisplasia Ossificans Progressive: A story of loneliness

Alza M.(1), Vera L.(2), Rojas V.(3), González D.(2), Fassrainer A.(2)
1) Children's Hospital 'J.M. de los Rios', Pediatric Residency, Caracas, Venezuela
2) Children's Hospital 'J.M. de los Rios', Department of Genetics, Caracas, Venezuela
3) Children's Hospital 'J.M. de los Rios', Department of Medicine I, Caracas, Venezuela

The Progressive Ossificans Fibrodysplasia (FOP), also called Münchmeyer Syndrome, formerly known as Progressive Myositis Ossificans, is a rare (1:2.000.000) autosomic dominant disease with heterotopic bone formation in the connective tissue of muscles and bone all over the body. Usually it appears in the first decade of life and is associated with brachydactily and hallux valgus of the first toe.
We report a seven years old boy who was treated with antibiotics for inflammation on the right scapula area after trauma but no response was observed. He was sent to the Children Hospital "J.M. de Los Rios" because of torticolis of unknown etiology. After evaluation in the Genetics Department, diagnosis of FOP was suspected because of light nodules over different skeletal areas, short first toes and no movement of the neck. He had a bony formation over a scar on the left forearm, because of a fracture eight months ago. By this presentation we want to spread the awareness of this illness and suggest care in handling it because of the heterotopic bone formation that is observed after medical invasive technique treatments.
Keywords: POF, Progressive ossificans fibrodysplasia, BMP4, heterotopic bone, Montelukast, Cromoglicate.

P099

Ataxia-telangiectasia as an adult-onset disease

Dörk T.(1), Wieland B.(2), Mavridou E.(1), Brinkhoff B.(1), Schürmann P.(1), Bendix-Waltes R.(2), Kolbe H.(3)
1) Hannover Medical School, Gynaecology Research Unit, Hannover, Germany
2) Hannover Medical School, Department of Radiation Oncology, Hannover, Germany
3) Hannover Medical School, Department of Neurology, Hannover, Germany

Ataxia-telangiectasia (A-T) is an autosomal recessive syndrome characterised by progressive neurodegeneration, immunodeficiency, infertility, cancer predisposition and increased sensitivity towards ionising radiation. Disease usually starts in early childhood and most patients become wheel-chair bound during adolescence. Many patients decease from infections or cancer before age 30. We here report on a 67-year-old woman who was recently diagnosed at our institution as having A-T. She is the mother of two children and has shown the first signs of mild limb ataxia by the end of her 30´s. At the time of diagnosis, she presents with marked gait and limb ataxia but is still able to walk with assistance. She has no history of recurrent infections or cancer. She was found to be compound heterozygous for the ATM missense mutation V2716A (exon 57) and the insertion 9021insA in exon 65, the last exon of the ATM gene. The frameshift mutation 9021insA leads to a different carboxyterminus without truncation of the protein. No ATM protein could be detected by immunoblotting in lymphoblastoid cells from the patient when an antibody against a carboxyterminal epitope was used, whereas some residual ATM became apparent with an antibody against pSer1981ATM after irradiation. Some residual kinase activity at about 10% of the wildtype level could be detected towards the ATM substrates p53 and MDC1 but not towards Nbs1/p95. In conclusion, we describe a case with an advanced age and an unusually late onset and mild expression of ataxia-telangiectasia indicating that variant forms of this disease manifest exclusively during adulthood.

P100

Diagnosis and treatment of DNA ligase IV deficiency

Gruhn B.(1), Seidel J.(1), Zintl F.(1), Varon R.(2), Tönnies H.(2), Neitzel H.(2), Bechtold A.(3), Hoehn H.(4), Schindler D.(4)
1) Department of Pediatrics, Jena, Germany
2) Department of Human Genetics, Berlin, Germany
3) Department of Human Genetics, Jena, Germany
4) Department of Human Genetics, Würzburg, Germany

We report on one of worldwide less than 10 patients with Ligase IV (LIG IV) deficiency who is the second child of young, non-consanguinous parents. Severe growth retardation and microcephaly were first noted during the 22nd week of pregnancy. Spontaneous delivery was at 35 weeks; birthweight 1.500g, length 42 cm, head circumference not recorded. Because of pronounced microcephaly, short stature, psychomotor retardation and a peculiar facial appearance ("bird-like" face) the diagnosis Seckel-syndrome was considered. Between ages 5 and 9 the child developed thrombopenia followed by neutropenia and erythropenia suggesting the diagnosis of the Dubowitz pancytopenia syndrome. There were recurrent episodes of ear and upper respiratory tract infections with low immunoglobulins. Karyotype was 46, XX, but exposure of peripheral blood mononuclear cells to the radiomimetic bleomycin yielded strongly increased breakage rates. Nijmegen breakage syndrome (NBS) was excluded by sequencing of the nibrin gene. Cell cycle analysis using bivariate BrdU-Hoechst ethidium bromide flowcytometry revealed reduced mitogen response and increased radiosensitivity reminiscent of ataxia telangiectasia (AT), but AFP was normal. The lack of convincing evidence for either AT or NBS in the presence of pronounced cellular radiosensitivity lead to the analysis of the DNA ligase IV gene and detection of compound heterozygosity for the mutations G469E and R814X. Because of increasing pancytopenia the patient received a bone marrow transplant from her HLA-identical brother at age 12 without prior irradiation and using a mild conditioning regimen. After transplantation the course was uneventful and without signs of acute or chronic GvH-disease. Stable and complete chimerism (100% donor cells) developed within 4 months. Now at age 15, the mentally retarded patient is in very good physical condition and attends a special education programme.

P101

Increased sister-chromatid-exchange (SCE) in non-neuronal cells of sporadic ALS (SALS) patients

Mehraein Y.(1), Meyer T.(2), Ludolph A.C.(3), König J.(4), Römer K.(5), Reichardt S.(1), Zang K.D.(1), Prudlo J.(6)
1) Institut für Humangenetik der Universität des Saarlandes, Homburg/Saar, Germany
2) Neurologische Universitätsklinik der Charite, Berlin, Germany
3) Neurologische Universitätsklinik, Ulm, Germany
4) Institut für Medizinische Biometrie, Epidemiologie und Medizinische Informatik der Universität des Saarlandes, Homburg/Saar, Germany
5) Institut für Virologie der Universität des Saarlandes, Homburg/Saar, Germany
6) Neurologische Universitätsklinik der Universität des Saarlandes, Homburg/Saar, Germany

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting motoneurons. Familial (FALS) and sporadic (SALS) ALS are known. About 20% of mainly FALS cases could be genetically characterized showing gene mutations in SOD1, Alsin (ALS2), or Dynaktin. The etiology of the vast majority of cases especially of SALS, however, is yet still unclear. Recently we demonstrated an increased prevalence of constitutional chromosomal rearrangements in SALS patients involving various different unrelated loci. Proceeding from this observation we now performed cytogenetic analyses including basic SCE analysis in peripheral blood lymphocytes (PBL) of 48 SALS patients and 19 age/sex-matched healthy controls to proof the hypothesis of an increased chromosomal instability in SALS. Beside SCE rates also chromosomal breakage events (CB) and mitotic activity (determined by 1. Mitosis/2. Mitosis-ratio following BrdU-Exposure) were analyzed. Increased SCE and CB may indicate either increased DNA-damage or impaired DNA repair. Thereby we could demonstrate a significantly elevated average SCE-level per cell in SALS of 15.52 versus 7.66 in controls (p=0,000002). While 18,8% of SALS patients displayed normal SCE values, 81.2% had elevated basic SCE (>8/cell) and amongst those 20.8% showed even strongly elevated SCE (20-55,3 SCE/cell). CB were 3.5 fold increased in SALS versus controls (p=0.001). 39.6% of SALS patients revealed CB-values >0,1. Among SALS a significantly reduced mitotic activty (M1/M2-ratio 12.94 vs. 1.34; p=0004) was observed. An increase of SCE and CB could be demonstrated in non-neuronal cells of SALS. Together with the reported finding of an elevated prevalence of constitutional structural chromosome aberrations, these observations further hint to a possible general genetic instability involved in SALS pathomechanisms.Continuing studies have to be performed involving mutagen induction testing to clarify a potential influence of impaired DNA repair

P102

LRP1/A2MR gene expression as a risk indicator for degenerative cell metabolism and diseases

Gläser C.(1), Birkenmeier G.(2), Schulz S.(1), Huse K.(3)
1) Institut für Humangenetik und Medizinische Biologie, Halle/Saale, Germany
2) Institut für Biochemie, Leipzig, Germany
3) Institut für Molekulare Biotechnologie, Jena, Germany

Disordered supply and disposal functions of cellular metabolism are characteristical for the final state of degenerative diseases like Alzheimer´s disease (AD) or coronary artery disease (CAD). An essential multi-ligand "cargo"-receptor for several components like A2M, ApoE, and APP is the LDL receptor-related protein 1 (LRP1/A2MR).
Subjects: We performed a comparative study about the in vivo LRP1 gene expression in 3 groups of probands, all from Germany and of Caucasian origin. The 1st group consisted of 9 individuals with Alzheimer´s disease (AD) (mean age 71y, SD 8.62; 4 males), the 2nd group consisted of 68 patients with coronary artery disease (CAD) (mean age 58.43y, SD 4.09; 49 males) and the 3rd group of 20 healthy controls (58.9y, SD 3.39; 18 males).
Methods: Ex vivo LRP1 gene expression was measured in freshly isolated monocytes from venous blood by real-time RT-PCR procedure.
Results: The expression level of the three groups of probands measured in monocytes differed significantly from each other (p=0.015). The highest LRP1 expression level was observed in AD patients (290.96 ag/cell) followed by CAD patients (174.49 ag/cell) and by the lowest expression in controls (158.79 ag/cell). There was no significant correlation between neither age nor gender and LRP1 expression in the subject group presented. The results demonstrate a disease-related increase of ex vivo LRP1 gene expression level in human circulating monocytes. In this way LRP1 appears to be not only a sufficient diagnostic halmark for AD as well as CAD but also a metabolic factor which could be important for sufficient strategy of therapy .

P103

Association of toll-interacting protein gene polymorphisms with atopic dermatitis

Schimming T.(1), Parwez Q.(2), Petrasch-Parwez E.(3), Falkenstein D.(1), Epplen J.T.(1), Hoffjan S.(1)
1) Ruhr-Universität Bochum, Humangenetik, Bochum, Germany
2) Praxis für Allgemeinmedizin, Gladbeck, Germany
3) Ruhr-Universität Bochum, Abteilung für Neuroanatomie und Molekulare Hirnforschung, Bochum, Germany

Atopic dermatitis (AD) is a common inflammatory skin disorder, affecting up to 15% of children in industrialized countries. Toll-interacting protein (TOLLIP) is an inhibitory adaptor protein within the toll-like receptor pathway, a part of the innate immune system that recognizes conserved molecular patterns of microbial pathogens, leading to an inflammatory immune response. In order to detect a possible role of TOLLIP variation in the pathogenesis of AD, we screened the entire coding sequence of the TOLLIP gene for mutations by SSCP in 50 AD patients. We identified one amino acid exchange in exon 6 (Ala222Ser) and one silent mutation in exon 4 (Pro139Pro) and subsequently genotyped these two variations and one additional promoter polymorphism (-526 C/G) in 297 AD patients and 214 healthy controls. The -526G allele was significantly associated with AD in our cohort (p=0.0048; pc=0.01). The 222Ser allele showed a weaker association with AD (p=0.01) that was lost after correction for multiple testing. Haplotype analyses revealed a significant association of a haplotype that was mainly discriminated by the promoter polymorphism. Yet, evaluation of mRNA expression by quantitative realtime PCR in 5 probands with the CC and 5 probands with the GG genotype at the -526 C/G locus did not show significant differences between genotypes. In conclusion, variation in the TOLLIP gene may play a role in the pathogenesis of AD, but the functional relevance of the associated promoter SNP remains to be elucidated.

P104

Periodic catatonia: mutation analysis of brain-expressed genes in a schizophrenia locus on chromosome 15q15

Ekici A.B.(1), Schanze D.(1), Stöber G.(2), Beckmann H.(2), Reis A.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) Department of Psychiatry and Psychotherapy, Würzburg, Germany

Periodic catatonia (MIM 605419) is a familial subphenotype of schizophrenic psychoses with a strong genetic component. The genetically heterogeneous disorder is characterized by psychomotor disturbances with akinetic and qualitative hyperkinetic episodes, grimacing facial movements and apathy. Parametric as well as haplotype analysis were consistent with an autosomal dominant inheritance with reduced penetrance and a morbidity risk of ~27% for first degree relatives. Previously we have identified a major disease locus on chr.15q15 in genome-wide linkage studies of 12 extended pedigrees. We also replicated the chromosomal locus in an independent set of four pedigrees. Linkage and haplotype analyses in three exceptionally large pedigrees linked to chr.15q15 disclosed an 11 cM critical region between markers D15S1042 and D15S659. Further extensive haplotyping studies refined the critical region to a 7.49 Mb interval, containing 189 annotated genes. In our efforts to reveal the underlying disease gene we prioritized all genes from the critical region on chr.15q15 by expression profiles and putative function from RT-PCR experiments and databases. We selected all 57 brain-expressed genes for mutation analysis by automated sequencing of DNA fragments in 8 individuals from linked families and 8 controls. To also cover variants beyond the coding region we sequenced generously flanking intronic sequences, the 3' and 5'-UTR as well as approximately 1500 bp upstream of the first exon of all genes. No disease-causing mutation was identified in the coding region of any of these genes, even though we detected 667 SNPs distributed in all regions including exons. These findings suggest, that variants in either i) genes or parts thereof currently not known to be brain-expressed or ii) in non-coding sequences such as regulatory elements may be the underlying genetic risk factor predisposing to periodic catatonia.

P 097

P105

Identification of common disease loci for gramulomatous diseases through a two-stage 100k genome wide association study in Sarcoidosis and Crohn Disease

Schürmann M.(1), Hampe J.(2), Kwiatkowski R.(2), Franke A.(2), Becker C.(3), Krawczak M.(4), Müller-Quernheim J.(5), Nürnberg P.(3), Schreiber S.(2)

1) Universität zu Lübeck, Institut für Humangenetik, Lübeck, Germany
2) Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany
3) Cologne Center for Genomics, University of Cologne, Köln, Germany
4) Institute of Medical Statistics and Biometry, Christian-Albrechts-Universität, Kiel, Germany
5) Department of Pneumology, University Hospital Freiburg, Freiburg, Germany

Background & Aims: Formation of granulomas is the pathogenetic hallmark in a group of autoimmune and infectious disorders. Increased co-morbidity has been observed for the two main non-infectious diseases that demonstrate granuloma formation, Crohn disease (CD) and sarcoidosis. While the primary sites of affection differ, both disease can manifest in almost all organs. The joint analysis of the genetic risk profiles will render common etiological pathways and could identify susceptibility loci for the overlapping "granuloma" trait.

Methods: Age- and sex-matched samples from 393 Crohn's disease patients, 400 sarcoidosis patients and 399 control individuals were genotyped on the 100K Affymetrix GeneChip system. Single point genotypic and allelic association tests were performed for markers fulfilling the following criteria in controls: i) no evidence of departure from Hardy-Weinberg-Equilibrium at the p=0.05 level, ii) minor allele frequency >1% and iii) Call rate >95%.

Results: Out of the 83,268 SNP meeting these criteria, 2466 SNPs showed significant association at the p=0.05 level in CD and 3135 in sarcoidosis. 364 markers overlapped between the two analyses, which is significantly more ($p<0.001$) than expected by random selection alone.

Conclusions: There is significant evidence of overlapping association leads between the two disorders ($p<0.001$). These might represent common susceptibility variants predisposing to granulomatous inflammation.

P106

Spasticity in multiple sclerosis (MS) is associated with polymorphisms of the FAAH and MGLL genes

Wiemann D.(1), Stuhrmann M.(1), Hardt C.(2)

1) Medizinische Hochschule, Humangenetik, Hannover, Germany
2) Universitätsklinikum Essen, Immunologie, Essen, Germany

Objectives: Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system. Several genetic factors contribute to the development and the course of MS. Severe muscle spasticity leads to considerable distress in many MS patients, while others have only mild or no spasticity. Treatment with cannabinoids shows a small effect on spasticity in MS patients and is highly effective in a EAE mouse model. Genetic factors of the cannabinoid system may contribute to the highly variable degree of spasticity in MS.

Methods: 41 MS patients with spasticity, 41 MS patients without spasticity and 50 healthy controls of the same age and gender distribution were included in the study. Polymorphisms of 8 different candidate genes were studied, including the cannabinoid receptor genes and genes which are involved in the metabolism of endogenous cannabinoids (anandamides).

Results: MS patients and controls showed similar frequencies of alleles and genotypes of all polymorphic markers studied. Homozygosity for the A alleles of two of the investigated single nucleotide polymorphisms (SNPs) was associated with spasticity among MS patients (rs1053627, OR 3.41, 95% CI 1.37-8.53, p=0.014; rs664910, OR 3.04, 95% CI 1.23-7.52, p=0.026). Homozygosity for both these SNPs (combined genotypes) was significantly more often seen among MS patients with spasticity than without (OR 7.99, 95% CI 2.41-26.53, p=0.0004).

Conclusion: The SNPs rs1053627 and rs664910 are genetic markers for the FAAH and MGLL genes, which both code for proteins involved in the cannabinoid system. Both proteins play an important role in the degradation of anandamides and may thereby act in concert. Variations of the FAAH and MGLL genes are possible genetic risk factors for spasticity in MS, possibly showing an additive effect.

P107

Association of variants in tumor necrosis factor receptor 1 (TNFRSF1A) with erosive joint disease in psoriatic arthritis

Lascorz J.(1), Hüffmeier U.(1), Böhm B.(2), Burkhardt H.(2), Reis A.(1)

1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany
2) Department of Internal Medicine III (Rheumatology) and Institute of Clinical Immunology, University Erlangen-Nuremberg, Erlangen, Germany

The cytokine tumor necrosis factor-α (TNF-α) is a major mediator of apoptosis, inflammation and immunity, with the majority of its activities signalled through the tumor necrosis factor receptor 1 (TNFRSF1A). Recently developed biological agents inhibiting TNF-α signalling improve psoriasis vulgaris (PsV) and psoriatic arthritis (PsA). To examine the possible relevance of TNFRSF1A variants in psoriasis patients we analysed two different German case-control cohorts: i. 374 patients with a rheumatologist´s diagnosis of PsA and ii. 375 single patients with PsV without any joint affection, as well as 376 healthy controls.

We assayed 6 haplotype tagging SNPs based on LD-data from HapMap of a 17 kb block encompassing most of TNFRSF1A. These SNPs capture 97% of haplotype variability in controls. We found only marginal association of two SNPs with PsV (p=0.032; rs767455), but none with PsA. However, when we compared disease severity in PsA patients with >3 years duration of arthritis (197 with erosive arthritis vs. 107 without erosive disease), we found strong association of all six htSNPs with severe disease course and erosive joint destruction. This was strongest for rs767455 (OR=1.83, p=0.0004). Homozygosity for the risk allele resulted in an OR=2.81 p=0,000178; Bonferroni corrected p=0.003 (22 tests). A haplotype encompassing rs767455 was less strongly associated (OR=1.53, p=0.017). Sequencing of coding regions and flanking sequences in 32 PsA patients with erosive disease and homozygous for the rs767455 risk allele identified no coding variants. A further variant flanking the LD-block showed association neither with PsO/PsA nor with erosive joint disease. Analysis with RT-PCR identified no alternative splicing variants in PsA patients homozygous for rs767455. Our data indicate that TNFRSF1A is a susceptibility factor for disease severity in psoriatic arthritis. Additional functional studies are required to elucidate the exact nature of this association.

P108

Lack of association between a potentially functional variant of the autoimmune regulator (AIRE) gene and alopecia areata

Pforr J.(1), Becker T.(2), Hua Y.(1), Hanneken S.(3), Eigelshoven S.(3), Cuyt I.(4), De Weert J.(5), Lambert J.(6), Kruse R.(3), Nöthen M.M.(7), Blaumeiser B.(4), Betz R.C.(1)

1) Institute of Human Genetics, University of Bonn, Bonn, Germany
2) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany
3) Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany
4) Department of Medical Genetics, University Hospital of Antwerp, Antwerp, Belgium
5) Department of Dermatology, University Hospital of Gent, Gent, Belgium
6) Department of Dermatology, University Hospital of Antwerp, Antwerp, Belgium
7) Life & Brain Center, University of Bonn, Bonn, Germany

Alopecia areata (AA) is a common dermatologic disease with patchy hair loss affecting approximately 1-2 % of the general population. AA can manifest in both sexes of all age groups preferentially in the second and third decade. The progression of AA can be extremely variable. A classification in three subtypes is established referring to the amount of hair loss. AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle, and may be associated with other autoimmune diseases. However, the etiopathogenesis of AA has not been completely understood so far but the susceptibility to AA is likely to follow a complex polygenic trait. At least one third of patients with the autoimmune polyendocrinopathy candidiasis ectodermal dysplasia syndrome (APEDEC), a rare autosomal recessive disorder, experience AA. Patients with this syndrome carry mutations in the autoimmune-regulator (AIRE) gene on chromosome 21q22.3. Therefore the AIRE gene can be regarded as an interesting candidate gene for AA. A recent study has suggested that a potential functional variant (rs1800520; S278R) of the AIRE gene contributes to susceptibility to AA, especially in the group with the severest form of AA, alopecia universalis (AU), and for disease of early age at onset ($\leq$ 30 years) (Tazi-Ahnini et al. 2002). In an attempt to replicate these findings we investigated the putative risk allele in a case-control sample of Belgian-German origin (273 patients/ 283 controls). Our results do not support a significant association of the risk allele in our AA patients collective. Furthermore, the analysis of subgroups of individuals with AU, with positive family history or early age of onset

did not reveal association of this polymorphism. We therefore suggest that the AIRE gene might not play a major role in the development of AA.

P109

Molecular characterisation of a balanced chromosome translocation identifies MGST2 as a candidate gene for psoriasis vulgaris

Tzschach A.(1), Hoffmann K.(1), Hoeltzenbein M.(1), Bache I.(2), Tommerup N.(2), Bommer C.(3), Körner H.(3), Kalscheuer V.(1), Ropers H.-H.(1)

1) Max Planck Institute for Molecular Genetics, Berlin, Germany
2) Wilhelm Johannsen Centre for Functional Genome Research, Copenhagen, Denmark
3) Charité - Institute of Medical Genetics, Berlin, Germany

Psoriasis vulgaris is a chronic inflammatory disease with a strong contribution of genetic factors. A recent meta-analysis of published linkage data has identified a major susceptibility locus (PSORS9) on chromosome 4q31. We report a male patient with a balanced chromosome translocation t(2;4)(p25;q31.1) and familial psoriasis vulgaris. The breakpoint in chromosome band 4q31 disrupts a non-coding RNA gene (CR742434) that overlaps, in antisense direction, the MGST2 gene. MGST2 encodes a microsomal glutathion S-transferase that plays a central role in the synthesis of leukotriene C4 (LTC4). Altered expression of this gene due to the chromosome rearrangement may influence the synthesis of LTC4 and change the balance of pro- and antiinflammatory mediators, thereby predisposing to disorders like psoriasis. Thus, MGST2 is a promising positional and functional candidate gene for psoriasis vulgaris.

P110

Analysis of polymorphisms of GBP28 (APM1) gene in the Polish subjects with obesity

Hoppe-Golebiewska J.(1), Kaczmarek M.(1), Owecki M.(2), Miczke A.(3), Bryll W.(3), Pupek-Musialik D.(3), Sowinski J.(2), Slomski R.(1)

1) Institute of Human Genetics, Polish Academy of Science, Poznan, Poland
2) University of Medical Sciences, Department and Clinic of Endocrinology and Internal Diseases, Poznan, Poland
3) University of Medical Sciences, Department and Clinic of Internal Diseases, Metabolic Disturbances and Hypertension, Poznan, Poland

Adiponectin is an adipocytes-specifically expressed protein regulating energy homeostasis, glucose and lipid metabolism. Plasma concentrations of adiponectin have been reported to be significantly lower in individuals with obesity. Recent work has suggested that adiponectin may be involved in mediation of the effects of body weight as a risk factor for coronary artery disease and type 2 diabetes mellitus. A proteolytic cleavage product of the adiponectin mouse homolog has been shown to stimulate skeletal muscle fatty acid oxidation and to limit weight gain in high caloric diet-fed animals. Adiponectin is encoded by GBP28 gene, mapped to chromosome 3q27. The GBP28 gene spans 16 kb and is composed of three exons from 18 bp to 4277 bp in size with consensus splice sites. The third exon of this gene contains a long 3'-untranslated sequence containing three Alu repeats. This gene organization is very similar to one of obese gene, encoding leptin.
The aim of the studies was studying correlation between adiponectin plasma level and occurrence of SNPs in Polish obese patients. In this purpose three fragments of GBP28 gene were chosen and PCR primers were projected. Analyzed fragments included SNP45 (325 bp) in exon 2 and SNP2808 (366 bp) in exon 3, reported as frequent in individuals with obesity, and another fragment of exon 3 of GBP28 gene (330 bp).
Blood samples from 200 individuals with diagnosed obesity and from 50 healthy controls were collected and DNA subjected to screening procedure of chosen fragments GBP28 gene. Using PCR-SSCP different SSCP band patterns were observed. The result of molecular analysis will be compared with plasma adiponectin values for obese patients and healthy controls.

P111

Exploration of the genetic architecture of idiopathic generalized epilepsies

Gehrmann A.(1), Taylor K.(1), Heils A.(2), Dietter J.(3), Sander T.(4)

1) Max Delbrück Center, Gene Mapping Center, Berlin, Germany
2) Rheinische Friedrich-Wilhelms-University of Bonn, Clinic of Epileptology and Institute of Human Genetics, Bonn, Germany
3) University Marburg, Institute of Medical Biometry and Epidemiology, Marburg, Germany
4) on behalf of the European Consortium on the Genetics of Idiopathic Generalized Epilepsy, Berlin, Germany

Idiopathic generalized epilepsy (IGE) accounts for approximately 25% of all epilepsies and affects about 0.2% of the general population. The etiology of IGE is genetically determined, but the complex pattern of inheritance suggests the involvement of several susceptibility genes. The objective of the present study was to explore the genetic architecture of common IGE syndromes and to dissect seizure type-related susceptibility loci. Genome-wide linkage scans were performed in 132 European IGE-multiplex families ascertained through a proband with either idiopathic absence epilepsy (IAE) or juvenile myoclonic epilepsy (JME), as well as at least one additional sibling affected by IGE. To differentiate seizure type-related susceptibility loci, linkage analyses were carried out in two distinct family subgroups, each containing two or more siblings affected by either typical absence seizures or myoclonic and generalized tonic-clonic seizures on awakening. Both non-parametric and parametric linkage scans revealed a complex and heterogeneous pattern of linkage signals (5q34, 6p12, 11q13, 13q31, 19q13) that differed in their composition, depending upon the predominant seizure type in the families. Susceptibility loci on 11p13 and 13q31 predisposed preferentially to absence seizures, whereas loci on 6p12 and 19q13 conferred susceptibility to myoclonic and generalized tonic-clonic seizures on awakening. Evidence for gene interaction could be demonstrated for the IAE loci on chromosomes 11q13 and 13q31, using Genehunter Two-Locus analyses. Our present linkage results suggest that different oligogenic configurations determine the phenotypic expression of either absence or myoclonic seizures in IGE-multiplex families.

P112

Molecular genetics of alopecia areata: characterization of a rat model

Nita A.-S.(1), Blech H.(1), Kleensang A.(2), Meyer B.(1), Kolberg S.(1), Ziegler A.(2), McElwee K.(3), Hoffmann R.(4), Hennies H.C.(1)

1) University of Cologne, Dermatogenetics, Cologne Center for Genomics, Koeln, Germany
2) University Clinics of Schleswig-Holstein, Inst. of Medical Biometry and Statistics, Lübeck, Germany
3) University of British Columbia, VCHRI and Div. of Dermatology, Vancouver, Canada
4) Albert-Ludwigs-Universität, Dept. of Dermatology, Freiburg, Germany

Alopecia areata is a non-scarring, inflammatory form of hair loss. It is characterized by the appearance of one or several circular patches completely devoid of hair on the head that may progress to universal alopecia. Males and females are similarly affected, and the phenotype may appear at any age. Investigation of humans and animal models demonstrated that alopecia areata is a multifactorial autoimmune disorder with a high recurrence risk for siblings between 20 and 60. The search for genetic factors of alopecia areata is made difficult by heterogeneity of the clinical course. In order to identify genes involved in the etiology of alopecia areata, we have embarked on the genetic analysis of an animal model. Up to 70% of Dundee experimental bald (DEB) rats spontaneously develop alopecia areata-like patchy hair loss associated with peri- and intra-follicular inflammation and presenting clinical heterogeneity. Using a crossing approach with PVG/Ola rats, we have obtained 130 affected F2 animals and, surprisingly, additionally 60 F2 animals with typical histopathology but without hair loss. To identify genes involved in the hair loss, we have analyzed the animals for linkage in a genome-wide scan with microsatellites. Using various tests, we have obtained hints at linkage on chromosomes 5 and 8 with non-parametric Z scores of 2.4 ($p<0.008$) and 2.5 ($p<0.006$), respectively, in animals with full but not in those with histologic phenotype. The Cochran-Armitage test showed slight association for markers on chromosome 14 ($p<0.02$). These regions are now being further characterized with saturated mapping. Identification of the gene(s) underlying hair loss in the rat model will provide candidate genes for alopecia areata in man and give insight into the pathophysiology of alopecia areata. A potential genotype/phenotype correlation in the DEB rat might also give a clue to different pathways implicated in the manifestation of Alopecia areata.

P113

Lack of association of a functional SLC6A4 promoter polymorphism with migraine with aura

Todt U.(1), Netzer C.(1), Goebel I.(1), Heinze A.(2), Heinze-Kuhn K.(2), Ramirez A.(1), Göbel H.(2), Kubisch C.(1)
1) Institute of Human Genetics, Cologne, Germany
2) Schmerzklinik, Kiel, Germany

Migraine with aura (MA) is an episodic neurological trait with complex inheritance. Although the molecular pathophysiology of MA is largely unknown, there is convincing functional and pharmacological evidence for a causal involvement of the serotonergic system in migraine pathogenesis. One important and well studied component of this system is the serotonin transporter (SLC6A4), in which promoter there is an insertion/deletion polymorphism (5-HTTLPR) that might influence serotonin uptake. Very recently, a significant association between the short allele (s-allele) of 5-HTTLPR and MA was reported in a small German sample of 96 MA patients and 115 controls. In order to replicate this finding, we analyzed this polymorphism in an independent large group of German individuals comprising 472 patients with MA and 506 healthy controls. The insertion/deletion polymorphism was genotyped by standard PCR-based techniques. To compare the polymorphism frequencies between cases and controls, we applied a standard chi2-test for allelic and genotypic association with the disease phenotype. The genotype distribution of the healthy controls was s/s 77 (15.2%), s/l 241 (47.6%), and l/l 188 (37.2%) and that of MA patients was 61 (12.9%), 223 (47.2%), and 188 (39.8%), respectively. The genotype distributions of both samples did not deviate significantly from the Hardy-Weinberg equilibrium as analyzed by the exact test (p>0.5). No significant differences could be detected in the allele or genotype distribution of 5-HTTLPR between MA patients and controls (p = 0.26, OR = 0.9 CI [0,749-1,080]). In conclusion, we found no evidence for a contribution of the 5-HTTLPR polymorphism to the genetic predisposition of complex inherited migraine with aura in our large German sample. As the genetic power of our sample is high, our data suggest that the reported genetic association may be a false positive finding possibly resulting from a small sample size.

P114

Genetics of retinal drusen formation in rhesus macaques

Singh K.K.(1), Ristau S.(1), Dawson W.(2), Krawczak M.(3), Schmidtke J.(1)
1) Hannover Medical School, Institute of Human Genetics, Hannover, Germany
2) University of Florida, J. H. Miller Health Sciences Center, Florida, United States of America
3) Christian-Albrechts University, Kiel, Germany

Retinal drusen is an important hallmark of ARMD (Age Related Macular Degeneration) in humans, but the underlying mechanism is not well characterized. It is known that rhesus (Macaca mulatta) present a natural animal model for retinal drusen, and in both, man and monkey, genetic factors are implicated. Aim of this study was to access the genetics implicated in drusen formation in rhesus macaques maintained by CPRC, USA, and to verify these findings in the macaques kept at the DPZ, Germany. A search using 42 microsatellite markers, linked to 7 different loci implicated in retinal pathology in humans, was performed in a single rhesus matriline. Several genes implicated in ARMD have been mapped to the 6q region in humans. Two of the candidate rhesus orthologs, ELOVL4 and IMPG1, were searched for mutation using PCR, SSCP and sequencing. An association between two alleles at heptallelic marker D6S1036 and the severity of drusen formation was found (P combined: 0.012). For ELOVL4, a polymorphism was found in the 3‘ UTR which was not associated with the drusen pathogenesis. In IMPG1, 6 SNPs were identified. Haplotype frequencies of these SNPs were found to differ significantly between affected and non-affected animals. This difference was mainly due to a risk haplotype which was found on 17% of chromosomes in affected animals but not in controls of CPRC group. However, a total absence of this risk haplotype and much lower drusen prevalence (53%), in comparison to CPRC group (72%), was observed in DPZ group. Our data demonstrate that one or several genes on the rhesus homologue of human 6q14-15 are likely to play a role in retinal drusen formation. The association of IMPG1 risk haplotype with drusen formation in CPRC animals and lack of this haplotype in DPZ animals fully explain the observed differences in drusen prevalence in both groups and indicate that IMPG1 variations can not be the only risk factor involved in drusen pathogenesis in rhesus macaques.

P115

Renal phenotype of heterozygous *Lmx1b* knockout mice (*Lmx1b*$^{+/-}$) after uninephrectomy

Endele S.(1), Molter T.(1), Richter S.(1), Klanke B.(2), Winterpacht A.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) Department of Medicine IV, Erlangen, Germany

The Nail-Patella Syndrome (NPS) is a rare autosomal-dominant disorder characterized by minor skeletal abnormalities, and, in some cases (~50%), nephropathy. Nephropathy is the most serious aspect of NPS and often results in complete renal failure. NPS is caused by heterozygous loss-of-function mutations in the transcription factor gene *LMX1B*. Despite of identical mutations only 50-60% of the NPS patients develop a renal phenotype, which suggests genetic modifiers in the outbred human genetic background. Lmx1b$^{-/-}$ knockout mice (Chen et al., 1998) show an NPS-like phenotype including skeletal defects and renal dysplasia but die within a few days after birth, while heterozygous Lmx1b$^{+/-}$ mice seem to be phenotypically normal.

We started to evaluate the Lmx1b knockout mouse as a possible model for the mapping of genetic modifiers of Lmx1b activity in the kidney. This requires the identification of a renal phenotype in heterozygous knockout animals. In order to induce renal damage and thus provoke a renal phenotype in Lmx1b$^{+/-}$ mice 11 Lmx1b$^{+/+}$ and 13 Lmx1b$^{+/-}$ mice (in C57BL6 genetic background) and 3 Lmx1b$^{+/+}$ and 4 Lmx1b$^{+/-}$ mice (in JF1 genetic background) underwent unilateral nephrectomy and received a salt diet for 6 weeks. Renal damage was evaluated following measurements of organ weights, urine albumin and total protein excretion as well as glomerular volume. Although we identified a slight, strain specific difference in kidney weight gain, no significant differences between the Lmx1b$^{+/+}$ and Lmx1b$^{+/-}$ mice could be detected. Interestingly, quantitative RT-PCR revealed that Lmx1b expression in Lmx1b$^{+/-}$ kidneys is still 70% of wildtype expression. This indicates a compensatory mechanism in the mouse probably by Lmx1b autoregulation, which would explain the missing phenotype in Lmx1b$^{+/-}$ knockout animals compared to the human situation.

P116

Interleukin-10 polymorphism and prematurity: an exploratory study

Kerk J.(1), Dördelmann M.(2), Dressler F.(2), Bartels D.B.(3), Brinkhaus M.J.(3), Dammann C.E.L.(2), Dörk T.(1), Dammann O.(3)
1) Hannover Medical School, Gynaecology Research Unit, Hannover, Germany
2) Hannover Medical School, Department of Pediatrics, Hannover, Germany
3) Hannover Medical School, Perinatal ID Epidemiology Unit, Hannover, Germany

Inflammation appears to play a role in preterm birth and neonatal disorders of preterm newborns, such as white matter damage (WMD), developmental delay (DD), cerebral palsy (CP), bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP). It is unknown how maternal and fetal contributions interact in this context. We investigated whether polymorphisms in the genes for IL-1, TNFA, TLR4 and IL-10 affect perinatal inflammatory responses and the risks for neonatal disorders in preterm infants. Four SNPs were genotyped using multiplex ARMS in 73 children whose gestational age at birth had been < 32 weeks, and in their mothers. Exploratory analyses were performed after stratification of the preterm infant group by WMD (defined as any periventricular echolucency or echodensity on postnatal ultrasound), BPD, ROP, CP, and DD at age > 2 years. Infants homozygous for the high IL-10 producer-1082*G allele (n = 15) were less likely to develop WMD (odds ratio 0.2, 95% C.I. 0.03-0.8). This reduced risk was not affected appreciably by adjusting for potential confounders. No child with two G-alleles developed CP compared to 6/58 with only one or no G-allele. Among G-homozygous children, the risks for BPD, ROP 3rd stage, and DD were also prominently, but not significantly, reduced. When the separate and combined effects of receptor single nucleotide polymorphisms (SNPs) of mothers and their fetuses towards risk of delivery before 29wks gestation (N=29) compared to 29-31 wks (N=25) were assessed, the presence of the high-producer IL-10 allele in either mother or fetus was associated with a five-fold increased risk for birth <29wks. The results provide evidence for a maternal-fetal (inter-individual) gene-gene-interaction in preterm delivery and indicate a prominently reduced risk and severity of cerebral damage in preterm newborns carrying the IL-10 -1082*G allele.

P117

A common variant within the KCNQ1 gene is associated with drug induced long QT-syndrome

Kääb S.(1), Pfeufer A.(2), Hinterseer M.(1), Näbauer M.(1), Jalilzadeh S.(2), Akyol M.(2), George Jr. A.(3), Norris K.(3), Bezzina C.(4), Wilde A.A.(4), Schulze-Bahr E.(5), Berthet M.(6), Denjoy I.(6), Guicheney P.(6), Wichmann H.-E.(7), Meitinger T.(2), Roden D.(3)
1) 1. Med. Klinik, Klinikum Grosshadern, Munich, Germany
2) Institute of Human Genetics, TU Munich and GSF Research Center, Munich, Germany
3) Dept. of Medicine and Pharmacology, Vanderbilt University, Nashville, TN, United States of America
4) Academic Medical Center, Dept. of Experimental Cardiology, University of Amsterdam, Amsterdam, Netherlands
5) Department of Molecular Cardiology, University of Münster, Münster, Germany
6) INSERM U.582 - Institut de myologie, Hopital Pitié-Salpêtrière, Paris, France
7) Institute for Epidemiology, GSF Research Center, Munich, Germany

Introduction: Rare and common variants of cardiac ion channel genes have been identified as causal in only a few patients with drug-induced long QT syndrome (diLQT). SNPs in established LQT disease genes have been shown to modify the QT interval in the general population.

Hypothesis: To identify common gene variants that modify patients´ risks towards diLQT we conducted a LD-based SNP association study of five selected candidate genes.

Methods: 137 caucasian patients with diLQT (60% female, age 63.9±19.0 years old) from North America and Europe and 702 controls from the population based KORA S4 survey (Bavarian caucasian, 50% female, age 57.7±12.4 years old) were genotyped for a total of 145 tagSNPs. tagSNPs were selected based on HapMap data to represent the LD structure within LQT disease genes KCNQ1 (61 SNPs), KCNH2 (39), SCN5A (24), KCNE1 and KCNE2 (21). The genomic control approach was chosen to adjust for potential population stratification and 61 SNPs randomly distributed across the genome were genotyped (Devlin & Roeder, 1999).

Results: The genomic control factor lambda was determined to be 2.08 which indicates the presence of significant population differences between the pancaucasian patient and the regional Bavarian control sample. A haplotype formed by 2 SNPs located in Exon 16 and in the 3'UTR of the KCNQ1 gene (KCNQ1_Y662Y and rs10766424) which were in high LD (D' = 0.939, r2= 0.179) was significantly associated with diLQTS (OR=2.36, p=0.000089) and exceeded the tablewide significance level. This variant is different from the QTL for general population QT interval in intron 1 of KCNQ1.

Conclusions: Although independent confirmation of this predisposing common variant is mandatory, evidence is accumulating that beyond monogenic mutations common gene variants also contribute to the genetic risk of patients to develop potentially lethal arrhythmias via the sequence of QT prolongation leading to TdP tachycardia.

P118

Confirmation of Matrilin-3 (MATN3) mutation (p.T303M) as a risk factor for hand osteoarthritis

Tagariello A.(1), Schweizer A.(1), Swoboda B.(2), Schaller P.(3), Pullig O.(2), Winterpacht A.(1)
1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany
2) Department of Orthopaedics, University Erlangen-Nuremberg, Erlangen, Germany
3) Klinik Dr. Erler, Nuremberg, Germany

Osteoarthritis (OA) is the most common musculoskeletal disorder, characterized by progressive degeneration of joint cartilage resulting in joint pain and stiffness. The disorder has a multifactorial etiology. Hand OA is a subtype of OA, for which a strong genetic basis is suggested. Recently, it has been reported that a single nucleotide polymorphism (SNP) in the matrilin-3 (*MATN3*) gene encoding T303M at a highly conserved position in an EGF-like domain is associated with an increased risk [mutation frequency of 2 %, relative risk of 2.1] of idiopathic hand OA in the Iceland population (Stefansson et al., 2003). We investigated the SNP in a small but heterogeneous German cohort of patients with hand OA [n = 50] by direct sequencing. In 4 out of the 50 patients the SNP was present in a heterozygous state (frequency = 8 %). In contrast, 4 out of 270 healthy control individuals showed this SNP again in a heterozygous state (frequency = 1,5 %). Although our cohort is fairly small, with a p-value of 0.023 in the Fisher´s exact test the data are significant.

In conclusion, the presented data strongly support the importance of this missense SNP for the generation of clinically well defined hand OA and the involvement of matrilin-3 in the etiology of osteoarthritis.

P119

Analysis of conserved non-genic sequences (CNG) in the schizophrenia locus SCZD10 on chromosome 15q15

Schanze D.(1), Ekici A.B.(1), Stöber G.(2), Beckmann H.(2), Reis A.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) Department of Psychiatry and Psychotherapy, Würzburg, Germany

The human genome contains approximately 1-2% single copy, highly conserved sequences that are not functionally transcribed (CNG). Since the human sequence is completely known and genomic sequences of other species become available, global alignment programs such as VISTA can be used to identify these conserved elements. Many studies have been performed but the functional attributes of CNGs remain largely unknown. The evolutionary depth of their conservation, though, indicates that genomic variation in conserved non-genic sequences may be associated with phenotypic variability and human disorders.

Previously we identified a schizophrenia locus on chr.15q15 with genome-wide linkage studies of 12 extended pedigrees. We refined the critical region to a 7.49Mb interval with further extensive haplotyping studies. In our efforts to reveal the underlying disease gene, we performed mutation analysis up to now for 79 candidate genes by automated sequencing of DNA from 8 individuals of linked families and 8 controls. Due to absent functional mutations, we analysed the CNGs in a 7Mb region of the schizophrenia locus SCZD10 on chromosome 15q15. We used VISTA to identify all highly conserved elements in the region with ≥100 bases. In total, we could identify 3099 CNGs with more than 70% identity, 65 of them with an identity of 100%. We could ascertain a negative correlation of the density of CNGs with the density of genes. 90% of the CNGs are located in gene deserts. We now sequenced 8 affected individuals for the 65 perfectly conserved CNGs and detected 6 SNPs within these CNGs. The genotyping of these SNPs in a large cohort of cases and controls as well as the functional analysis of the affected CNGs is ongoing.

P120

Chromosome morphology and the decondensation defect in MCPH1 deficiency: Implications for the diagnosis of the disorder

Trimborn M.(1), Olejniczak K.(1), Sobczynska A.(1), Hirano T.(2), Neitzel H.(1)
1) Institut für Humangenetik, Charité, Berlin, Germany
2) Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, United States of America

Mutations in MCPH1 (microcephalin) cause autosomal recessive primary microcephaly with misregulated chromosome condensation. The hallmarks of the disease are a pronounced microcephaly and an elevated proportion of prophase-like cells (PLCs) in routine cytogenetic preparations (up to 20 %). The latter is due to premature chromosome condensation in the G2 phase of the cell cycle and delayed decondensation in G1 phase post mitosis. Another characteristic of the disorder is a poor banding resolution even on high resolution metaphase spreads (<250 bands per haploid complement). As shown here, this is due to shortening of the chromosome length axis. The average length of chromosomes of MCPH1 patients with early truncating mutations is reduced to approximately 75 % of normal controls. The central chromatid axes show a strong curling when labelled with antibodies against condensin I - a morphological effect that could possibly explain the shortening of the the chromosomes and the poor banding resolution observed in MCPH1 patients. Recently, we reported a new patient with the disorder showing an extraordently mild clinical and cellular phenotype with only 3% of PLCs in lymphocytes and lymphoblastoid cell cultures. Here, we demonstrate, that the decondensation defect accounts for nearly 50 % of the prophase-like cells and as a consequence the use of spindle poisons distinctly decreases the number of PLCs. In patients with mild mutations this reduces the amount of prophase-like cells to a normal level. If poor banding resolution is observed in the diagnostic process of patients with primary microcephaly, we therefore suggest that screening for prophase-like cells without colcemid treatment should be performed, since the application of the spindle poison attenuates the condensation defect by masking the postmitotic effect in early G1.

P121

Molecular variability within cytogenetic similar inversions of chromosome 2 and 9

Fickelscher I.(1), Schmidt S.(1), Claussen U.(1), Starke H.(1), Liehr T.(1), Weise A.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany

Inversions are the most common rearrangement in human constitutional karyotype. The constitutional inversions of chromosome 2 and 9 occur with a frequency of about 2% in the general population and seem to appear within the same cytogenetic region. Although these constitutional inversions are frequently observed in cytogenetic diagnostics only little is known about their formation and molecular characteristics. This might be due to the fact that in general heterozygote individuals are phenotypically normal even though associations with different syndromes have been suggested (summarized in Starke et al., 2002, Europ J Hum Gen). Here, we present the breakpoint mapping of the constitutional inv(2)(p11q13) and inv(9)(p11q13) with a molecular analysis of the corresponding breakpoint region. Analysis was done on peripheral blood lymphocytes derived from 30 healthy persons showing an inv(2)(p11q13) and 20 carriers of an inv(9)(p11q13). Breakpoint mapping was done by BAC-FISH and sequence analyses. One main and three minor breakpoint sites in 2p11 could be defined. For 2q13 all breakpoints were located within a 1 Mb region that has homologues sequences to the 2p11 breakpoint region; only one case showed a different breakpoint in 2q13. Investigations for inv(9) are in progress presently. The breakpoint regions are known to be hemiheterochromatic. Thus, they have a 'cross hybridising character' that makes it more difficult to define breakpoints. Nevertheless, until now 2 variant breakpoints for 9p11 were found. The finding of different molecular breakpoints for inv(2) and inv(9) confirmed and specified our previous findings (Schmidt et al., 2005, Hum Genet). Moreover, this is the prerequisite for a genotype–phenotype correlation of the different molecular breakpoints.

Supported by the Dr. Robert Pfleger-Stiftung and in parts by the Deutsche Krebshilfe/Mildred Scheel Stiftung für Krebsforschung (70-3125-Li1) and the IZKF together with a grant from the university of Jena.

P122

Molecular cytogenetic characterization of the mouse cell line WMP2 by spectral karyotyping (SKY) and multicolor banding applying murine probes (mcb)

Karst C.(1), Trifonov V.(2), Romanenko S.A.(2), Claussen U.(1), Mrasek K.(1), Michel S.(1), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Institute of Cytology and Genetics, Novosibirsk, Russian Federation

The Moloney murine leukemia virus-transformed suspension cell line WMP2 is derived from wild mice (Mus musculus) of the WMP/WMP strain. These mice carry nine pairs of metacentric Robertsonian translocation chromosomes. As the chromosomes of the wild-type mouse are all acrocentric, metaphase spreads of the WMP2 cells seam to be highly suited for physical gene mapping. Here we studied the WMP2 line using spectral karyotyping (SKY) combined with new established mouse specific multicolor banding (mcb) probes for the chromosomes X, 3, 4, 6 and 18. SKY revealed that the WMP2 cell line developed further four derivative chromosomes. After application of mcb five previously unrecognizable intrachromosomal rearrangements with 9 breakpoints were detected for the studied chromosomes: a translocation-chromosome including parts of the X-chromosome could now be described as der(9)t(9;X)(?;C); mcb 4 revealed in the dic(4;6) a deletion and an inversion in those two chromosomes; mcb 18 proved the presence of two dic(7;18) and a dic(8;8)t(8;8;18) and of two different variants of the dic(7;18). Moreover, the results obtained with the new established mcb probe sets for chromosomes 1 and 19 will be presented.

Supported in parts by the the DFG (436 RUS 17/49/02, 436 RUS 17/135/03, 436 RUS 17/48/05), the INTAS (2143), the Evangelische Studienwerk e.V. Villigst and the Deutsche Krebshilfe (70-3125-Li1). The cell line WMP2 was kindly provided by Dr. M. Rocchi (Bari, Italy) and Dr. H. Hameister (Ulm, Germany).

P123

Towards the molecular basis of the cytogenetic co-localization of fragile sites, evolutionary conserved breakpoints and cancer associated breakpoints

Mrasek K.(1), Reichelt F.(1), Claussen U.(1), Liehr T.(1), Weise A.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany

A possible association of fragile sites, evolutionary conserved breakpoints (bp) and cancer associated bp is discussed since a publication of Dutrillaux in 1979 suggesting a common mechanism in their formation. Fragile sites are specific chromosomal regions that tend to result in breaks and gaps on metaphase spreads. Similar to LCRs/segmental duplications they can play a role in pathogenesis especially in tumorigenesis. Nevertheless, only a few such sites are already investigated on a molecular level. In contrast, evolutionary conserved bp are well defined for great apes, especially for the chimpanzee, since the release of its complete genomic sequence. This fact made it possible now to choose BAC clones for these evolutionary conserved bp regions and test them directly on aphidicolin induced fragile sites as well as on cancer bp that map within the same cytogenetic region. By now we compared the evolutionary conserved bp in 5q15 (present in chimpanzee) using the corresponding BAC clone RP11-526D16 (Gross et al., 2006) to the FRA5D site in 5q15. The FRA5D site was studied in aphidicolin induced metaphase spreads of a normal donor. It could be shown, that the BAC clone RP11-526D16 mapped more proximal than the FRA5D site. The same held true for BAC clones RP11-1134K14 (present in chimpanzee; Gross et al., 2006) and RP11-577H5 (present in gorilla; Müller et al., 2005) which are co-localized cytogenetically with the FRA7F site in 7q22. Thus, for FRA5D and FRA7F a molecular co-localization with evolutionary conserved bp could be excluded. The studies concerning the cancer associated bp as well as those on other evolutionary conserved bp are in progress and will provide more insights whether there exists a formation mechanism in common or not.

Supported by the Evangelische Studienwerk e.V. Villigst, the IZKF together with a grant from the university of Jena, the Deutsche Krebshilfe (70-3125-Li1) and the IZKF / TMWFK (TP 3.7 / B307-04004).

P124

Rare sex chromosome mosaicism causing Ulrich-Turner Syndrome (UTS)

Ehresmann T.(1), Wimmer R.(1), Anthuber S.(2), Ochsenkühn R.(2), Steinlein O.(1)
1) Institut für Humangenetik, München, Germany
2) Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Klinikum der Universität München - Großhadern, München, Germany

The incidence of UTS is approximately 1:3000 newborn girls. About 50% of the cases are non mosaic 45,X. 40-50% are mosaic with an additional normal or structurally rearranged X chromosome. Less than 10% show mosaicism with a cell population containing a normal or an aberrant Y chromosome. These cases have an increased risk of gonadoblastoma due to the presence of the male determining gene SRY.
Our patient, a Turkish female, came to observation at the age of 17 years because of primary amenorrhoea and short stature. Surprisingly, the cytogenetic analysis of blood lymphocytes revealed three different cell lines, 46,X,der(Y)[65]/45,X[24]/46,X,+mar[16].
The der(Y) chromosome was of average size and none-fluorescent. By FISH-analysis the structure of the derivative Y chromosome and the origin of the marker chromosome were analysed.
The der(Y) chromosome turned out to be a pseudodicentric idic(Y)(pter?q11.2::q11.2?pter), thus it contains two copies of SRY. The marker chromosome consists of the Y centromere and surrounding euchromatic sequences, supposedly forming a ring chromosome r(Y)(p11.2q11.2). The Y chromosomal origin of both markers and the absence of a second X chromosome allows the conclusion, that a paternal meiotic error and consequent mitotic rearrangements caused our patient's karyotype.
Despite the increased percentage of SRY-containing cells in the lymphocytes, the patient had no signs of virilisation. Because of the high risk to develop a gonadoblastoma a gonadectomy was performed. The histological examination showed a non-malignant Leydig cell proliferation. Further studies of cultured fibroblasts will help to investigate the contribution of the different cell lines in gonadal tissues. Currently, the patient is treated with hormones to induce puberty.

P125

Small supernumerary ring chromosome 20 in dysmorphic child: identification of mosaic extra marker chromosome by centromere-specific multicolor FISH

Polityko A.(1), Rumyantseva N.(2), Jaroshevich E.(2), Naumchik I.(2), Mrasek K.(3), Liehr T.(3)
1) Republican Medical Center "Mother and child", Cytogenetics, Minsk, Belarus
2) Republican Medical Center "Mother and child", Minsk, Belarus
3) Institute of Human Genetics and Anthropology, Jena, Germany

Small supernumerary marker chromosome (sSMC) is structurally abnormal chromosome that can not be characterized unambiguously by conventional banding approaches alone due to its size (equal or smaller than chromosome 20). The prenatal and postnatal ascertainment of sSMC origin is the important task of molecular cytogenetics, this knowledge provides the exact complete cytogenetic diagnosis, as well as gives the understanding of structure and mechanisms of sSMS formation, helps to study the phenomenon of phenotypic „silence" or manifestation of euchromatic/heterochromatic material sSMC contains, uniparental disomy and another effects of sSMC presence. As such abnormal chromosomes carry centromere usually, centromere-specific multicolor FISH (cenM-FISH) technique was developed for simultaneous characterization of all human centromeres in order to clarify the origin of centromeric sSMC (Nietzel 2001). We report on the 8-years old male having moderate developmental delay, facial dysmorphies and mosaic karyotype 47,XY,+mar/46,XY according to conventional GTG analysis. Supernumerary sSMC was characterized as a small ring. Molecular cytogenetics. CenM-FISH was applied for marker chromosome study, and the origin of extra ring was determined as r(20). The second step using single color FISH with alpha satellite probe for chromosome 20 was performed to confirm this result and to examine the degree of mosaicism in blood lymphocytes and in skin fibroblasts, the ratios of abnormal and normal cells were 45/54 and 217/520 correspondently. Subsequently hybridization using subcenM-FISH - special probe mixture for chromosome 20 containing pcp probes and subcentromeric p and q BACs (Starke 2003) allowed to establish that marker includes subcentromeric euchromatic material. Finally the marker was characterized as ish r(20)(p11.2q11.2)(96L6+,243J16+). Data concerning genotype-phenotype correlation in the presence of mosaic imbalance due to small r(20) are presented and discussed.

P126

A new case of Dup(1q)(q42→qter) syndrome: molecular cytogenetic characterisation

Richter R.(1), Neumann L.M.(1), Klopocki E.(2), Neitzel H.(1), Tönnies H.(1)

1) Charité - Universitätsmedizin Berlin, Institut für Humangenetik, Berlin, Germany
2) Charité - Universitätsmedizin Berlin, Institut für Medizinische Genetik, Berlin, Germany

We report on a 2-year-old male patient with small stature, macrocephaly, a large fontanelle and a profound developmental retardation. High resolution GTG banding showed additional chromosomal material on the short arm of chromosome 1. The parental karyotypes were normal. Comparative genomic hybridisation (CGH) revealed a partial trisomy for 1q – the distal segment (1)(q42→qter) was translocated to the terminal region of the short arm of one chromosome 1. Therefore the karyotype can be described as: 46,XY,der(1)(qter→q42::pter→qter). Findings were proved by FISH using probes for subtelomere regions of 1p and 1q demonstrating an interstitial subtelomere 1p within the derivative chromosome 1. The translocation to 1p did not coincide with a loss of material in this region. Pure partial trisomy 1q is rare and only few cases with imbalances of (1q)(q42→qter) are reported. This is, to our knowledge, the first description of a translocation to the distal end of 1p, resulting in a chromosome with three subtelomeric regions without deletion in 1p.

P127

Insertional partial trisomy 9q due to maternal five –break double reciprocal translocation t(1;14)t(9;21)ins(14;9): confirmation by FISH findings and suggestion for a clinical diagnosis

Puttinger R.(1), Rittinger O.(1), Kronberger G.(1)

1) Paracelsus Medizinische Privatuniversität Salzburg, Universitätsklinik für Kinder-und Jugendheilkunde, Salzburg, Austria

Objectives: Reciprocal non-Robertsonian translocations are the most frequent structural chromosomal aberrations. Balanced carriers are at risk for variable unbalanced rearrangements with different viability in the offsprings. Four-break or more complex rearrangements have an even smaller chance for vertical transmission in a balanced state. We report on a 18 months old girl who was admitted with a history of intrauterine growth retardation, hypertrophic pyloric stenosis and surgery due to dislocation of the hip, which was apparently not detectable in the neonatal period. Physical exam revealed severe growth failure (- 4.75 SDS), flat occiput, unusal fair complexion, small ears and hyperextensible joints of the hands. Mental retardation seemed to be only mild but was not investigated in detail. Cytogenetic studies revealed a translocation t(1;14) with the possibility of additional material in one of the derivative chromosomes. Lymphocyte culture of the parents confirmed the girl's translocation to be a consequence of double reciprocal translocation t(1;14)t(9;21) in the mother. By means of wcp and subtelomeric FISH investigation, the additional material was shown to be an inserted part of 9q. Therefore, the karyotype in the child was described as 46,XX,der(1) t(1;14)der(14)t(14;1)ins(14;9). Karyotypes of the mother's parents and the patient's father were normal.

Conclusions: An unbalanced viable segregation of a complex maternal rearrangement is described. Interestingly, at least 2 further examples of insertional trisomy 9q are cited in Schinzel's catalogue (2001). Moreover, from the clinical point of view, pyloric stenosis appears to be common in cases with proximal 9q duplications, as reviewed by Yamamoto (1988). Therefore this feature might be a meaningful clinical sign especially in female retarded patients with dysmorphic features

P128

Search for a potential common genetic denominator predisposing to the development of cytogenetically unrelated chromosome 22 rearrangements in two brothers

Moritz A.(1), König M.(1), Müller I.(2), Dauber E.-M.(3), Ullmann R.(2), Haas O.A.(1)

1) Children's Cancer Research Institute (CCRI), Vienna, Austria
2) Max Planck Institute for Molecular Genetics, Berlin, Germany
3) Medical University Vienna, Department of Blood Group Serology, Vienna, Austria

Large-scale polymorphisms and repetitive sequence elements can promote illegitimate recombination between homologous and non-homologous chromosomes and thereby contribute significantly to the generation of a variety of chromosome rearrangements. These in turn are responsible for a multitude of genomic disorders and clinical diseases. With this notion in mind, we set out to investigate whether any and, if so, which potential common genetic features and mechanisms might have contributed to the formation of two intriguing and apparently unrelated chromosome 22 rearrangements in two brothers. The first cytogenetic analysis of the older brother, which was already performed in 1992 soon after birth because of holoprosencephaly, atrial septal defect and cleft palate, revealed a 45,XY,der(22)(q10;q10). Since the karyotype of both parents was normal, this abnormality was considered to have occurred de novo. Following the birth of a healthy girl, another boy was born in 2000, who intriguingly also suffered from a cleft palate as well as an atrial septal defect. He had an apparently normal karyotype. FISH analysis also ruled out a 22q11.2 deletion syndrome in both boys. However, to our surprise we found a triplication of the chromosome 22 subtelomer control probe in the younger brother. It was due to a cryptic unbalanced t(16;22)(p13.3;q13.3) with a loss of the corresponding 16p subtelomer. Detailed investigations with a 4590 BAC CGH array confirmed the 22q13.3 duplication and narrowed down the 16p13.3 deletion to the last three telomeric BACs. Subsequent PCR analyses of short tandem repeat polymorphisms uncovered that both the maternal and paternal chromosomes contributed to the formation of the der(22). Since the 22q duplication as well as the16p deletion affected only the paternal homologues in the other boy, both a meiotic or postzygotic mitotic origin of this rearrangement still remains possible.

P129

Polymorphic micro-inversions contribute to the genomic variability of humans and chimpanzees

Szamalek J.M.(1), Cooper D.N.(2), Schempp W.(3), Minich P.(1), Kohn M.(1), Hoegel J.(1), Goidts V.(1), Hameister H.(1), Kehrer-Sawatzki H.(1)

1) University of Ulm, Human Genetics, Ulm, Germany
2) Cardiff University, Medical Genetics, Cardiff, United Kingdom
3) University of Freiburg, Human Genetics and Anthropology, Freiburg, Germany

A comprehensive classification of the differences between human and chimpanzee genomes is required to identify the key genetic changes responsible for the morphological, physical and behavioural differences between these two species. We performed gene order comparisons of 11,518 human and chimpanzee orthologous gene pairs in order to detect regions of inverted gene order that are potentially indicative of small-scale rearrangements such as inversions. By these means, a total of 71 potential micro-rearrangements were detected, 9 of which were considered to represent micro-inversions encompassing more than three genes. These putative inversions were then investigated by FISH and/or PCR analysis and the authenticity of 5 of the 9 inversions, ranging in size from ~800-kb to ~4.4-Mb, was confirmed. These inversions

mapped to 1p13.2-13.3, 7p22.1, 7p13-14.1, 18p11.21-11.22, and 19q13.12 and encompass 50, 14, 16, 7 and 16 known genes, respectively. Intriguingly, four of the confirmed inversions turned out to be polymorphic: 3 were polymorphic in chimpanzees and one in humans. Functional analyses of the genes encompassed by these micro-inversions revealed that two clusters of genes with related functions have been disrupted by the breakpoints. These are a cluster of GSTM1-5 genes on HSA 1p13.2-13.3 and a ZNF (zinc finger protein gene) cluster on HSA 19q13.12. We conclude that micro-inversions make a significant contribution to genomic variability in both humans and chimpanzees and inversion polymorphisms may be more frequent than previously realised.

P130

Maternal inherited inverted duplication Xq28 involving MECP2 and L1CAM genes in two brothers with severe mental retardation and motor developmental delay

Vrábel K.(1), Bauer P.(1), Bonin M.(1), Feuchtinger T.(2), Tzschach A.(3), Singer S.(1), Altug-Teber Ö.(1), Enders H.(1), Riess O.(1), Dufke A.(1)

1) University of Tuebingen, Medical Genetics, Tuebingen, Germany

2) Paediatrician in Private Practice, Salem, Germany

3) Max Planck Institute for Molecular Genetics, Berlin, Germany

Distal Xq duplications of varying size including the MECP2 and L1CAM genes have recently been associated with severe mental retardation (MR) and neurological symptoms in males. While impaired function of MECP2 and L1CAM due to intragenic mutations are leading to classical Rett syndrome in females and X-linked-hydrocephalus in males, respectively, duplication of the MECP2/L1CAM region seems also to cause a distinct MR-phenotype. However, the clinical description is based on few patients only. In familial cases asymptomatic carrier females showed complete skewing of X-inactivation. We describe two brothers, aged 5 and 10 years, with severe mental and motor developmental delay. Retardation became apparent within the first year of life. Both brothers achieved independent sitting and crawling, but have no speech development. They both show muscular hypotonia and developed contractures of the lower limbs. Seizures were not observed.
Screening for subtelomeric rearrangements revealed a deletion of Xq in both patients and the mother, however, on GTG-banding the der(X) was elongated compared to a normal X chromosome. The precise nature of the rearrangement has been investigated by SNP-microarray, multicolour-FISH, locus specific BAC clones, and MLPA. The combination of these techniques revealed an intrachromosomal rearrangement with an inverted duplication of ~7Mb in Xq28. The centromeric breakpoint lies between exons 1 and 3 of the FMR2 gene, the telomeric deletion affects the PARII region in Xqter. The duplication includes the MECP2/L1CAM region. Since deletions of PARII are innocuous, we strongly suggest that the duplication is responsible for the patients phenotype. Furthermore, X-inactivation studies in the mother showed complete skewed X-inactivation.
The phenotype of our patients is very similar to previous reports on patients with smaller duplications of the MECP2/L1CAM region, suggesting a minor impact of other genes in the duplicated region on the phenotype.

P131

Submicroscopic unbalanced translocation 10p/13q detected by subtelomere-FISH

Roos A.(1), Rudnik-Schöneborn S.(1), Eggermann K.(1), Senderek J.(1), Zerres K.(1), Schüler H.(1)

1) Institute of Human Genetics, Aachen, Germany

Cryptic subtelomeric rearrangements resulting in segmental aneuploidy and gene-dosage imbalances represent a significant cause of MR associated with multiple congenital anomalies.
We report on the clinical findings as well as the cytogenetic and molecular results in a patient with a cryptic partial monosomy 10p15.3 and trisomy 13q34 as the consequence of an unbalanced de novo translocation which was identified by subtelomeric FISH. Clinically, our patient showed a marked growth retardation (3cm < 3rd centile) and a head circumference on the 3rd centile as well as cryptorchidism, bilateral inguinal hernias and a pyloric stenosis. The craniofacial phenotype comprises frontal bossing a long philtrum and a thin upper lip. His psychomotor development was significantly delayed. The patient fulfills consequently three items of the checklist published by De Vries et al. (2001). Among (sub)telomeric rearrangements, deletions of 10p have rarely been described in the literature. The smallest deletion affected 10p14-pter and was reported in 4 cases. The family history was unremarkable. By means of microsatellite typing using 10p and 13q specific markers we showed that the 10p deletion as well as the 13q duplication affected paternal chromosomes. The size of the partial trisomy 13q34 could be determined to comprise 0,7Mb. The partial monosomy 10p15.3 spanned approximately 4,8Mb. No comparable cases with corresponding deletions and duplications have as yet been published. In conclusion, the contribution of both partial aneuploidies to the phenotype in our patient is difficult to estimate but apparently the terminal 13q contributed to the facial gestalt while the 10p deletion caused the congenital malformations.

P132

Molecular cytogenetic analysis identifies a candidate gene for corpus callosum agenesis in a patient with pericentric inversion 3

Wolf A.(1), Großmann B.(1), Zechner U.(1), Brandt C.A.(2), Haaf T.(1), Beyer V.(1)

1) Universitätsklinikum Mainz, Institut für Humangenetik, Mainz, Germany

2) Country Hospital of Vejle, Department of Clinical Genetics, Vejle, Denmark

We report on a 13-year old boy with severe psychmotoric retardation and epileptic seizures but without dysmorphic features and congenital malformations. A CT scan revealed agenesis of the corpus callosum. Cytogenetic analysis demonstrated an apparently balanced pericentric inversion,
46,XY,inv(3)(p11q13.3)or(p12.2q21)or(p12.2q13.3) in the patient and his mother. So far no CT scan or detailed clinical information is available from the mother. FISH with region-specific large-insert clones identified a breakpoint spanning BAC, RP11-402E20, on chromosome 3q13.31. The molecular cytogenetic analysis of the breakpoint on 3p is still in progress. The breakpoint spanning BAC on 3q is located between exons 1 and 2 of the LSAMP gene. This gene encodes a neuronal surface glycoprotein found in cortical and subcortical regions of the limbic system. During development of the limbic system, the LSAMP protein is detected on the surface of axonal membranes and growth cones, where it acts as a selective homophilic adhesion molecule, and guides the development of specific patterns of neuronal connections. Further molecular studies, in particular mutation analysis of the second allele on the normal chromosome 3, are underway to determine whether the patient´s phenotype results from a disruption of the LSAMP gene.

P133

A mosaic karyotype 47,XX,+r(Y)(p?11.1q11.2)/46,XX in a female patient referred to ICSI due to male factor infertility

Dechend F.(1), Pabst B.(2), Miller K.(2), Schubert S.(2), Wilke G.(1), Graf N.(1)

1) Gemeinschaftspraxis Algermissen, Justus, Wilke, Graf, Klaßen, Hildesheim, Germany

2) Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover, Germany

We report a case of a mosaic karyotype 47,XX,+r(Y)(p?11.1q11.2)/46,XX in a 27 year old female nullipara, who was cytogenetically diagnosed before performing ICSI. The woman is phenotypically and gynaecologically normal with the exception of an endometriosis uteri externa, but her 25 year old husband exhibits an OAT-syndrome. Chromosome analysis of lymphocytes from her subfertile husband revealed a normal 46,XY karyotype, while we found in about 60% of her lymphocyte cells a 46,XX karyotype and in 40% of her cells an additional ring chromosome Y. We were able to confirm these findings in fibroblasts from different tissues (ovar, skin and buccal mucosa). From FISH and PCR analysis it is suggested that the breakpoints of the ring chromosome Y are in p11.1, lacking most of the short arm, including SRY and the TSPY clusters; and in q11.2 between the AZFb and AZFc region. Although the TSPY clusters were deleted we could show by PCR and sequence analysis that at least one functional full-length TSPY copy is present.
Whether the patient carries a risk for gonadoblastoma or has an increased risk for i.e. ovarian cancer is difficult to predict. To our knowledge, this is the first reported case of a chromosomal mosaicism with two X chromosomes and one ring Y chromosome. To elucidate the origin of the ring chromosome Y further studies are under progress.

P134

Different sizes of the duplicated region in partial proximal trisomy 10q syndrome might be caused by rearrangements affecting intrachromosomal repeats

Caliebe A.(1), Fryns J.-P.(2), Martin-Subero J.I.(1), Heidemann S.(1), Siebert R.(1)
1) Institut für Humangenetik, Kiel, Germany
2) Centre for Human Genetics, Leuven, Belgium

The mechanisms leading to chromosomal deletions and duplications are poorly understood. Proximal trisomy 10q syndrome is a rare chromosomal duplication syndrome. A common pathogenetic mechanism of this syndrome might be suggested by the fact, that all hitherto described patients carry direct rather than inverted duplications. We investigated two patients with direct duplications dup(10)(q11.2-22.3) and dup(10)(q11.2-23.3). The phenotype of the first patient, a 15-months-old girl, has been published recently (van Buggenhout et al., Genet Couns., 1996). The second propositus was a 13-months-old boy with dystrophy and developmental delay. G-Banding chromosome analyses revealed a male karyotype with a direct duplication 10q11.2q23.3. Chromosome analyses of both parents showed this aberration to have arisen de novo. At the age of 26 months he was of small stature (-4SD) and had a head circumference in the lower normal limit (–1,8SD). Facial dysmorphism was compatible with previously published patients and included small deep set eyes, protruding pinnae, hypoplastic maxilla, and downturned corners of the mouth. Hands and feet were short. He could walk unaided since age 23 months and spoke two words. FISH mapping of the duplicated regions in both patients revealed a different size of the trisomic material. Whereas the centromeric breakpoints were located in 10q11.2 in both patients, the telomeric breaks were assigned to 10q22.3 and 10q23.2~23.31. Remarkably, all breakpoints were located within regions strongly enriched for intrachromosomal repeats and phylogenetic breakpoints. Our findings suggest that repetitive regions proximal and distal to the duplicated region and unequal crossing over in meiosis may be a mechanism leading to proximal trisomy 10q syndrome. The size of the duplicated regions varies depending on the affected repeat region. Whether this influences the clinical phenotype has to be investigated in larger series of patients.

P135

A contiguous gene syndrome with Greig cephalopolysyndactyly syndrome through an interstitial del(7)(p14.2p13)

Bauer I.(1), Menzel C.(2), Viertel P.(2), Müller-Navia J.(3), Krüger G.(1)
1) Universität Rostock, Abteilung Medizinische Genetik, Rostock, Germany
2) MPI für Molekulare Genetik, Berlin, Germany
3) Institut für Klinische Genetik, Mainz, Germany

We report on a 13 years old girl with craniofacial dysmorphism, broad terminal thumb phalanges, syndactyly of hands, polydactyly of feet, and mental retardation. No agenesis or dysgenesis of corpus callosum was found.
An interstitial deletion within chromosome arm 7p was seen by conventional cytogenetic banding techniques. FISH analyses using a panel of YAC and BAC probes from the critical regions and reverse FISH after microdissection and DOP-PCR narrowed down the breakpoints to 7p14.2 and 7p13, respectively. Phenotype-genotype correlations suggest a GLI3 haploinsufficiency.

P136

Characterization of the human-specific pericentric inversion that discriminates human chromosome 1 from the homologous chromosomes in great apes

Szamalek J.M.(1), Goidts V.(1), Hameister H.(1), Kehrer-Sawatzki H.(1)
1) University of Ulm, Human Genetics, Ulm, Germany

Human and chimpanzee genomes are distinguished by 10 gross karyotype differences that include 9 pericentric inversions and one chromosomal fusion. Seven of these large pericentric inversions were fixed in the chimpanzee lineage and comparative breakpoint analyses showed that the two sister species, the common chimpanzee (Pan troglodytes) and the pygmy chimpanzee (Pan paniscus) share the same breakpoints. Thus these inversions predate the separation of the two chimpanzee species 0.86-2 Mya. Two inversions however, those of human chromosomes 1 and 18 were fixed in the human lineage after the split of humans and chimpanzees. We performed detailed molecular and computational characterization of the breakpoint regions of the human specific inversion of chromosome 1. FISH analyses as well as sequence comparisons revealed that the pericentric region of HSA 1 contains extensive segmental duplications of high sequence similarity between the p and q chromosome arms. Careful analyses of these regions allowed us to map the p-arm breakpoint proximal to the 120.4-Mb of the HSA 1 sequence and the q-arm breakpoint to the 851-kb region between 145.7 and 146.5-Mb. Both these regions contain human specific segmental duplications as determined by array CGH. We propose that the pericentric inversion of HSA 1 was mediated by intrachromosomal non-homologous recombination between these segmental duplications of inverted orientation and 99 % sequence homology, which arose specifically during the evolution of the human genome, by duplicative transposition events.

P137

Global brain dysmyelination with above-average verbal skills in a patient with a terminal deletion del(18)(21.33)

Ehrbrecht A.(1), Kornblum C.(2), Helmstaedter C.(3), Engels H.(1), Schwanitz G.(1), Urbach H.(4), Schröder R.(2), Weber R.G.(1), Netzer C.(1)
1) Institute of Human Genetics, Rheinische Friedrich-Wilhelms University, Bonn, Germany
2) Department of Neurology, Rheinische Friedrich-Wilhelms University, Bonn, Germany
3) Department of Epileptology, Rheinische Friedrich-Wilhelms University, Bonn, Germany
4) Department of Radiology, Rheinische Friedrich-Wilhelms University, Bonn, Germany

Patients with 18q- syndrome commonly display cerebral dysmyelination and developmental delay. To our knowledge, all reported cases characterized by molecular analysis who had no mental retardation as confirmed by neuropsychological testing had a chromosomal breakpoint within the two most distal bands, 18q22 or 18q23.
To improve the karyotype-phenotype correlation in 18q- syndrome, we thoroughly analyzed the deletion size, the mental and radiologic status in a 23-year-old woman with a terminal 18q deletion. Our patient was diagnosed with a developmental delay at the age of one year. Motor and language development were retarded throughout childhood. Motor and mental development greatly improved around the age of 11-12 years and growth as well as language skills in puberty. Our patient successfully graduated from school with a “Realschulabschluss”. She completed a professional training and is presently employed as an office clerk. We performed conventional cytogenetic and FISH analysis on peripheral blood lymphocytes and buccal mucosa smears, structural brain MRI, and extended neuropsychological testing. Molecular karyotyping revealed a 17Mb deletion of terminal 18q with a breakpoint in 18q21.33 and no evidence for mosaicism in two tissue types. Whereas brain MRI demonstrated severe global dysmyelination, the patient showed normal verbal intelligence and skills, i.e. a neuropsychological pattern that allowed for normal psychosocial and job achievement. However, visual, visual-spatial, visual-constructional, and executive functions were found to be severely impaired. Here, we present a patient with one of the largest terminal 18q deletions reported in an individual without obvious mental retardation. Our analysis highlights the fact that deletion size and structural brain MRI abnormalities may not sufficiently predict cognitive capabilities and that detailed neuropsychological testing is beneficial.

P138

Molecular cytogenetic characterisation of the DNA-degradation process in erythrocytes indicates similarities to marker chromosome formation

Lemke J.(1), Felka T.(1), Michel S.(1), Lemke C.(2), Gruhn B.(3), Liehr T.(1), Claussen U.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Institut für Anatomie I, Jena, Germany
3) Kinderklinik, Jena, Germany

The mechanism of DNA- and chromosome degradation during marker chromosome formation is not well understood so far. It might be possible that during maturation of erythrocytes a similar stem cell mediated process takes place leading to Howell-Jolly-Bodies (HJB) known as nuclear fragments in erythrocytes, which often appear in patients with pernicious anaemia and after splenectomy. Here, we analysed the DNA-content of HJB in detail. We selected formaldehyde-fixed erythrocytes of 5 splenectomised male patient with the help of an extended Pasteur pipette and characterised the DNA of the HJBs with molecular cytogenetic techniques. For that, we amplified and labelled the DNA by DOP PCR and performed reverse painting on normal metaphase spreads. At present, more

than 100 HJBs were characterised which led to specific centromeric signals preferentially on the chromosomes 1 and 7, 8 and 18. The centromeric regions of chromosomes 2, 3, 4, 9 and 10 were not frequently involved. Single HJBs consisted DNA from euchromatic and non-centromeric heterochromatic regions. In summary, we suppose that HJBs preferentially are consisted of centromeric heterochromatin of different chromosomal origin. Furthermore, we assume that HJBs are formed during a normal DNA degradation process which eliminates euchromatic material first. This degradation process might be stem cell specific and similar to that of marker chromosome formation during early embryonic development.

P139

Evolution of Y chromosomal segmental duplications in higher primates

Batz C.(1), Kirsch S.(2), Münch C.(1), Bernardi C.(1), Schempp W.(1)
1) Institute for Human Genetics and Anthropology, Freiburg, Germany
2) Institute of Human Genetics, Heidelberg, Germany

FISH analysis of BAC and cosmid clones spanning the human Y chromosomal euchromatin/heterochromatin transition regions in Yq11.23/Yq12 and Yq12/PAR2, respectively identified segmental duplications at 15 paralogous loci predominantly located in pericentromeric regions of 10 different chromosomes in the human karyotype. To elucidate the evolutionary fate and possible origins of these segmental duplications we comparatively hybridised these human Y chromosomal clones to metaphase spreads of great apes, gibbon and various Old World monkeys as well as a New World monkey as an outgroup species. Apart from the gorilla karyotype, where a highly amplified burst-like distribution pattern of signals was observed, in all other great apes a human- like distribution of signal pattern could be detected especially on their autosomes. The signals on the respective Y chromosomes however reflect the well known species-specific Y-chromosomal rearrangements in human and great apes. While the segmental duplications derived from the DNA clone of the Yq12/PAR2 transition region first appeared in the orangutan, those from the Yq11.23/Yq12 transition were first detected in the genomes of Old World monkey and gibbon being restricted to two autosomal pairs. Most interestingly, one of these autosomal pairs corresponds to the rDNA-containing "marker- chromosome" in gibbon and macaques. No signals appeared on the chromosomes of the New World monkey outgroup species, the Geoffroy´s marmoset. Our results fit well the view that the initial process of segmental duplication was directed to the rDNA-containing "marker- chromosome" of the macaques providing the target modules for a further burst of segmental duplications in great apes and human (see abstract from Münch et al.).

P140

Model of the evolution of segmental duplications in higher primates

Münch C.(1), Kirsch S.(2), Batz C.(1), Bernardi C.(1), Rappold G.(2), Schempp W.(1)
1) Institut für Humangenetik und Anthropologie, Freiburg, Germany
2) Institut für Humangenetik, Heidelberg, Germany

About 5% of the human genome consists of segmental duplications, which are large, highly homologous (>95%) fragments of sequences and are predominantly located in euchromatin/heterochromatin transition regions. It has been estimated that these segmental duplications emerged during the past ~35 million years (Myr) of human evolution and that they correlate with chromosomal rearrangements and genomic variability and plasticity. It is assumed that a first wave of segmental duplications was initiated by AluS-elements about 35 – 25 Myr ago, after an ancestor of New World monkeys branched off the Old World monkey lineage, and a second wave initiated by AluY-elements occurred some 8 – 4 Myr ago after separation of the human from the chimpanzee lineage. These periods of segmental duplications coincide with important steps in human evolution, and thus gene sequences within these duplications could have provided the genetic raw material for the evolution of adaptive processes in great apes and human. Our comparative FISH analyses in higher primates using clones from three euchromatin/heterochromatin transition regions of the human Y chromosome long arm fit with this evolutionary time course of segmental duplication. While there are no signals visible in New World monkeys, FISH-signals for segmental duplications are predominantly localised on the rDNA-containing „marker chromosome" in Old World monkeys. Only in great apes and human a complex chromosomal distribution of segmental duplications can be observed. In conclusion our evolutionary model of segmental duplications assumes that in a first step the acquisition of „attractor-modules" mediated by mobile elements and initial duplication on the rDNA-containing „marker chromosome" in Old World monkeys provided the target modules for a further process of segmental duplications in great apes and human.

P141

The median relative distance of the 5'BCR and 3'ABL FISH spots in tumour cells is a sample specific parameter

Bernhardt A.(1), Gloria A.(1), Spies-Weißhart B.(1), Jochannes T.(2), Lörch T.(2), Claussen U.(3), Loncarevic I.(1)
1) Institut für Humangenetik und Anthropologie des UKJ, Tumorgenetik, Jena, Germany
2) MetaSystems GmbH, Altlussheim, Germany
3) Institut für Humangenetik und Anthropologie des UKJ, Jena, Germany

Fluorescence in situ hybridization (FISH) is capable to identify tumour cells by a co-localisation of a 5'BCR and a 3'ABL FISH signal in cell nuclei (nuc ish). In some tumour samples we noted a higher proportion of cells with a significant distance between the co-localised signals. This prompted us to use the automatic image analysis system Metafer-MetaCyte (MetaSystems, Germany) to generate more objective data. At least 1000 cells per sample were subjected to 3-dimensional spot distance analysis. To be independent from the nuclei size, the relative distance (RD) measurement was introduced. From the distribution of the relative distances found in an individual sample we could determine the median relative distance (RD-M). Within the samples analyzed, the RD-M ranged from 0,06 to 0,13. The dynamic range of the analysis was 0.04 - 0,22. We observed a variance of only about 4% in repeated, independent experiments. Moreover the RD-M varied less than 2% when different conditions in cell preparation, e. g. ambient humidity, were used. No correlation was found for RD-M with distinct BCR-ABL subtypes (M-BCR or µBCR). Similar analyses were performed for other close located sequences (300kb to 1Mb). These experiments also showed no correlation of the RD-M and the genomic distance of the respective DNA sequence. Although, a high reproducibility of the RD-M was also found in these analyses. Despite the open questions, RD-M is found to be a sample specific constant. This result reveals interesting aspects, e.g. for the discussion about the ´cut off´ levels.

P142

Prenatal diagnosis of a de novo small supernumerary marker chromosome 4

Wehner L.-E.(1), Liehr T.(2), von Eggeling F.(2), Osmers R.(3), Zoll B.(1), Bartels I.(1)
1) Institut für Humangenetik, Georg-August-Universität, Göttingen, Germany
2) Institut für Humangenetik und Anthropologie, Friedrich-Schiller-Universität, Jena, Germany
3) Frauenklinik, Städtisches Krankenhaus, Hildesheim, Germany

Small supernumerary marker chromosomes (sSMC) are present in about 0.05% of newborn children. Prenatal detection of an sSMC presents a special difficulty in genetic counselling, as a karyotype-phenotype correlation has so far not been established for most sSMCs. We report here on the prenatal detection of an sSMC derived from chromosome 4. Indication for amniocentesis was an increased nuchal translucency of 3.9mm at 13 weeks of gestation. An sSMC was detected in 68 of 111 metaphases (61%) of three independent cultures. The sSMC was also present in chorionic villi in 60% of metaphases. Chromosome analyses of the parents were normal. Using M-FISH-, subcenM-FISH- and MCB analysis we characterized the sSMC as min(4)(:p13->q11.1:). To the best of our knowledge no other comparable sSMCs have been reported to date. A uniparental disomy for chromosome 4 was excluded. Detailed ultrasonography at 20 weeks of gestation revealed a polyhydramnion and a possible esophageal stenosis with tracheoesophageal fistula. All other parameters in ultrasonography were normal. The parents decided to continue the pregnancy. At 39 weeks of gestation a girl of 3740g was spontaneously delivered with an Apgar-score of 9/10/10. Detailed clinical examination revealed no abnormalities. Postnatal chromosome analysis is currently underway. Detailed molecular cytogenetic analysis of all cases of sSMCs and detailed clinical follow-up are needed in the future in order to establish a comprehensive karyotype-phenotype correlation. This would allow for a better genetic counselling in cases detected prenatally.

P143

Intrachromosomal triplication of chromosome 2p: molecularcytogenetic characterization and phenotype

Kron A.(1), Müller-Navia J.(2), Hilal A.(3), Decker J.(1), Decker J.(4), Steinberger D.(5)
1) Bioscientia, Zentrum für Humangenetik Ingelheim, Ingelheim, Germany
2) Institut für klinische Genetik, Mainz, Germany
3) Armed Forces Hospital, Department of Pediatrics, Khamis Mushayt, Saudi Arabia
4) Johannes Gutenberg Universität, III. Medizinische Klinik und Poliklinik, Hämatologie, Onkologie und Pneumologie, Mainz, Germany
5) Bioscientia, Zentrum für Humangenetik Ingelheim und, Justus-Liebig Universität, Institut für Humangenetik, Giessen, Germany

Triplications of chromosome 2p have not been described in the international literature so far. Here we present the first case with such a chromosomal aberration and describe the molecular-cytogenetic and clinical findings in a 3 month old girl.

The patient was born preterm after 31 weeks of pregnancy in breech presentation by spontaneous vaginal delivery. Soon after birth she developed a respiratory distress syndrome with pneumothorax. She presented multiple dysmorphic features including frontal bossing, wide metopic suture and anterior fontanelle, low set and posteriorly rotated ears with abnormal helix and antihelix, peri-auricular pits, antimongoloid palpebral fissure, abnormal retinal pigmentation, micrognathia, cleft palate, short neck and small hands and feet. The echocardiogram indicated a small atrial septal defect.

The postnatal conventional chromosome analysis revealed in all cells analysed a triplication of proximal parts of the short arm of chromosome 2 with the suspected breakpoints 2(p13p21) respectively 2(p11.2p15). Further delineation of the breakpoints using microdissection analyses are ongoing. FISH analysis confirmed that the chromosomal origin of the additional material is completely derived from chromosome 2.

P144

Trisomy 6 mosaicism associated with holoprosencephaly

Hempel M.(1), Schramm T.(1), Minderer S.(1), Högel B.(2), Daumer-Haas C.(1)
1) Pränatal-Medizin München, München, Germany
2) Rotkreuzkrankenanstalten, Pathologie, München, Germany

We report a fetus with trisomy 6 mosaicism and complex brain malformations. Microcephaly was suspected in the 27th week of the first pregnancy of a 30-year-old healthy woman. In addition to microcephaly alobar holoprosencephaly with fusion of thalami, an abnormal facial profile and overlapping fingers were found. Amniocentesis was performed with normal FISH results (13, 18, 21, X and Y). The pregnancy was terminated because of the severe brain malformations with no possibility of cognitive development. Fetal examination revealed microcephaly, microphthalmia with fused eyelids, large and flat nose with broad nares, short and simple philtrum, small mouth with thin lips, small mandible, dysplastic ears, retrognathia, oligodactyly of the right hand with camptomelia and overlapping fingers, syndactyly of second and third toes of the right foot.

Pathological examination showed abnormal cranial bones, alobar holoprosencephaly, cerebellar abnormalities, anophthalmia, facial dysmorphism, abnormal pulmonary segmentation, small stomach, single pelvic kidney with one ureter and uterus bicornis. Trisomy 6 mosaicism was found in two independent amniotic fluid cell cultures (mos 47,XX,+6[12]/46,XX[38]).

Trisomy 6 mosaicism is a rare chromosomal aberration. Up to now only eight cases with trisomy 6 mosaicism were reported in the literature. Four of these cases resulted in normal liveborns. In two cases the patients had multiple malformations (without brain malformation) but relatively normal mental development. Two reported pregnancies with trisomy 6 mosaicism were electively terminated.

To our knowledge our case is the first report of trisomy 6 mosaicism associated with severe brain malformations (including holoprosencephaly) leading to poor outcome.

P145

Conventional CGH versus array-CGH: genome-wide detection of euchromatic imbalances in diagnostic cases – a comparison

Tönnies H.(1), Gerlach A.(1), Trotier F.(2), Matthäi A.(3), Schröck E.(3), Ullmann R.(4), Mundlos S.(2), Neitzel H.(1), Klopocki E.(2)
1) Charite, Universitätsmedizin Berlin, Institut für Humangenetik, Berlin, Germany
2) Charité, Universisätsmedizin Berlin, Institut für Medizinische Genetik, Berlin, Germany
3) TU Dresden, Institut für Klinische Genetik, Dresden, Germany
4) Max Planck Institut für Molekulare Genetik, Berlin, Germany

Today, conventional cytogenetics (CC) is the main technique in routine genetic diagnostics for the detection of chromosomal abnormalities. During the last decades, fluorescence in situ hybridization (FISH) of specific probes to patient metaphases has proven to be useful for the characterization and validation of aberrations found in CC. In our laboratory comparative genomic hybridization (CGH) is a well established routinely used whole genome FISH-based approach. Conventional CGH detects genomic imbalances with cytogenetic resolution on normal metaphase spreads without prior knowledge of the aberrant chromosomal region. Using conventional CGH for the identification and validation of chromosome aberrations in several hundred hybridizations before, we are now investigating the capacity and sensitivity of microarray-based CGH (array-CGH). In our array-CGH experiments we used arrays with different resolutions (1Mb and 150kb) for the precise and fast detection of chromosomal imbalances. Here we present some outstanding cases in routine diagnostics investigated by conventional and array-CGH in order to demonstrate the differences of both techniques in analyzing and characterizing de novo chromosomal rearrangements. The higher BAC-clone based resolution of array-CGH compared to conventional CGH allows us to define the size of deletions and/or duplications more precisely. Additionally, array-CGH refined the description of the aberrant chromosome segments by enabling direct mapping of imbalances to the genome sequence data. Thus simplifying further validation of the detected imbalances by FISH on patients' metaphases and allowing us to characterize the underlying gene(s) involved in the patients' phenotype.

P146

Individual risk estimates for the occurrence of unfavorable pregnancy outcomes in two pedigrees of t(1;11)(p36.22;q12.2) and t(12;14)(q15;q13) carriers

Midro A.T.(1), Panasiuk B.(1), Gajêcka M.(2), Lecœniewicz R.(1), Schwanitz G.(3), Schaffer L.(2)
1) Department of Clinical Genetics Medical Academy, Bialystok, Poland
2) Health Research and Education Center, Washington State University, Spokane, Wa, United States of America
3) Institute of Human Genetics, Bonn, Germany

Families with balanced chromosomal translocations frequently ask for genetic risk of unfavourable pregnancy outcome and infertility. Empirical data of 60 pregnancies from 10 families of t(1;11)(p36.22;q12.2) of one pedigree and empirical data of 11 pregnancies from 3 families of t(12;14)(q15;q13) of a second pedigree were collected. Probability rates for the occurrence of an unfavourable pregnancy outcome were calculated according to the method of Stengel-Rutkowski and Stene. Chromosome investigations were done by combining at least two different banding techniques. Additional FISH techniques were applied for breakpoint identification. FISH mapping on chromosome 1 revealed that the breakpoint is located between BAC 575L21 (AL096841) and AP001098 (RP11-874A11) giving a distance between 10,311,592-10,503,096 bp from the telomere. FISH mapping on chromosome 11 revealed that the breakpoint is covered by BAC AP003721 (RP11-881M11). M-FISH investigation of family 2 confirmed the breakpoint position obtainted by banding techniques. Based on indirect analysis of pedigree one it was found, that the probability rate for the birth of a child with unbalanced karyotype (monosomy 1p36.22→pter combined with trisomy 11q12.2→qter after 2:2 disjunction and adjacent 1 segregation) is <0.9% (0/50) (low risk), for a stillbirth 2,0 ± 1,9% (1/50) and for miscarriages 34,0 ± 6,7% (17/50). For pedigree two there was found no risk for an unbalanced progeny at birth, and low risk for miscarriages of about 1% (1/11) after ascertainment correction. The data were offered for genetic counselling of the families.

(BMBF project POL 03/025 and DPJ 05/005. KBN Polish-German project no 5253)

P147

Re-investigations in patients with the 22q11.2 microdeletion

Lischke C.H.(1), Köhler A.(1)
1) Institut für Humangenetik, Gießen, Germany

Microdeletion analysis in 22q11.2 is a frequently requested test in the Human Genetics laboratory. The test is easy to perform using the commercially available FISH probes TUPLE1 or N25 (eg. Vysis). Both probes derive from within the common 3Mb deletion representing 84-90% of

the deletions found in patients with DiGeorge syndrome and VCFS, respectively. Another, smaller deletion is found in 7-14% of cases and is also detected by probes TUPLE1/N25 (Rauch et al 2005; Kerstjens-Frederikse et al 1999).
We recently started a re-investigation of our TUPLE1/N25 deletion patients using DNA from 7 BAC-clones kindly provided by Dr. A. Tzschach (MPI for Molecular Genetics, Berlin). Two probes (CTD-2367L15, CTD-2536F14) were proximal and two (RP11-22M5, RP11-659D9) were distal flanking markers of the commonly deleted region. An additional three (CTD-2522F24, RP11-138C22, RP11-54C2) were from within this region. After DOP-PCR we labelled the probes with Spectrum Orange (Vysis) and Spectrum Green (Vysis), respectively by nick translation and hybridized them to metaphases of our TUPLE1/N25 deletion patients. So far we have analysed 16 deletion patients and the study is ongoing. In 15 cases the common deletion was demonstrated while one patient displayed the shorter proximal deletion. In this patient only clones CTD-2522F24 and RP11-138C22 were deleted. Her clinical features were comparable to those of the other 22q11.2 deletion patients.
Literature: Kerstjens-Frederikse et al 1999; J Med Genet 36: 723-4
Rauch et al 2005; J Med Genet42: 871-6

P148

Array based comparative genomic hybridization in the diagnosis of idiopathic mental retardation

Martin T.(1), Weber G.(1), Oehl-Jaschkowitz B.(1), Haupert M.(2), Christmann A.(1)
1) Praxis für Humangenetik Homburg/Saar, Homburg, Germany
2) Zentrum für innovative genetische Diagnostik IGD-Saar GmbH, Homburg, Germany

Chromosomal aberrations are a common cause of inborn syndromes including growth and developmental delay and dysmorphism. Conventional chromosomal analysis on a at least 550 bands per haploid genome level is still the golden standard for the analysis of these children although there is a resolution of only about 10 to at most 5 Mb. Especially the screening for subtelomeric rearrangements, mostly using FISH based assays, has improved these resolution but only for the subtelomeric regions. In children with idiopathic mental retardation and additional dysmorphic features a detection rate of about 7% for chromosomal aberrations has been achieved. Now novel high resolution, whole genome technologies as array based comparative genomic hybridisation (array-CGH) are leading to a new quality step in chromosomal diagnostic with, according to serious estimations, a doubled detection rate. With the new techniques a resolution of at least 1 Mb is possible concerning not only the subtelomeric regions but the whole genome. All interstitial microduplications or -deletions within this size causing chromosomal imbalances can be revealed. We have launched a microarray based CGH in our clinical diagnostics of mentally retarded and dysmorphic children when conventional chromosomal analysis and a subtelomeric screening have failed. We use a commercially available microarray from ArrayGenomics containing 3400 BAC clones spaced at approximately 1 Mb intervals across the genome. We had to improve the labelling protocol and hybridisation conditions to achieve reproducible and reliable results. The hybridisations are done as duplicates including a dye switch which gives an internal control. Suspicious results are checked by FISH using the concerning BAC clones and controlled for familiarity by testing the parents. First results will be presented.

P149

Fanconi anemia with a high rate of spontaneous and MMC-induced chromosomal translocations – diagnostic screening strategy

Müller A.-S.(1), Schindler D.(2), Neitzel H.(1), Tönnies H.(1)
1) Institut für Humangenetik, Charité, Universitätsmedizin Berlin, Chromosomendiagnostik und Molekulare Zytogenetik, Berlin, Germany
2) Institut für Humangenetik, Biozentrum, Würzburg, Germany

Fanconi anemia (FA) is a rare, heterogeneous recessive chromosome instability disorder with at least 12 complementation groups (FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM). FA patients are characterized by congenital abnormalities, bone marrow failure with a high risk for the transformation into acute myeloid leukemia (AML). In addition, FA patients have an increased risk for the development of some types of solid tumors such as squamous cell carcinomas. The underlying defect in DNA-crosslink repair in FA forms the basis of high chromosome breakage rates.
Gold-standard for the diagnosis of FA is the characteristic hypersensitivity of FA cells to agents such as mitomycin C (MMC). Most common chromosomal aberrations in FA are chromatid breaks and radial figures. Here, we propose a case of FA uncommon by an extreme high spontaneous chromosome breakage rate as judged by the number of chromosomal translocations compared to other FA patients. Chromosomal translocations, rarely detectable by conventional chromosome breakage tests after Giemsa staining, were detected using a three whole chromosome painting (wcp) assay established in our lab recently. Automated capturing of up to 500 metaphase spreads was performed using the Metafer scanning system.
For complementation group assignment, FANCD2 immunoblotting showed D2-S and D2-L bands excluding FANCD2 and the upstream genes of the FA core complex as mutated. Two complementation groups of FA are known to act downstream of FANCD2: FANCJ was present at the protein level excluding group FA-J. BRCA2 mutations are currently searched for classifying this patient as belonging to the rare group FA-D1.

P150

Cytogenetic and molecular characterization of an azoospermic male with a pseudodicentric (Yq) Isochromosome

Javad K.Z.H.(1), Gebauer J.(2), Omrani M.D.(3), Kalscheuer V.(4), Kulawik A.(2), Royer-Pokora B.(2)
1) Institut of Human Genetics und Anthropology, Heinrich-Heine-University of Duesseldorf, Prenatal and Postnatal Diagnostics, Duesseldorf, Germany
2) Institut of Human Genetics and Anthropology, Heinrich-Heine-University of Duesseldorf, Duesseldorf, Germany
3) Department of Genetic, Urmia Medical Science University, Urmia, Iran, Islamic Republic Of
4) Max-Planck-Institute of Molecular Genetics Berlin, Berlin, Germany

We describe a 38-year-old male with a pseudodicentric Y chromosome. The testis of this man are small and hard. Biopsy and pathocytologic examination of the left testicle revealed a complete absence of germ cells. Analysis of the ejaculate showed azoospermia, which is defined as the complete absence of sperms in a spermiogram. The androgen hormonal level values were in normal range. Chromosome analysis, using G, Q, C banding and FISH techniques revealed a gonosomal mosaicsm between a monosomy X (51%) and a pseudodicentric Y isochromosome (49%) : mos 45,X/46,X,psu dic(Y)(qter→p11.31::p11.2→qter)
Molecular genetic studies with 20 STS-markers (Polymerase chain reaction: PCR) did not confirm a absence of the SRY-gene and the whole scanned AZFabc-regions, which could partly be reconfirm by molecular cytogentic methodes (Fluoresence In Situ Hybridization: FISH). Combined FISH- analysis with a SRY-DNA-probe and a special DNA-marker (Marker 200kb) shows only one SRY-gene in the pseudodicentric Y-chromosome. Moreover, there was no indication for any translocation between the aberrant Y chromosome and other autosomal chromosomes. Therefore, the azoospermia could be the result of the monosomy X (45,X) cell line, which may affect the normal gonadal development.

P151

Chromosomal instability as a genetic mechanism of leukemogenesis in congenital bone marrow failure syndromes

Michaelsen G.(1), Kratz C.(2), Zeidler C.(3), Niemeyer C.(2), Welte K.(3), Schlegelberger B.(1)
1) MHH, Institut für Zell- u Molekularpahtologie, Hannover, Germany
2) Univeristätsklinikum Freiburg, Kinderklinik Hämatologie und Onkologie, Freiburg, Germany
3) MHH, Pädiatrische Hämatologie und Onkologie, Hannover, Germany

Patients with congenital bone marrow failure syndromes (BMFS) carry an increased risk for hematological neoplasias.
Using cytogenetic and molecular genetic methods, within the framework of a subproject supported by the German Federal Ministry of Education and Research, the course of diagnosis of bone marrow failure syndromes right up to tumor development is being studied. The aim of the project is to better characterize the mechanism of development of hematological neoplasias, in order to use the results for improved diagnostics and possibly for new therapies. A major point of interest is the chromosomal instability, which leads to aneuploidy or structural changes of the chromosomes. Since the start of the project in January 2004, we have examined material from 89 patients with BMFS, whereby alongside the banding analysis, we have carried out fluorescence in situ hybridization with specific probes for chromosomes 7 and 8. Clonal aberrations

have been identified in 5 of the patients examined. In one child with congenital thrombocytopenia, we were able to diagnose a Jacobsen syndrome due to a deletion in 11q; in a second child with congenital thrombocytopenia, a constitutional translocation t(11;13)c was detected. In a female patient diagnosed with Shwachman-Diamond syndrome, a complex aberrant chromosome set was found with an isochromosome i(7)(q10), monosomies 5 and 13 as well as a deletion in 20q, whereby the bone marrow morphology showed a simultaneous transition into an MDS. The brother of this patient, who was also diagnosed with Shwachman-Diamond-Syndrome, showed an isochromosome i(7q) as a single aberration. In a patient with congenital neutropenia, a 21q+ marker containíng a low-level amplification of the AML1 gene was identified at the point of transition into an AML.
A further aim is to characterize by extensive analysis the clonal aberrations in more detail, and to clarify whether cytogenetic findings give an indication of evolving tumor development.

P152

Cytogenetic Quiz - Test and train your abilities to recognize chromosome aberrations

Mau-Holzmann U.A.(1), Singer S.(1), Enders H.(1), Dufke A.(1), Rieß A.(1), Rieß O.(1)
1) Institute of Human Genetics, Department of Medical Genetics, Tuebingen, Germany

Several karyotypes with various chromosome aberrations are displayed. Different levels of difficulty might be interesting for beginners as well as for experienced cytogeneticists. The viewer is invited to test and train herself/himself if she/he is able to recognize the represented aberrations. Additionally, some clinical aspects of the different cases are presented.

P153

Regulation of neural crest cell migration in the trunk: The axolotl as a model system

Heß K.(1), Steinbeisser H.(1), Epperlein H.-H.(2)
1) Institut für Humangenetik, Sektion Entwicklungsgenetik, Heidelberg, Germany
2) Institut für Anatomie, TU Dresden, Dresden, Germany

The neural crest (NC) is an exclusive feature of vertebrate embryonic development that is found transiently during neurulation and gives rise to a wide variety of cell types and structures in the body such as connective and supportive tissue of the skull, neuronal and glial cells of the peripheral nervous system, endocrine cells, and pigment cells. Defects in the distribution and differentiation of NC cells, so called 'neurocristopathies', may lead to various malformations of the heart and face (e.g. 'Otocephaly'), to disorders of the PNS (e.g. Hirschsprung's disease), or to melanomas.
We use the Mexican axolotl (Ambystoma mexicanum) to dissect the molecular mechanisms governing migration along the dorsolateral pathway that is followed by pigment cells. This urodele has several advantages for studying developmental processes such as slow development, large eggs and a high regenerative capacity. We have developed a new approach to study dorsolateral NC cell migration in the living axolotl embryo. Implantation of Xenopus animal cap cells expressing secreted signalling molecules under the control of a heat shock promoter allowed us to control the expression of growth factor molecules in a spatially and temporally regulated manner. One of the signalling molecules we analyzed is bone morphogenetic protein-4 (BMP-4). This TGFß growth factor has a number of important functions during early embryogenesis and differentiation of the neural crest. BMP-4 acts as a morphogen and patterns the developing mesoderm. It also controls NC cell delamination, regulates apoptosis of cranial NC cells and differentiation of sympathetic neurons. Using the animal cap implantation assay, we show that BMP-4 and its antagonist Noggin can modulate pigment cell migration in vivo specifically without interfering with NC delamination or differentiation.

P154

Functional analysis of murine Brunol4, a member of Elav/Bruno RNA binding protein family

Dev A.(1), Shoukier M.(1), Nayernia K.(1), Engel W.(1)
1) Institut für Humangenetik, Göttingen, Germany

RNA binding proteins are involved in posttranscriptional mechanisms and control gene expression at many regulatory levels including RNA splicing, stabilization and transport. Although numerous RNA binding proteins have been identified, very few have been studied extensively in the context of developmental processes. We focused our study on Brunol4, a novel mouse gene closely related to elav-type family of genes encoding for RNA-binding proteins. The subfamily was named according to Bruno gene of Drosophila.The murine Brunol4 gene contains 13 exons and encodes a protein containing 495 amino acids. Brunol4 expression is detectable from two-cell stage embryo to all the stages of developing embryo. In postnatal stage its expression is restricted to cerebral structure, in particular the cerebellum where it persists in the adult organism. However, the function and potential targets of Brunol4 beyond RNA binding are unknown. To elucidate the function of Brunol4 in vivo we generated knockout mice using homologous recombination. A region of 800 bp of exon1 including ATG was replaced by GFP/Neo cassette. While Brunol4 heterozygous mice were apparently healthy and fertile, no Brunol4 homozygous mice were identified. These results reveal that homozygous embryos are lethal. Currently we are determining the stage when development of homozygous embryos is arrested. Taken together these observations suggest that Brunol4 plays a crucial role during early embryonic development in mouse.

P155

***Sox9* is necessary for inner ear development in the mouse**

Barrionuevo F.(1), Naumann A.(2), Neubüser A.(2), Scherer G.(1)
1) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
2) Institute of Biology I, University of Freiburg, Freiburg, Germany

SOX9, the transcription factor gene mutated in the semilethal skeletal malformation syndrome campomelic dysplasia (CD; OMIM 114290), is involved in many developmental processes. Surviving CD patients can suffer from sensorineural hearing loss. In mice, *Sox9* is expressed in the otic placode, the inner ear anlagen. To study the role of *Sox9* during during inner ear development, we conditionally inactivated both *Sox9* alleles using the Cre/*loxP* recombination system, whereby *Cre* is under control of the *Foxg1* locus. Histological analysis at day 11.5 of embryonic development (E11.5) revealed that in *Sox9* mutant embryos, all the structures of the developing inner ear are absent. During the course of inner ear development, the ectodermally derived otic placode invaginates around E9.5 to form the otic vesicle, which subsequently differentiates to form the inner ear. In *Sox9* mutant embryos, the initial formation of the otic placode is normal, but this structure remains as a non-invaginated layer of epithelial cells, which is eliminated by apoptosis starting at E9.5. Expression of inner ear molecular markers *Pax8, Pax2, Dlx5, Bmp7, Pea3* and *Erm* during the otic placode stage (E8.0 – E8.5) shows no difference between wild-type and *Sox9* mutant embryos. In contrast, other molecular markers as *Sox10*, *Tbx2* and *Col2a1* are either absent or show reduced levels of expression, placing them downstream of *Sox9*. Furthermore, using the *Ck19:Cre* line, mosaic inactivation of *Sox9* reveals that only SOX9-positive areas of the otic placode can invaginate. Our results thus reveal an essential and early role of *Sox9* for otic development, already before differentiation of the inner ear proper occurs that shows dysfunction in surviving CD patients.

P156

Regulation of cell movements by Wnt/Frizzled signaling in early vertebrate embryo

Swain R.(1), Steinbeisser H.(1)
1) Institut für Humangenetik, Heidelberg, Germany

Wnts are secreted glycoproteins that regulate diverse processes during vertebrate development and are implicated in human diseases. These Wnt signals are mediated by seven-transmembrane proteins of Frizzled family and by intracellular components such as β-catenin, cJun-N-terminal kinase (JNK), small GTPases Rho and Rac and the calcium regulated kinases such as Protein kinase C (PKC) and CamKinase II. The formation of the anteroposterior body axis in vertebrate embryos is dependent on tightly regulated Wnt signaling and perturbation of these signals causes defects in morphogenesis and results in dysmorphic phenotypes. We are using Xenopus as a model organism to study the role of Wnt/Frizzled signaling in regulating vertebrate gastrulation movements. Wnt signaling is essential for convergent extension (CE) movements of the mesoderm and the neuroectodem. Hyperactivation or inhibition of Wnt signaling prevents neural tube closure causing a 'spina bifida-like' phenotype, which is also a major defect during human embryogenesis.
Our comprehensive analysis of the role of Wnts, Frizzleds and the secreted Wnt antagonists during Xenopus gastrulation aims to elucidate the molecular mechanisms underlying the coordinated cell movements responsible for proper vertebrate morphogenesis.

P157

Mutational analysis of a conserved zebrafish *sox9* notochord enhancer

Heß I.(1), Rastegar S.(2), Ertzer R.(2), Strähle U.(2), Scherer G.(1)
1) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
2) Institute of Toxicology and Genetics, Forschungszentrum Karlsruhe, Karlsruhe, Germany

Mutations in the transcription factor gene SOX9 cause campomelic dysplasia, a rare skeletal malformation syndrome. The large 5' intergenic region of SOX9 contains several cis-regulatory elements highly conserved between human, mouse, Fugu and zebrafish. One of these elements, E1, which directs reporter gene expression to the notochord in transgenic mice (Bagheri-Fam et al., in press), was analysed in more detail by using zebrafish as a model system. Due to a genome duplication event around 300 million years ago, zebrafish has two sox9 homologues, termed sox9a and sox9b, both of which contain the E1 element. The 130 bp core sequence of the sox9a E1 (E1a) element was shown to direct expression of the green fluorescent protein (gfp) reporter gene to the notochord in 24 h-old transient transgenic zebrafish embryos. By in situ hybridisation, the endogenous sox9a and sox9b gene were shown to be expressed in the notochord in a time window between 17 hpf (hours post fertilization) and 20 hpf, while the E1a enhancer shows expression between 11.5 hpf and 20 hpf. We have previously shown that mutation of the winged helix transcription factor FoxA2 binding site within E1a leads to loss of notochord expression, indicating that sox9a might be a direct target of FoxA2. In fact, EMSA assays proof that bacterially expressed FoxA2 binds to the conserved E1a FoxA2 binding site, but not to a mutant site. In addition to mutation of the FoxA2 site, mutation of a second conserved sequence in the E1a core enhancer also results in loss of notochord expression, indicating that FoxA2 alone is not sufficient to direct the complete expression pattern. Presently, we test the importance of this second motif as a potential and necessary transcription factor binding site. Sequence comparison of conserved enhancer elements from other notochord-expressed genes may allow us to define a common motif structure of notochord-specific enhancers, which will be tested in transient transgenic zebrafish.

P158

Pax3 and Pax7 expression during myogenesis in the mouse embryo

Horst D.(1), Ustanina S.(2), Sergi C.(1), Mikuz G.(1), Jürgens H.(3), Braun T.(2), Horst J.(4), Vorobyov E.(4)
1) Medizinische Universität Innsbruck, Institut für Pathologie, Innsbruck, Austria
2) Max-Planck-Institut für Herz- und Lungenforschung, Bad Nauheim, Germany
3) Universitätsklinikum Münster, Klinik und Poliklinik für pädiatrische Hämatologie/Onkologie, Münster, Germany
4) Universitätsklinikum Münster, Institut für Humangenetik, Münster, Germany

The transcription factors Pax3 and Pax7 are known to play a role in myogenic precursors of the developing trunk. However, it is not yet clear whether these genes are required for the myogenic cell specification in the head and for the post-somitic myogensis per se. In part, this uncertainty is due to the scarce information about their normal time course and pattern of expression. Using immunohistochemical in situ analysis we detected spatiotemporal characteristics of Pax3 and Pax7 protein expression in comparison to that of MyoD and myogenin in the developing trunk and head muscles. The observed patterns of expression suggest that Pax3 is not involved in myogenesis in the head, and its post-somitic expression in the trunk and limb muscles is mostly repressed after stage E13.5. In contrast, Pax7 expression is shared among all striated muscles and exhibits a uniform pattern. Pax7 is expressed only in mononucleated cells that either differentiate into myotubes or later form satellite cells. During development of head muscles, expression of Pax7 follows expression of MyoD and myogenin, implying that Pax7 is not required to induce the initial steps of the myogenic program in the head. In Pax7 homozygous mutants, in which muscle development proceeds normally, expression of Pax3 is indistinguishable from its wild-type pattern (i. e. absent), suggesting that after stage E13.5 myogenesis does not require Pax3 and Pax7. These data challenge the concept that Pax3 and Pax7 determine a persistent lineage of myogenic precursors in pre-natal and post-natal muscle development. Based on these results, it is tempting to speculate that Pax3 and Pax7 allow the formation of only provisory myogenic structures, in which the myogenic process itself is initiated as a distinct program independent of Pax genes.

P159

Differential DNA replication and amplification of the *Igf2* gene in mouse trophoblast giant cells

Leuchs S.(1), Haaf T.(1), Hemberger M.(2), Zechner U.(1)
1) Universitätsklinikum Mainz, Institut für Humangenetik, Mainz, Germany
2) Babraham Institute, Laboratory of Developmental Genetics and Imprinting, Babraham, Cambridge, United Kingdom

Mouse trophoblast giant cells (TGCs) differentiate from trophoblast cells by endoreduplication, that is successive rounds of DNA replication without cytokinesis. The endoreduplicated homologous chromosomes in TGCs do not appear to be closely associated. In most endoreduplicating cells from other tissues and organisms, the parameters of the S phase can be altered to cause over- or underreplication of specific chromosome regions. So far little is known about differential DNA replication in mouse trophoblast giant cells. We performed interphase FISH with gene-specific BAC probes to analyze the copy number and spatial organization of genes that are essential for trophoblast development versus control genes in TGCs differentiated from two trophoblast stem (TS) cell lines, TS-G28 and TS-GFP. Analysis of the TS-G28 cell line revealed a partial trisomy of the central and distal part of mouse chromosome 7 containing the *Igf2* gene in undifferentiated TS cells, leading to three independent *Igf2* gene clusters in differentiated TGCs. Several BAC probes showed a scattered signal distribution in TGC nuclei suggesting a polyploid rather than a polytene arrangement of chromosomes, whereas other BAC probes exhibited two signal clusters which would be consistent with polyteny. Analysis of the TS-GFP cell line revealed differential DNA replication of several genes, e.g. an amplification of the *Igf2* gene on distal mouse chromosome 7 and an under-replication of the *Snrpn* gene on central mouse chromosome 7. Since IGF2 has been reported to promote differentiation of TS cells into TGCs, the amplification of the *Igf2* gene may provide a selective advantage for the differentiation process.

P160

Expression pattern of *WHSC3* (Wolf-Hirschhorn syndrome candidate gene 3) during neocortex and cerebellum development suggests a role in neuronal migration

Boerdlein A.(1), Schlickum S.(2), Winterpacht A.(1), Endele S.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) Rudolf-Virchow-Zentrum, Würzburg, Germany

Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation syndrome which is caused by partial deletion of the short arm of one chromosome 4. WHS is thought to be a contiguous gene syndrome with a yet unknown number of genes contributing to the phenotype. We focused on the neurological features of the syndrome (mental retardation, seizures) and further investigated a recently cloned gene (*WHSC3*) from the Wolf-Hirschhorn syndrome critical region (WHSCR1) which is strongly expressed in brain and testis. Human *WHSC3* encodes a protein of 90 amino acids, which probably encodes a preprohormone or neuropeptide precursor.
Here, we present a detailed expression study of *Whsc3* during murine neocortex and cerebellum development by RNA-*in situ*-hybridization of mouse embryos. Expression in the cerebral cortex starts after the preplate stage (> E12). At E14.5 to E17.5 *Whsc3* is expressed in the intermediate zone and in the marginal zone (most probably in the Cajal Retzius cells) of the developing cortex. In newborn mice, we could detect signals in neurons of the cortical plate and in Cajal Retzius cells (marginal zone) whereas in adult brain signals were present in all cortical and subcortical regions of the brain. In the developing cerebellum, expression could be detected in the intermediate zone at stages E.13 to 17.5, in the molecular layer in newborn mice and in the granular and molecular layer in adult mice (most probably in the interneurons).
In conclusion, the expression pattern suggests a potential role of this gene in neuronal migration processes, and thus in the pattern formation of human cerebral and cerebellar structures.

P161

Multicolor banding studies on the 'Barr-body' in 3D-preserved human lymphocyes and on chromosomal orientation in human sperm interphase nuclei

Hunstig F.(1), Pellestor F.(2), Steinhaeuser U.(1), Starke H.(1), Ziegler M.(1), Claussen U.(1), Liehr T.(1)
1) Institut für Humangenetik und Anthropologie, Jena, Germany
2) Institute of Human Genetics, CNRS, Montpellier, France

Human cytogenetic preparations obtained by the airdrying procedure of chromosome preparations are good targets for molecular cytogenetics, in general. However, this standard procedure leads to a flattening of the originally spherical interphase and metaphase nuclei. Thus, when interphase or metaphase architecture shall be studied this flattening leads to questionable results. Recently we reported an approach called suspension-FISH (S-FISH) where the whole FISH-procedure is performed on cell suspension. We proved that it is possible to do 3-D-analyses on totally spherical interphase nuclei or even on three-dimensional metaphases (Steinhaeuser et al., J Histochem Cytochem 2002, 50:1697-1698). Here we present first multicolor banding (MCB-)results on 3D-preserved interphase of human lymphocytes to study position, size and orientation of the inactivated X-chromosome (Barr-body) compared to the active one. Active and inactive X-chromosome can be distinguished by S-FISH combined with MCB. Preliminary data provide evidence for the fact that the Barr-body is located more at the interphase periphery, while the active one is located more central. Additionally, we will present data on the orientation of the X-chromosome in the human sperm. The studies are presently in progress, however, initial results show that the X-chromosome seems to be orientated along the sperm axis.

Supported in parts by the "Förderverein des Klinikums der FSU Jena e.V." and the INTAS (AISbl 03-51-4060).

P162

Molecular signatures allow diagnosis of benign human tumors with highest specificity and sensitivity

Comtesse N.(1), Ludwig N.(1), Keller A.(2), Lenhof H.-P.(2), Meese E.(1)
1) Universität des Saarlandes, Humangenetik, Homburg, Germany
2) Universität des Saarlandes, Zentrum für Bioinformatik, Saarbrücken, Germany

Molecular signatures promise early detection of human tumors and offer new therapeutic targets. This study was aimed to define signatures in meningioma as a benign human tumor.
We assembled a panel of 57 meningioma-expressed proteins that show reactivity with serum antibodies of meningioma patients, including 41 newly identified meningioma antigens that were detected by screening of a fetal brain expression library.
We tested this panel for reactivity with 63 sera of patients with common-type, atypical, and anaplastic meningioma, respectively, and 40 sera from individuals without known disease. We detected 11 proteins exclusively with patient sera. The natural killer-tumor recognition sequence (NKTR) was recognized by more than 44% of meningioma patients, SRY (sex determining region Y)-box2 (SOX2) by more than 31% and KIAA1344 by more than 30%. Using statistical learning methods we demonstrate that our approach allows differentiation between meningioma patients and healthy individuals with a specificity of 96% and a sensitivity of 90%.
This is so far the best discrimination between cancer patients and controls by a noninvasive diagnostic approach.

P163

Functional analyses of DAZ genes plasticity in Y chromosomes of different haplogroups

Falcao C.(1), Enders C.(1), Härkönen K.(2), Tyler-Smith C.(3), Strowitzki T.(1), Vogt P.(1)
1) University Women Hospital, Dept. Gynecol. Endocrinol.& Infertility, Heidelberg, Germany
2) University of Turku, Dept. Medical Genetics, Turku, Finland
3) The Wellcome Trust Sanger Institute, Hinxton, United Kingdom

After the human Y sequence has been finalised, not only the classical AZFa,b,c microdeletions but more rearrangements (deletions, duplications, inversions) in- and outside of the three AZF-intervals have been elucidated and intrachromosomal recombinations of homologous repetitive sequence blocks were identified as their major causative agent (Vogt, 2005; Hum. Reprod. Update, 11, 319). These include duplications in AZFa, AZFb, AZFc and partial AZFb-AZFc deletions summarized under the pseudonym of "gr/gr deletions". At least some of these rearrangements were associated with distinct Y-chromosomal lineages resulting in a different AZF gene content (AZF gene plasticity). Since the AZF deletions are known today as the most prominent genetic lesion causing male infertility it is now important to reveal which of the deleted AZF genes are functional redundant and which of them are essential for the male germ cell maturation process. For this purpose we first concentrated on a functional analysis of the DAZ1 and DAZ2 genes in proximal AZFc encoding a highly conserved class of RNA binding proteins expressed only in male germ cells (Reijo, 2000; Biol. Reprod. 63, 1490). In men from haplogroup R containing four DAZ genes we found that their transcription is functionally controlled by a unique bipartite promoter structure of which one domain contains a distinct CpG island. To analyse whether the same promoter structure was also conserved on Y haplogroups with only the DAZ1 and the DAZ2 gene we established STS/SNV markers along ~4Mb in distal Yq11 to distinguish putative ampliconic rearrangments in AZFb/c associated with distinct Y lineages and to identify fertile and infertile men from Y haplogroup N. This Y chromosome was recently shown to contain only the DAZ1/DAZ2 gene doublet (Fernandes et al. 2004; Am. J. Hum. Genet. 74, 180). At the meeting we will present first results of the associated promoter regions functionally mapped by appropriate Luciferase reporter assays.

P164

Direction bias in processing the targeted nucleotide exchange mediated by specific modified oligonucleotides

Wuepping M.(1), Kenner O.(1), Vogel W.(1), Kaufmann D.(1)
1) University-Ulm, Human genetics, Ulm, Germany

Gene editing mediated by specific single-stranded oligonucleotides is a possibility to change genomic sequences. We worked with a hprt-system in V79 cells. Editing rates are very low, so we studied the influence of different modified oligonucleotides especially in view of the 5` or 3` modifications. We designed oligonucleotides with so called GC-clamps or TA-clamps on the 5` site or 3` site of the oligonucleotides We found that oligonucleotides with a TA-clamp had higher corrections efficiencies than GC-clamp modified oligonucleotides. Additional findings showed that the clamp on the 5` site is also better in inducing the conversion than clamps on the 3` site. Furthermore it is unknown in which phase of the cell cycle the editing takes place. Using mechanical synchronisation and temporary defined selection, we were able to allocate one phase of the cell cycle.

P165

Whole genome association study: strategy, workflow, documentation, and data analysis of a 500K genotyping project

Saar K.(1), Patone G.(1), Esparza J.(1), Rueschendorf F.(1), Wahn U.(2), Lee Y.-A.(3), Huebner N.(1)
1) Max Delbrueck Center for Molecular Medicine, Berlin, Germany
2) Clinic of Pediatrics, Charite, Berlin, Germany
3) Max Delbrueck Center for Molecular Medicine and Clinics of Pediatrics, Charite, Berlin, Germany

Mapping genes that cause complex disorders has proven to be difficult, in parts due to the low contribution of single genes to the overall effect. Hence, genetic association studies in humans are a powerful approach for the identification of modest effect genes. The completion of the human sequence and access of sequence information to public databases have led to a rapid technical development in genotyping single nucleotide polymorphisms (SNPs). The need to perform whole genome association studies, where a large dense set of SNPs is genotyped across the whole genome in a considerable number of individuals, also requires careful handling of huge sets of genotyping data. In a whole genome association study frequencies of alleles or genotypes of variants between affected cases and healthy controls are compared. Alternatively, when using family-based controls, no knowledge of potential candidate genes influences the search for genes contributing to a specific phenotype, and a potential population stratification of data is avoided.
We currently establish hightroughput genotyping of arrays with 500.000 Single Nucleotide polymorphisms (SNPs) with the Affymetrix technology. We met the challenge of workflow and analysis of data generated with more than 1000 samples by setting up a database-free laboratory information management system that meets the need to control sample workflow. Our Sample Flow Documentation (SaFD) system stores sam-

ple information, prints barcodes and controls automated laboratory work steps. All data are easily transferred to downstream systems. Also, data storage and backup strategies are managed with the help of the SaFD tool. The output of the produced data is in the gigabytes magnitude. Quality control and data conversion are performed with the help of the Alohomora tool developed by Rueschendorf. Adopting appropriate analysis tools to perform familial based whole genome association, we will present our first results.

P166

DetecTiff©: A universal image analysis software package for high content screening microscopy

Gilbert D.(1), Pepperkok R.(2), Runz H.(3)
1) Zoologisches Institut, Universität Heidelberg, Heidelberg, Germany
2) EMBL, Cell Biology/Biophysics Programme, Heidelberg, Germany
3) Institut für Humangenetik, Heidelberg, Germany

Biological data acquired by microscopic imaging is often complex and difficult to interpret. Compared to pure visual inspection, automated systems for the acquisition and analysis of phenotypic data offer important advantages such as a high reproducibility of structure recognition and interpretation, extraction of quantitative parameters as well as high-throughput performance. Here we describe the establishment of the novel image analysis software DetecTiff©, which allows fully-automated structure recognition and quantification from digital images. Core module of the LabView©-based software DetecTiff© is an algorithm for structure recognition, which employs alternating intensity thresholding and size-dependent particle filtering in microscopic images. Detected structures are converted into templates, which are used for quantitative image analysis. DetecTiff© enables processing of multiple detection-channels and provides functions for template organisation. A module for data display permits fast interpretation of acquired data. The simple program architecture and the intuitive user interface allow short training periods and fast reconfiguration for various applications. Thus, DetecTiff© is a high performance and low cost alternative to other image analysis software.

P167

Transgenic studies on physiological function of testicular insulin

Shirneshan K.(1), Meinhardt A.(2), Sancken U.(1), Engel W.(1), Adham I.M.(1)
1) University of Göttingen, Institute of Human Genetics, Göttingen, Germany
2) University of Giessen, Department of Anatomy and Cell Biologie, Giessen, Germany

Transgenic studies in our group revealed that the Leydig insulin-like hormone (Insl3) produced in pancreatic β-cells is able to restore the cryptorchidism phenotype in Insl3- deficient mice. These results demonstrate regulated secretory mechanisms of processed proInsl3 to mature Insl3 by proteolytic cleavage of C-peptide in β-cells of the pancreas. In this report, we investigated the efficiency of testicular Leydig cells to secrete mature insulin and the functional consequences of overexpression of human insulin in Leydig cells. To address that, we have generated three transgenic mouse lines expressing the human insulin gene under the control of the Insl3 promoter (I3I2). Northern blot analysis showed that more than ten times insulin mRNA is present in testis than in pancreas of transgenic mice. Immunhistochemical analysis revealed that the translation of the human insulin is restricted to Leydig cells. To determine the efficiency of the testicular human insulin to rescue diabetes development in Pax4-deficient mice resulting from the developmental impairment of β-cells, we introduced the I3I2-transgenic allele in the genome of Pax4-/- mice. Analyses of I3I2: Pax4 double transgenic mice revealed that testicular human insulin failed to compensate the deficiency of pancreatic insulin. We have then determined the level of the human proinsulin in testis and pancreas and the level of human C-peptide in serum of transgenic mice. The level of secreted human C-peptide in serum was found to be significantly lower than that of murine C-peptide in serum of wild-type mice. These results suggest that Leydig cells are not able to process the proinsulin efficiently and/or to regulate insulin secretion.

P168

Highly parallel SNP genotyping with affymetrix human mapping arrays

Wagner F.(1), Rieck T.(1), Radelof U.(1)
1) RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin, Germany

General Information: SNP genotyping with DNA microarrays is a cutting-edge technology offering several advantages compared to conventional SNP genotyping technologies. Hundreds of thousands of SNPs can be typed simultaneously on a single array, which significantly increases speed and resolution of disease gene localization and requires only 250 ng of genomic DNA as starting material. SNPs have been validated for Caucasian, African-American and Asian populations. Extensive, up-to-date annotations are delivered along with the genotypes. RZPD´s SNP Genotyping Service offers the complete portfolio of Affymetrix´ Human Mapping Arrays. 10 K 2.0 Array 100K Set 500K Set Number of SNPs 10,200 116,000 500,000 Mean intermarker distance 258 kb 23.6 kb 5.8 kb Average heterozygosity 0.38 0.30 0.29
Applications: Linkage analyses: SNP-Array-based linkage analysis offers several clear advantages over e.g. microsatellite analysis. It is significantly faster, requires only 250 ng of genomic DNA per assay in comparison to several micrograms with microsatellite analysis, and the information content of the 10K 2.0 array is significantly higher than the information content of standard panels of 400 or 1,000 microsatellites.
Association studies: The 100K and 500K Sets enable to perform association studies in a relatively short time, as a single technician can determine millions of genotypes per day. With the 500K set, costs per SNP are reduced to 0.3 Euro-Cent.
Copy Number Analysis: Affymetrix as well as several academic groups have developed software tools to determine gain or loss of chromosomal copy number. With the increasing SNP density of the 100K and 500K sets, regions of copy number changes can be narrowed down.
Loss-of-heterozygosity (LOH): Naturally, SNP genotyping technology allows to determine LOH regions. Furthermore, array-based SNP genotyping is the only technology which can detect copy-neutral LOH or uniparental disomy in a single experiment.

P169

Services on Highly Flexible NimbleGen Microarrays

Rieck T.(1), Radelof U.(1), Wagner F.(1)
1) RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin, Germany

Oligonucleotide microarrays by NimbleGen Systems are synthesized in situ by Maskless Array Synthesizer (MAS) technology, which is a synthesis of digital projection and combinatorial chemistry. At the heart of the MAS is the Digital Micromirror Device (DMD), which contains about 786,000 tiny aluminum mirrors on a computer chip, each of which is individually addressable and freely programmable. UV light is projected onto the DMD and - dependent on micromirror position - decouples a defined protection group of a growing oligonucleotide on the array, making it ready for the next coupling step.
MAS technology is extremely flexible and allows rapid changes in array design. With up to 390,000 features per array and oligonucleotide lengths from 24 to 85 nt, researchers can choose from more than 300 prokaryotic and eukaryotic catalog arrays or have new arrays designed and produced within a few weeks at minimal design fees and no minimum package volume.
These features make the MAS technology superior for customized applications in comparison to conventional photolithographic DNA microarray synthesis which uses chromium masks to generate light patterns for the activation of oligonucleotide synthesis sites. Mask fabrication is a costly and time-consuming process, thereby making the production process inexpedient for small-scale custom array applications or applications requiring frequent chip re-design.
Services on NimbleGen microarrays are available at RZPD as its exclusive distributor in Germany and Austria. Current applications include:
Gene Expression Profiling: in addition to the standard format, 12-well NimbleScreen™ arrays allow for the parallel processing of 12 independent samples.
ChIP-chip™ Assays for rapid identification of protein-DNA binding sites and studies on chromatin remodelling.
Comparative Genomic Hybridization (Array CGH) for the detection of chromosomal aberrations.
Comparative Genome Sequencing (Array CGS): a combination of CGH and resequencing to identify DNA polymorphisms between haploid genomes.

P170

Automated yeast two-hybrid screening: from single screens to proteomes

Schwarz F.(1), Schick M.(1), Haas J.(2), Korn B.(1), Kögl M.(1)
1) RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH, Heidelberg, Germany
2) Max-von-Pettenkofer Institute, München, Germany

Large scale protein interaction networks play an increasingly important role in functional proteomic research. Application of the yeast two-hybrid method has already allowed the analysis of the proteomes of several model organisms. We have streamlined and automated the method to dramatically increase its throughput and reliability. In a pilot project focussing on nuclear receptors as a family of drug targets, we have performed 425 screens yielding 6425 interacting fragments that form 1613 interaction pairs (Albers et al., Mol Cell Proteomics 2005, *4*:205-213). Statistic analysis has allowed to select a subset of high-confidence interactions containing 61% of validated interactions. Methods to reproduce yeast two-hybrid based protein interactions on protein microarrays are under development in our laboratory. In a current project, the complete set of viral proteins of Varicella Zoster Virus, the causative agent of chicken pocks and shingles, is analysed for interactions with host cell protein. In this project, we will perform close to 300 screens with 96 different bait proteins. The automated system has now been opened for collaborations and service projects(contact: koegl@rzpd.de).

P171

The simultaneous consideration of gene and environment may enhance the power to identify environmental factors associated with cancer risk

Lorenzo Bermejo J.(1), Hemminki K.(2)
1) German Cancer Research Centre, Molecular Genetic Epidemiology, Heidelberg, Germany
2) German Cancer Research Centre, Molecular Genetic Epidemiology, Heidelberg, Germany and, Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden

The identification of environmental factors that influence the risk of cancer represents the greatest opportunity for cancer prevention. The present study assessed the relevance of the simultaneous consideration of genotype and environment to analyze the impact of environmental risk factors on disease.

We modeled hypothetical environmental exposures, genotypes and phenotypes according to reasonable parameter settings. Different models of gene-gene-environment interaction were considered. For each parameter combination, the simulated data were analyzed using the case-only and the case-control study designs. We calculated the proportions of cases attributable to the simulated gene-environment interactions. We also estimated the power to detect the environmental risk factor and the probability of identifying a dependence of the environmental effect on genotype. A complicated relationship between the power and the proportion of cases attributable to gene-environment interactions was found. When only rare genotypes were at increased risk due to environmental exposure, and the marginal effect of the environmental factor on cancer risk was relatively weak (e.g., OR=1.2), the likelihood of detecting the modulation of the environmental effect by the genotype was generally higher than the power to identify the environmental risk factor.

We conclude that the simultaneous consideration of both genes and environment may be critical in identifying environmental risk factors. The possible modification of the effect of environmental exposures by heritable traits should be kept in mind when determining health impact and priorities of genetic research.

P172

Investigation of polymorphisms in genes involved in GH1 release and breast cancer risk

Wagner K.(1), Hemminki K.(2), Grzybowska E.(3), Klaes R.(4), Burwinkel B.(1), Bugert P.(5), Schmutzler R.K.(6), Wappenschmidt B.(6), Butkiewicz D.(3), Pamula J.(3), Pekala W.(3), Försti A.(2)
1) DKFZ, Molecular Genetic Epidemiology, Heidelberg, Germany
2) Karolinska Institute, Department of Biosciences at Novum, Huddinge, Sweden and DKFZ, Molecular Genetic Epidemiology, Heidelberg, Germany
3) Centre of Oncology, Maria Sklodowska-Curie Institue, Department of Molecular Biology, Gliwice, Poland
4) University of Heidelberg, Insitute of Human Genetics, Heidelberg, Germany
5) University of Heidelberg, Faculty of Clinical Medicine, Institute of Transfusion Medicineand Immunology, Red Cross Blood Service of Baden-Württemberg-Hessia, Mannheim, Germany
6) Clinical Center University of Cologne, Germany; Center of Molecular Medicine Cologne (CMMC), University Hospital of Cologne, Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Cologne, Germany

Breast cancer is the most common cancer in females in Europe and after lung cancer the second most common cancer worldwide with 1.05 million new cases each year. Breast cancer represents 14% of cancer deaths and 28% of all cancer cases in women. The development of the normal breast and the initiation and progression of breast cancer are under the influence of endocrine hormonal growth factors, that, when produced locally, can act in an autocrine/paracrine fashion. During the last decade research has revealed an important influence of the growth-hormone 1 (GH1)/ insulin-like-growth factor-1 (IGF-1)-axis on cell proliferation, differentiation and apoptosis and further on breast cancer development. GH1, which stimulates the production of IGF-1, is produced under the regulation of growth-hormone-releasing hormone (GHRH) and ghrelin (GHRL) whereas the influence of somatostatin (SST) inhibits its production. By binding to their respective receptors, these hormones control signalling in a GH1/IGF-1 dependent manner.

We screened the gene regions of the GHRH, GHRHR, GHRL, GHSR, SST and SSTR2 genes for published polymorphisms. The ones with a potential functional influence were selected for further analyses in a Polish and a German cohort of totally 798 breast cancer cases and 1011 controls. Our study identified a novel TC repeat polymorphism in the SST promoter that was in strong linkage disequilibrium with a single nucleotide polymorphism in intron 1. Both polymorphisms revealed an association with a decreased breast cancer risk in the Polish study population. Furthermore, we identified a protective effect of the GHRHR C-261T polymorphism in both populations (joint analysis CC vs. CT+TT: OR 0.80, 95% CI 0.65-0.99, p = 0.04). This effect was carried to a haplotype containing the protective allele. Thus, our study concludes an influence of distinct polymorphisms in genes involved in GH1 release on breast cancer risk.

P173

Association of the CASP10 V410I variant with reduced familial breast cancer risk and interaction with the CASP8 D302H variant

Frank B.(1), Hemminki K.(2), Wappenschmidt B.(3), Meindl A.(4), Klaes R.(5), Schmutzler R.K.(3), Bugert P.(6), Untch M.(7), Bartram C.R.(5), Burwinkel B.(1)
1) Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany
2) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden and Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg, Germany
3) Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Clinical Center University of Cologne and Center of Molecular Medicine Cologne (CMMC), University Hospital of Cologne, Cologne, Germany
4) Department of Gynaecology and Obstetrics, Klinikum rechts der Isar at the Technical University, Munich, Germany
5) Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany
6) Institute of Transfusion Medicine and Immunology, Red Cross Blood Service of Baden-Württemberg-Hessia, University of Heidelberg, Faculty of Clinical Medicine, Mannheim, Germany
7) Department of Gynaecology and Obstetrics at the Ludwig-Maximilians-University, Munich, Germany

Dysregulation of apoptosis plays a crucial role in carcinogenesis. As part of death receptor- and mitochondrion-mediated apotosis, the homologues caspase-10 and caspase-8 may act as low-penetrance breast cancer susceptibility genes. In death receptor-mediated apotosis, engagement of death receptors by their ligands involves the assembly of the death inducing signalling complex (DISC). In mitochondrion-mediated apoptosis, the release of cytochrome C into the cytosol results in apoptosome formation. Recruitment of both caspase-10 and-8 to DISC and apoptosome leads to their activation by dimerization. We investigated the influence of the coding caspase-10 (CASP10) variant V410I (G1228A) by performing a case-control study - using 511 familial breast cancer cases and 547 control subjects - on breast cancer risk and revealed a significant association of V410I with a reduced risk (OR = 0.62, 95% CI = 0.43 to 0.88, P = .0076) related to the number of variant alleles (Ptrend = .0039). As CASP10 and CASP8 functionally co-operate during apoptosis, we analysed the mutual effect of both CASP10 V410I and CASP8 D302H, resulting in a significant association between the number of the variant alleles I410 and H302 and a highly decreased familial breast cancer risk (OR = 0.35, Ptrend = .007), pointing to the interaction between the CASP10 and CASP8 polymorphisms in breast carcinogenesis.

P174

Association of prolactin and its receptor gene regions with familial breast cancer

Vaclavicek A.(1), Hemminki K.(2), Bartram C.R.(3), Wappenschmidt B.(4), Meindl A.(5), Schmutzler R.K.(4), Klaes R.(3), Untch M.(6), Burwinkel B.(1), Försti A.(2)
1) DKFZ, Molecular Genetic Epidemiology, Heidelberg, Germany
2) Karolinska Institute, Department of Biosciences at Novum, Huddinge Sweden and, Division of Molecular Epidemiology, DKFZ, Heidelberg, Germany
3) University of Heidelberg, Insitute of Human Genetics, Heidelberg, Germany
4) Clinical Center University of Cologne, Germany; Center of Molecular Medicine Cologne (CMMC), University Hospital of Cologne, Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Cologne, Germany
5) Klinikum rechts der Isar at the Technical University, Department of Gynaecology and Obstetrics, Munich, Germany
6) Ludwig-Maximilians-University, Department of Gynaecology and Obstetrics, Munich, Germany

The contribution of prolactin (PRL) to the pathogenesis and progression of human mammary tumors has received recent attention. In addition to the pituitary, the PRL gene is expressed in other tissues, including the breast, where it is differentially regulated by an alternative promoter. The actions of PRL are mediated by its receptor (PRLR). The PRL/PRLR complex activates several signaling pathways involved in cell proliferation, differentiation and survival. In this study, we investigated whether genetic variation in the PRL and PRLR genes is associated with breast cancer (BC). We screened the gene regions, including the promoters, for single nucleotide polymorphisms (SNPs). A total of 7 SNPs were genotyped in a series of 497 German familial BC cases and 552 controls. Two SNPs (rs1341239, rs12210179) within the PRL distal and proximal promoter regions, respectively, were significantly associated with increased risks in homozygotes for the variant alleles (OR 1.67, 95% CI 1.11-2.50, P = 0.01; OR 2.09, 95% CI 1.23-3.52, P = 0.005). The PRL haplotype TGTG, containing the variant alleles of the promoter SNPs, increased significantly the risk of BC (OR 1.42, 95% CI 1.07-1.90, P = 0.02). For the PRLR, a haplotype was associated with a significant decrease in BC risk compared to the wild type haplotype (OR 0.69, 95% CI 0.54-0.89, P = 0.004). An increasing number of PRL and PRLR risk haplotypes lead to a significant trend of increasing risk for BC ($\chi 2$ = 12.15, P = 0.007). Thus, genetic variation in the PRL and PRLR genes was shown to influence BC risk.

P175

Biochemical data in ornithine transcarbamylase deficiency (OTCD) carrier risk estimation. Logistic discrimination and Bayesian combination with genetic information.

Oexle K.(1)
1) Institut für Klinische Genetik, TU Dresden, Medizinische Fakultät, Dresden, Germany

One fifth of the gene mutations causing ornithine transcarbamylase deficiency (OTCD) cannot be detected. In such cases carrier risk estimation must refer to biochemical results such as increased plasma glutamine concentration or increased orotidine excretion after allopurinol load although these parameters do not yield a definite diagnosis. I have derived likelihood ratios (odds) for carrier risk estimation from published data, i.e., from mean and standard deviation of glutamine concentrations in carriers and non-carriers assuming normal distributions and from allopurinol test data in individual carriers and non-carriers using logistic regression. I show how such biochemical information may be combined with genetic information. The necessity to assess individual data in larger proband groups and to consider possible correlations between different parameters is indicated.

P176

Association of genetic variants in the Rho guanine nucleotide exchange factor AKAP13 with familial breast cancer

Wirtenberger M.(1), Tchatchou S.(1), Hemminki K.(2), Klaes R.(3), Schmutzler R.K.(4), Bermejo J.L.(1), Chen B.(1), Wappenschmidt B.(4), Meindl A.(5), Bartram C.R.(3), Burwinkel B.(1)
1) German Cancer Research Center (DKFZ), Division of Molecular Genetic Epidemiology, Heidelberg, Germany
2) Karolinska Institute, Department of Biosciences at Novum, Huddinge, Sweden and Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
3) University of Heidelberg, Institute of Human Genetics, Heidelberg, Germany
4) Clinical Center University of Cologne, Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Cologne, Germany
5) Ludwig-Maximilians University, Department of Medical Genetics, Munich, Germany

The A-kinase anchor protein 13 (AKAP13, alias BRX and lbc) tethers cAMP-dependent protein kinase to its subcellular environment and catalyses Rho GTPases activity as a guanine nucleotide exchange factor. The crucial role of members of the Rho family of GTPases in carcinogenesis is well established and targeting Rho proteins with antineoplastic compounds has become a major effort in the fight against cancer. Thus, genetic alterations within the candidate cancer susceptibility gene AKAP13 would be expected to provoke a constitutive Rho signalling, thereby facilitating the development of cancer. Here, we analysed the potential impact of four polymorphic non-conservative amino acid exchanges (Arg494Trp, Lys526Gln, Asn1086Asp and Gly2461Ser) in AKAP13 on familial breast cancer. We performed a case-control study using genomic DNA of BRCA1/2 mutation-negative German female index patients from 601 unrelated families, among a subset of 356 high-risk families, and 1053 German female unrelated controls. The newfound Lys526Gln polymorphism revealed a significant association with familial breast cancer (OR = 1.58, 95% CI = 1.07 - 2.35) and an even stronger association with high-risk familial breast cancer (OR = 1.85, 95% CI = 1.19 - 2.88). Haplotype analyses were in line with genotype results displaying a similar significance as analyses of individual polymorphisms. Due to the pivotal role of AKAP13 in the Rho GTPases signalling network, this variant might affect the susceptibility to other cancers as well. Carcinogenesis in press.

P177

Association of death receptor 4 haplotype 626C-683C with an increased breast cancer risk

Shanmugam K.S.(1), Frank B.(1), Hemminki K.(1), Meindl A.(2), Klaes R.(3), Schmutzler R.K.(4), Wappenschmidt B.(4), Bartram C.R.(3), Burwinkel B.(1)
1) German Cancer Research Center, Division of Molecular Genetic Epidemiology, Heidelberg, Germany
2) Klinikum rechts der Isar at the Technical University, Department of Gynaecology and Obstetrics, Munich, Germany
3) University of Heidelberg, Institute of Human Genetics, Heidelberg, Germany
4) Clinical Center University of Cologne, Division of Molecular Gynaeco-Oncology,Department of Gynaecology and Obstetrics, Cologne, Germany

Dysregulation of apoptosis plays a crucial role in carcinogenesis. Tumour necrosis factor-related apoptosis-inducing ligand TRAIL stimulates the extrinsic apoptotic pathway by binding to death receptor 4 (DR4). Thus, genetic alterations within the candidate tumour suppressor gene DR4 would be expected to provoke a deficient apoptotic signalling thereby facilitating the development of cancer. The DR4 variants Thr209Arg and Glu228Ala were genotyped in a series of 521 breast cancer cases and 1100 control subjects from Germany determining their impact on breast cancer risk. Neither Thr209Arg (626C>G) nor Glu228Ala (683A>C) alone were significantly associated with breast cancer risk (OR = 0.84, 95% CI = 0.65-1.08, P = 0.18 and OR = 0.89, 95% CI = 0.72-1.12, P = 0.30). Haplotype analysis, however, revealed a 3.5-fold risk for carriers of the 626C-683C haplotype (OR = 3.52, 95% CI = 1.45-8.52, P = 0.003). Carcinogenesis in press.

P178

Hereditary prosopagnosia is also very common in Hong Kong Chinese – First prevalence data outside the Caucasian population

Kennerknecht I.(1), Ho T.(2), Wong V.(2)
1) Institut für Humangenetik, Münster, Germany
2) The University of Hong Kong, Queen Mary Hospital, Hong Kong, China

Prosopagnosia or face blindness refers to the deficit in face recognition in the presence of intact sensory and intellectual function. The acquired form of prosopagnosia is a rare condition and the congenital form is generally considered to be even less common. In the German population we could recently asses a very high prevalence of 2•47 % (CI 95% 1•33 – 3•80). We could further show that this congenital anomaly has almost always familial recurrence which is compatible with simple autosomal dominant inheritance. We therefore introduced the term hereditary prosopagnosia (Kennerknecht et al. 2002, Grüter et al. 2005). The high frequency of this cognitive deficit - hitherto only described in the Caucasian population - prompted us to extend our search to other ethnic groups. A questionnaire based screening was performed on 534

medical students at The University of Hong Kong. Data were collected on the following features: integrity of visual input; frequency of contact with other people; sense of orientation in cities, buildings, and nature; differentiation of common animal and plant species (inter/intra class object differentiation); recognition of other people in a variety of situations; behaviour in meetings with known and unknown people. 119 candidates suspicious for prosopagnosia were selected by this approach and underwent a detailed semi-structured interview. Finally the diagnosis of prosopagnosia was assessed in 9 subjects. This gives a minimal prevalence of 1•69% (CI•95% 0•59 – 2•79). Three of the index probands allowed us to examine their families. In all these cases we found further affected first degree relatives supporting the concept of autosomal dominant hereditary prosopagnosia.

P179

Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer

Chandra S.(1), Hemminki K.(1), Hoffmeister M.(2), Brenner H.(3), Chang-Claude J.(4), Försti A.(1)
1) German Cancer Research Center, Division of Molecular Genetic Epidemiology, Heidelberg, Germany
2) German Cancer Research Center, Division of Clinical Epidemiology and Aging Research, Heidelberg, Germany
3) The German Center for Research and Aging, Department of Epidemiology, Heidelberg, Germany
4) German Cancer Research Center, Division of Clinical Epidemiology, Heidelberg, Germany

Colorectal cancer (CRC) is the second to fourth most common cancer in industrialized countries. There are several factors that regulate and control cell growth and which play important roles in the etiology of cancer. It has been reported that the growth hormone1 (GH1)/insulin-like growth factor1 (IGF1) pathway plays a significant role in both the normal and malignant cell growth. IGF1 binds to type 1 IGF receptor and starts a signalling cascade that regulates cell proliferation, differentiation and apoptosis. The interaction between IGF1 and its receptor is mainly regulated by a binding protein, IGFBP3. Any disturbance in the expression of IGF1 or IGFBP3 can affect cell division and apoptosis. A CA repeat polymorphism, which is located 969 bp upstream of the transcription start site, in the IGF1 gene and A-202C and Ala32Gly single nucleotide polymorphisms (SNPs) in the IGFBP3 gene have been associated with the serum levels of the respective proteins and CRC, but the results are controversial. We wanted to study if these polymorphisms or any haplotype/s of the IGF1 and IGFBP3 genes are associated with CRC. A high linkage disequilibrium is seen in these gene regions; therefore, a CA repeat along with 2 tagging SNPs in the IGF1 gene and A-202C, Ala32Gly and 2 tagging SNPs in the IGFBP3 gene were selected to cover the whole gene region. A case-control study was carried out using 661 German CRC cases and 607 age and sex matched controls. We did not find any association between the CA repeat length and the other SNPs in IGF1 and the risk of CRC. In IGFBP3, G-806A showed a trend for a decreased risk of CRC with an increasing number of the A allele (Ptrend = 0.006). There was no association between the other SNPs in the IGFBP3 gene and CRC risk. There was also no association between the haplotypes of the IGF1 and the IGFBP3 genes and the risk of CRC. Thus, our study excludes a major role of the genetic variation within the IGF1 and IGFBP3 genes in CRC risk.

P180

Autozygosity mapping – an effective method for the identification of recessive disease genes in consanguineous families

Fischer C.(1), Rüschendorf F.(2), Zschocke J.(1)
1) Universität Heidelberg, Institut für Humangenetik, Heidelberg, Germany
2) Max-Delbrück-Center, Bioinformatics and Gene Mapping Center, Berlin, Germany

Inherited disorders in children from consanguineous couples are frequently caused by homozygosity for recessive disease mutations. However, the exact diagnosis is often uncertain or genetic tests are deemed impossible as the causative gene is unknown. In this situation, autozygosity mapping may be an effective tool for the identification of candidate genes. The approach has become much more feasible with the advent of high-resolution SNP chips in the last years. Affected pedigree members are expected to share a very rare haplotype around the disease causing gene and the determination of this haplotype allows much more powerful calculations than the mere identification of homozygous DNA markers in affected individuals. We systematically calculate the requirements for successful autozygosity mapping projects with regard to family structure, number of affected and unaffected siblings, required DNA samples, expected shared length of haplotypes, and resulting optimal SNP chip resolution. The expected proportion and length of autozygous regions may be calculated from the imbreeding coefficient of the parents. We also report on the development of the software tools HDSlinkW and Alohomara for planning and execution of autozygosity studies using commonly available SNP chip platforms. Autozygosity mapping is also possible when the exact relationship of carrier parents is uncertain. Even if the results fail to reach genome-wide significance, a-posteriori probabilities in the genome provide essential data for molecular gene identification. The approach was evaluated in several families with recessively inherited neuropaediatric and dental disorders, with degrees of kinship ranging from simple first cousin marriage to a large family with affected children in two related branches of which the common ancestor lived six generations ago.

P181

Combined haplotype estimation and association analysis using a Markov Chain Monte Carlo approach

Marquard V.(1), Beckmann L.(1), Nothnagel M.(2), Rohde K.(3), Chang-Claude J.(1)
1) Deutsches Krebsforschungszentrum, Klinische Epidemiologie, Heidelberg, Germany
2) Medizinische Informatik und Statistik der Universität zu Kiel, Kiel, Germany
3) Max-Delbrück-Centrum, Bioinformatics, Berlin, Germany

Haplotype analysis requires haplotype estimation from phase-unknown multi-locus genotypes. An asssociation test can be based on the most likely – or "best" – haplotype pair configuration for each individual in the sample. However, this does not account for the uncertainty in haplotype estimation. Alternatively, the test can be sampled over all possible haplotype pair configurations according to their probability. We recently proposed a Markov Chain Monte Carlo approach in combination with the Metropolis Hastings algorithm, in which we start a Markov Chain at the most likely state, and try stepwise transitions to other randomly chosen states. In a case-control association study, the control group is drawn without any bias from the population. The case group is expected to have a bias in the direction of the predisposing haplotype. The question therefore arises, whether to estimate the haplotypes in cases and control separately or pooled together.
Goals of the study presented here are:
1. Comparison of the "best" haplotype configuration with sampling over all possible haplotype pairs using a Markov Chain Monte Carlo algorithm.
2. Comparison of haplotype estimation in cases and controls separatedly with estimation in the pooled sample.
We used two haplotype-based association tests:
(i) a Mantel statistic based on haplotype sharing, which we recently proposed for gene mapping, and
(ii) an association test based on contingency tables for haplotype pairs.

P182

Haplotype reconstruction in population individuals using coalescent trees

Qian D.(1), Beckmann L.(2)
1) City of Hope National Medical Center, Duarte, CA, United States of America
2) Deutsches Krebsforschungszentrum, Klinische Epidemiologie, Heidelberg, Germany

We present a two-stage algorithm to reconstruct haplotypes in population individuals using hierarchical clustering and coalescent tree analysis. First, haplotypes are grouped into clusters, and each cluster contains similar haplotypes that are likely descended from a common ancestor. Second, haplotypes within each cluster are fitted to a coalescent tree structure. Each tree is then quantified with a tree distance, and the haplotype configuration with the minimum sum of tree distances is defined as the optimal one under the identified trees. The similarity between haplotypes as well as the tree distances are calculated based on haplotype sharing, i.e. the shared chromosomal segments identical by descent. The approach allows for multiple origins of haplotypes within a population. The plausibility of a haplotype is quantified using similar haplotypes within the same cluster, and the effect of random similarities between clusters has been excluded. The algorithm is applicable for both SNPs and multiallelic microsatellites.
We analyzed three real data sets with known molecular haplotypes. The impact of missing data was analyzed in a simulation study by imputing randomly 3% and 6% missings into the data sets. For comparison, we used three commonly used algorithms (PHASE, HAPLOTYPER, SNPHAP).

Preliminary results indicate similar performance as the Bayesian approach implemented in PHASE. Our approach outperformed HAPLOTYPER and SNPHAP in the presence of missing data in two data sets.

P183

Gene-environment interactions in human genetics: what it means and how to measure it

Dempfle A.(1), Beckmann L.(2), Hein R.(2), Scherag A.(1), Chang-Claude J.(2), Schäfer H.(1)
1) Universität Marburg, Institut für Medizinische Biometrie und Epidemiologie, Marburg, Germany
2) DKFZ Heidelberg, Unit of Genetic Epidemiology, Division of Clinical Epidemiology, Heidelberg, Germany

Genetic and environmental risk factors and their interactions contribute to the development of complex diseases. While there are several important examples of interactions, the full extend of their relevance is not clear from available data. Moreover, the meaning of interaction varies between statistical and biological sciences, as precise definitions are often omitted. We argue that the investigation of gene-environment (G x E) interaction is mostly sensible in advanced stages of genetic research, for the detailed characterization of identified disease genes or the stratified analysis of environmental effects by genotype. The widespread use of G x E interaction for targeted intervention or personalized treatment (pharmacogenetics) is still beyond current means due to unconvincing evidence or low predictive and discriminative power. Valid results on G x E interactions require studies that include large sample sizes, corrections for multiple testing and replication. A brief overview of suitable study designs with their respective advantages and pitfalls is given in relation to the aim of a study.

P184

Direct SNP haplotyping of apo(a) alleles for analyses in evolutionary genetics

Schmidt K.(1), Khalifa M.(1), Kraft H.G.(1), Utermann G.(1)
1) Department für Medizinische Genetik, Molekulare und Klinische Pharmakologie, Sektion für Humangenetik, Innsbruck, Austria

Plasma Lipoprotein(a) (Lp(a)) is a quantitative trait associated with atherothrombotic disease. Lp(a) concentrations vary widely within and between populations with Africans exhibiting on average two- to threefold higher Lp(a) levels and a different distribution compared to Europeans. The trait is highly heritable both in Africans and Europeans. The apolipoprotein(a) (apo(a)) gene locus on chromosome 6q26-27 (LPA, MIM 152200) has been identified as the major quantitative trait locus for Lp(a) concentrations. A size polymorphism of apo(a), resulting from a copy number variation of a 5.5 kb Kringle IV-2 repeat (KIV-2-VNTR), is inversely correlated with Lp(a) concentrations in all populations, although KIV-2 allele of identical size may be associated with markedly different Lp(a) concentrations. On average, alleles with high and medium numbers of KIV-2 copy repeats are associated with higher Lp(a) concentrations in Africans than in Europeans.The physiological function of apo(a)/Lp(a) is still unknown, and the marked differences in both Lp(a) plasma concentrations and the frequency distributions of KIV-2 size alleles between different world populations have risen the question whether these observed differences are due to genetic drift or might reflect selection. We therefore have performed an in-depth analysis of apo(a) haplotype evolution. All 27 non-repetitive exons (4109 nt) and flanking intron regions (app. 6300 nt) in 40 apo(a) alleles each from three different world populations (represented by Gabonese, Austrians, and Chinese) were sequenced and the population frequencies of a total of 58 different SNPs were assessed. The KIV-2-VNTR allowed to separate individual apo(a) alleles by PFGE, enabling us to directly assign SNP haplotypes. Our results indicate much higher haplotype diversity in Africans than in other populations, including the occurrence of a cluster of triallelic SNPs only in Africans, and give clues to the evolutionary history of apo(a) in humans.

P185

Trisomy 21 in Oman: A case-control study on environmental and genetic factors

Neitzel H.(1), Näthe J.(1), Khandekar G.(2), Goud M.(2), Al-Harrasi S.(2), Jaffer J.(3), Mohammed A J.(3), Sperling K.(1), Rajab A.(2)
1) Universitätsmedizin Charité, Institut für Humangenetik, Berlin, Germany
2) Ministry of Health, Genetic Unit, Muscat, Oman
3) Ministry of Health, Directorate General of Health Affairs, Muscat, Oman

Trisomy 21 is a main cause of human prenatal and postnatal morbidity and mortality. There is good evidence that maternal meiosis is an error prone process, susceptible to predisposing genetic and to exogenous factors. In this respect, the extremely high frequency of trisomy 21 among Omani newborn is of special interest. Moreover, all diagnoses are recorded at one institution only (ascertainment > 80%) which is a most favorable setting for an epidemiological study.
Here, we have performed a case-control study based on a structured questionnaire. It covers socio-demographics, family history of the woman and her partner, and work-place history so that all suspected confounders and risk factors are covered. The „cases“ are the mothers (parents) of the trisomy 21 children (100 cases), the „controls“ are mothers chosen at random from the same clinical department (100 cases). In addition, the parental origin of the extra chromosome 21 as well as the number and chromosomal distribution of recombinational events have been analyzed.
The annual incidence of trisomy 21 in Oman with 1:454 newborn is, perhaps, the highest reported so far. There is a clear maternal age effect. This, however, is less expressed than in Berlin and does not explain the high mutation rate. All cases were due to maternal non-disjunction (> 70% are MI errors), the chromosomal distribution of crossing-over events differed between younger and older women. There was no difference between „trisomy 21“ and control mothers with respect to reproductive behavior and the high frequency of consanguineous marriages, however, the education levels differed significantly.
The results are discussed with respect to the significant regional and seasonal differences in the frequency of trisomy 21 in Oman, the unusual frequencies of polymorphisms in genes affecting the folate and methyl metabolisms and under the aspect of risk avoidance and primary prevention.

P186

Hybrid two-stage sampling for genomewide association scans.

Scherag A.(1), Schäfer H.(1)
1) Philipps-Universität, Institut für Med. Biometrie und Epidemiologie, Marburg, Germany

The imminent availability of ultra-high-volume genotyping platforms (on the order of 100,000-1,000,000 genotypes per sample) at a manageable cost, raises the interest in conducting genomewide association studies for detecting susceptibility loci of complex traits.
In a review on recent developments in genomewide association scans Thomas et al. (2005) highlight the importance of multistage sampling designs in terms of expected efficiency gains. Although several multistage designs for association studies have been suggested since the two-stage design considerations by Sobell et al. (1993), most of these designs rely on nominal markerwise p-values for marker selections at the interim stage. Often either independent markers are assumed or Bonferroni type adjustments are applied for testing. Moreover little effort is put in developing designs which combine information from the multiple stages ascertained under different sampling schemes (e.g. case control design in the first stage, familiy based design in the second stage).
We present preliminary data on two-stage designs which allow for different sampling schemes in the two-stages. Unlike many previous design considerations marker selection at a first stage will be based on conditional power evaluations for the second stage.

P187

TGFBR1 and TGFBR2 mutations in patients with features of Marfan Syndrome and Loeys-Dietz Syndrome

Arslan-Kirchner M.(1), Singh K.(1), Rommel K.(1), Schmidtke J.(1)
1) Medizinische Hochschule Hannover, Institut für Humangenetik, Hannover, Germany

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder characterized by manifestations in the cardiovascular, skeletal, ocular and other organ systems. MFS type1 (MFS1) is caused by mutations in the gene encoding fibrillin (FBN1). Recently, the transforming growth factor-ß receptor-2 gene, TGFBR2, has been shown to be associated with a second type of disorder with typically mild or absent ocular involvement (MFS type 2; MFS2). Several point mutations were found in the highly conserved serine/threonine kinase domain of TGFBR2. Mutations in both, TGFBR1 and TGFBR2 are associated with Loeys-Dietz aortic aneurysm syndrome (LDS).
We have searched for TGFBR1 and TGFBR2 mutations in 41 unrelated patients fulfilling the diagnostic criteria of Ghent nosology or with the

tentative diagnosis of Marfan syndrome but with absence of mutations in the FBN1 coding region. In TGFBR1, a total of three (two novel) exonic and one 3'UTR sequence alterations were detected. In TGFBR2, a total of 4 sequence alterations in the 5' upstream region (2 novel) and 7 exonic sequence alterations (4 novel) were found. There is extensive clinical overlap between patients with MFS1, MFS2 and LDS.

P188

Novel mutations in the ENG and ACVRL1 genes causing hereditary hemorrhagic teleangiectasia – towards a genotype – phenotype correlation.

Argyriou L.(1), Twelkemeyer S.(1), Panchulidze I.(1), Wehner L.-E.(1), Teske U.(1), Engel W.(1), Nayernia K.(1)
1) Institut für Humangenetik Göttingen, Göttingen, Germany

Hereditary haemorrhagic teleangiectasia (HHT) is an autosomal dominantly inherited disorder characterised by cutaneous and mucosal telangiectasias, epistaxis and arteriovenous malformations in lung, liver, central nervous system and gastrointestinal tract. Until today, mutations in the genes for endoglin (ENG) and for activin A receptor type II-like kinase 1 (ACVRL1) have been identified to cause HHT. We performed molecular diagnosis in clinically affected probands of 52 HHT families and detected mutations in 34 cases. We report on a total of 19 novel disease-causing mutations, 7 in ENG and 12 in ACVRL1. Three of the novel mutations affected acceptor splice-sites in the ENG gene. RNA analyses in these three patients and in two further patients described before resulted in reduction of the transcript or in a shortened transcript. This could be confirmed by RT – PCR and / or Quantitative – RT – PCR. Furthermore, we identified a family with the mutation c.199C>T in the ACVRL1 gene with liver AVMs. This is the fifth family with this mutation and liver AVMs, clearly indicating a genotype – phenotype correlation for this mutation. This is the first HHT – causing mutation for which such a correlation is possible.

P189

Spectrum of molecular defects and mutation detection rate in patients with severe, mild and moderate haemophilia A

Bogdanova N.(1), Markov A.(2), Nowak-Göttl U.(3), Eisert R.(4), Wermes C.(5), Pollmann H.(6), Todorova A.(7), Dworniczak B.(1), Eigel A.(1), Horst J.(1)
1) Institut für Humangenetik, Münster, Germany
2) Institut für Medizinische Biochemie, Münster, Germany
3) Pädiatrische Hämatologie und Onkologie, Münster, Germany
4) Medical School, Gerinnungsambulanz, Hannover, Germany
5) Medical School, Pädiatrische Hämatologie und Onkologie, Hannover, Germany
6) Hämophilie-Zentrum, Münster, Germany
7) Laboratory of Molecular Pathology, Sofia, Bulgaria

Haemophilia A (OMIM 306700) is the most frequent X-linked bleeding disorder affecting 1 to 2 per 10 000 males worldwide. The amount of residual factor VIII (FVIII:C) determines the clinical variability of haemophilia A. About 50% of the patients have severe haemophilia A with a FVIII:C activity less than 1% of normal. Moderate (FVIII:C 2-5% of normal) and mild (FVIII:C >5% of normal) haemophilia A occur in about 10% and 30-40% of patients, respectively. Recently we showed that the mutation detection rate in severely affected male patients is virtually 100% when testing for the common intron 22- / intron 1- inversions and big deletions, followed by genomic sequencing of the F8 gene. We also showed that protein truncating molecular defects are prevalent in those patients [Bogdanova, Markoff et al., 2005, Hum Mutat 26(3):249-54]. Here we report on the spectrum of mutations and their distribution throughout the F8 protein in 136 moderately (n=24) or mildly (n=112) affected patients with haemophilia A. The performed sequencing analysis revealed a molecular defect in 121 (89%) of the patients, whereas 15 (11%) had no mutation in the coding region of the F8 gene, in the exon/intron borders or in the promoter region. All negative patients were mildly affected with exception of one patient with FVIII:C 2-3%. Thirty six of the mutations identified are novel. The vast majority of the detected mutations were missense mutations (n=104). Two molecular changes in the promoter region of the factor VIII gene were detected in two patients with mild haemophilia A. To our knowledge this is the first report on promoter mutations in the F8 gene. Our data show that, in contrast to severe haemophilia A, the analysis on the genomic level fails to detect the molecular defect in about 4% of the moderately and in 12.5% of the mildly affected patients. These patients could have mutations in the intronic regions or, alternatively, the reduction of the F8 activity is due to other reasons.

P190

Expression of the gamma-glutamyl carboxylase containing the Arg485Pro mutation found in two unrelated VKCFD1 patients

Rost S.(1), Fregin A.(1), Mutucumarana V.(2), Stafford D.(2), Oldenburg J.(3), Müller C.R.(1)
1) Department of Human Genetics, University of Würzburg, Würzburg, Germany
2) Department of Biology, University of North Carolina, Chapel Hill, United States of America
3) Institute of Experimental Haematology and Transfusion Medicine, University of Bonn, Bonn, Germany

The vitamin K-dependent gamma-glutamyl carboxylase (GGCX) catalyses the posttranslational modification of vitamin K-dependent proteins involved in blood coagulation (FII, FVII, FIX, FX, protein C, S and Z), bone and tissue metabolism (BMP and MGP) and cell growth (Gas6). A defect in the GGCX gene results in a very rare bleeding disorder, called "familial multiple coagulation factor deficiency type 1" (VKCFD1). So far, only two different mutations in the GGCX gene (Leu394Arg and Trp501Ser) could be proven to be causative for the VKCFD1 phenotype by recombinant expression in insect cells and subsequent measurement of the GGCX activity.
Here we report on the expression of a GGCX variant comprising the Arg485Pro mutation which has been detected in two unrelated patients and was shown to arise from a common origin (founder effect).
After recombinant expression of the GGCX wild-type protein and the Arg485Pro variant, the GGCX activity assay was performed by adding vitamin K hydroquinone and either the artificial substrate FLEEL in combination with the propeptide ProFIX19, or FIXproGla as a more physiological substrate. Kinetic studies were carried out by adding various concentrations of one of these substrates while keeping the others constant. The kinetic data show that the Arg485Pro mutation has no effect on FLEEL carboxylation and on vitamin K binding. But it strongly affects propeptide binding which could be demonstrated by an increased half maximal stimulation concentration for ProFIX19 and a higher Michaelis-Menten constant for FIXproGla kinetics in the Arg485Pro variant. Furthermore, the mutation is located in a highly conserved region of GGCX between amino acid residues 438 and 507 which could previously be demonstrated to comprise the propeptide binding domain. Our results confirm previous reports on the location of the propeptide binding site of the GGCX and verify the Arg485Pro mutation as causative for the VKCFD1 phenotype.

P191

Somatic mosaicism of APC mutations causing attenuated or atypical familial adenomatous polyposis (FAP)

Aretz S.(1), Stemmler S.(2), Uhlhaas S.(1), Stienen D.(1), Propping P.(1), Pagenstecher C.(1), Mangold E.(1), Friedl W.(1)
1) Institut für Humangenetik, Universität Bonn, Bonn, Germany
2) Abteilung für Molekulare Humangenetik, Ruhr-Universität Bochum, Bochum, Germany

Germline mutations in the tumour suppressor gene APC (including point mutations and large deletions) are identified in approximately 80% of patients with classical FAP and in about 30% of patients with attenuated FAP. During APC mutation screening in 1168 unrelated FAP patients we identified five cases with a de novo mutation, in whom analytical data pointed to the presence of somatic mosaicism: In three of the five patients a faint additional band was detected in the PTT pattern (exon 15) while in two patients a hardly detectable deviation from the normal pattern was observed by DHPLC.
Sequencing of the corresponding fragments revealed very weak mutation signals that would not have been recognized in the sequencing runs without the prescreening data and therefore had to be confirmed by other methods: The mutation c.3379C>T; p.Gln1127X was confirmed in the venous blood sample by a weak new Alu restriction site, and was found to a much larger extent in DNA extracted from an adenoma. The mutation c.3925G>T; p.Glu1309X was confirmed by an allele-specific PCR. The mutation c.2107dupG in codon 703 was hardly detectable by sequencing of DNA extracted from venous blood or buccal swabs, but it was readily detected in DNA extracted from adenomatous tissue. Similarly, the mutations c.3454C>T; p.Gln1152X and c.646C>T; p.Arg216X were detected as faint signals by sequencing. A consistent genotype-phenotype correlation has been observed in FAP: most patients with mutations within codons 168-1444 exhibit a classical or severe polyposis. The five mutations are localised in this APC region, however, all of the patients presented with an attenuated or atypical disease. Our data

demonstrate that deviations from the expected genotype-phenotype correlation can be explained in part by the presence of somatic mosaicism. Moreover, in a fraction of FAP patients the causative APC mutation might not be detected due to somatic mosaicism.

The study was supported by the Deutsche Krebshilfe.

P192

Distinctive FMR-1 expression profiles in patients with POF syndrome

Schuettler J.(1), Peng Z.(1), Janssen B.(2), Steinbach P.(3), Strowitzki T.(1), Vogt P.H.(1)
1) University Women Hospital, Dept. Gynecol. Endocrinol.& Infertility, Heidelberg, Germany
2) University of Heidelberg, Human Genetics, Heidelberg, Germany
3) University of Ulm, Human Genetics, Ulm, Germany

The POF (Premature Ovarian Failure) syndrome is a secondary hypergonadotrophic amenorrhoea affecting about 1% of females younger than 40 years. In 30% of them POF is caused by genetic defects of which some are based on X chromosomal gene or structure abnormalities mapped to one of the two POF loci (Vogt, 2003; in: Nature Encyclop. of the Human Genome, vol. 3, 458-464). Most prominent is the FMR-1 gene in POF1 causing familial POF in 16% of pedigrees with an expansion of the CGG triplet in exon 1 (Sullivan et al., 2005; Hum.Reprod. vol. 20, 402). We therefore explored whether we can use the FMR-1 expression profile of POF patients in their leukocytes as a diagnostic tool for indicating dysfunction of the FMR protein in their folliculogenesis. Blood samples from 80 clinically carefully evaluated POF-patients were collected for RNA and DNA extraction and a quantitative RT-PCR analysis for FMR-1 gene expression was performed on a Light Cycler machine using the SYBR Green I fluorescent staining assay. We found two different FMR-1 transcripts in all RNA samples analysed. Additionally, a third FMR-1 transcript variant (III) was found in a subgroup of them. Sequence analysis revealed that FMR-1-III transcripts have skipped exon 11 and 12; not disrupting the coding frame for the FMR-1 protein but reducing its molecular weight significantly. Comparison of the quantitative FMR-1 expression profiles in the leukocytes of POF patients with that of our normal control group revealed three distinctive expression patterns only in the patients´ leukocytes. Since we assumed that these patterns are probably associated with a different expansion and/or different methylation pattern of the FMR-1 CGG-repeat in exon 1 we examined the length of the CGG-repeat and its methylation status in patients with different FMR-1 expression profiles by Southern blotting. The results presented and discussed at the meeting will help to evaluate the need of the FMR-1 protein for human folliculogenesis.

P193

Recombinant expression and 3-D modelling of C1-Inhibitor protein variants

Förster T.(1), Schleinkofer K.(2), Oldenburg J.(3), Müller C.R.(1)
1) Department of Human Genetics, University of Würzburg, Würzburg, Germany
2) Department of Bioinformatics, University of Würzburg, Würzburg, Germany
3) Institute of Experimental Haematology and Transfusion Medicine, University of Bonn, Bonn, Germany

C1 inhibitor (C1INH) is a single-chain glycoprotein of 105 kDa that belongs to the family of serine protease inhibitors (Serpins). Serpins are suicide inhibitors and have a unique mechanism of inhibition that involves a conformational change of the protein after binding to its target protease. Hereditary Angioedema (HAE) (OMIM: 106100), an autosomal dominant disease with incomplete penetrance occurs due to mutations in the C1INH gene. C1INH deficiency may result in recurrent episodes of acute, local, circumscribed edema of the skin or mucosa.
Five years ago, we started a routine genetic testing protocol for HAE. Over the years, we have analysed DNA samples from almost 400 patients suspicious for HAE type I or HAE type II, respectively. About 30% of our patients present with amino acid substitutions. Since there are some known polymorphisms in the C1INH gene which also lead to the substitution of amino acids (e.g. V458M) it is impossible to predict the causality of the alterations offhand. Therefore, we have tested for the functional effect on C1INH activity of a series of C1INH mutations in a recombinant expression system (HEK 293 cells). Most substitutions studied so far resulted in an almost complete loss of inhibitor activity of the recombinant proteins while some mutations (e.g. A-21V and S233T) retained a reduced activity when compared to wildtype protein. Since the crystal structure of C1INH has not been elucidated yet, we used data from other serpins to build an analogous 3-D model of the protein. This model was used to predict whether the observed amino acid substitutions introduce significant changes in the protein structure.
In summary, recombinant expression of mutated C1INH protein is a useful tool to characterize the role of individual amino acid residues for C1INH activity and hereditary angioedema.

P194

Expanding the mutational and clinical spectrum in *LAMB2*-associated disorders

Matejas V.(1), Hasselbacher K.(2), Hinkes B.(2), Schumacher V.(3), Royer-Pokora B.(3), Reis A.(1), Hildebrandt F.(2), Zenker M.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) University of Michigan, Departments of Pediatrics and of Human Genetics, Ann Arbor, United States of America
3) Institute of Human Genetics, Duesseldorf, Germany

LAMB2 encodes the laminin beta 2 chain, which is expressed in basal laminae of specific tissues, including glomerulus, neuromuscular synapse, lens capsule, and intraocular muscles. We have recently identified *LAMB2* mutations as the cause underlying Pierson syndrome (OMIM 609049). This disorder comprises congenital nephrotic syndrome (CNS) with diffuse mesangial sclerosis, ocular abnormalities including microcoria, abnormal lens and retinal changes, as well as neurodevelopmental deficiencies. With one exception, the previously reported patients harboured truncating mutations (presumable null alleles) on both *LAMB2* alleles. Herein we report on 8 novel unrelated patients with the Pierson phenotype. The four long-term survivors showed considerable discrepancies in their neurological development. Biallelic *LAMB2* mutations, mostly truncating ones, were found in all patients with Pierson syndrome as defined by the presence of CNS and microcoria. In addition, we present a consanguineous family with isolated CNS and linkage to the *LAMB2* locus, and another family with CNS and mild ocular anomalies (nystagmus, fundus abnormalities), rather corresponding to the disorder reported by Barakat et al. (Pediat Nephrol 1982; 3: 33-35). Novel *LAMB2* missense mutations affecting highly conserved amino acid residues were identified in both families. These findings expand the clinical spectrum of *LAMB2*-associated disorders from Pierson syndrome with neurological deficits as the most severe phenotype to isolated nephrosis at the mild end of the spectrum. Moreover, we suggest that the disorder reported by Barakat et al., listed as “diffuse mesangial sclerosis with ocular abnormalities” (OMIM 249660), is allelic to Pierson syndrome. The molecular findings argue in favour of a close genotype phenotype correlation. We conclude that *LAMB2* mutations have to be considered in CNS even in the absence of features defining the Pierson syndrome.

P195

Genotype-phenotype characterization in hereditary multiple exostosis

Leube B.(1), Hardt K.(1), Westhoff B.(2), Portier S.(2), Krauspe R.(2), Royer-Pokora B.(1)
1) Institute of Human Genetics, UKD, Düsseldorf, Germany
2) Clinic of Orthopedics, UKD, Düsseldorf, Germany

Hereditary multiple exostosis is an autosomal dominant disorder, characterized by numerous cartilagineous exostosis (osteochondroma), which develop primarily on the long bones from early childhood until puberty. It is caused by germ line mutations in several genes, especially in EXT1 or EXT2. Subclinic symptoms as well as severe symptoms such as bone deformation with disturbance of movements, shortening of extremities, reduction of height and even malignant transformation to chondrosarcoma can be observed.
The purpose of the study was to discover a possible genotype-phenotype correlation. Patients were clinically investigated and the mutated gene was identified by linkage analysis (indirect genotyping) and if possible by direct mutation analysis. We investigated 38 patients of 12 families. 14 patients of 6 families were linked to EXT1 and 14 patients of 3 families to EXT2. Two families could not be linked successfully. In one family, linkage to all known EXT- and EXTL-loci was excluded. 4 of 5 affected members of this family had very short stature (below the 3. percentile). Patients of EXT1-linked families had more exostoses and more often a short stature. In contrast to a previous study, neither short stature was correlated to mutations in exon 1 nor chondrosarcoma were restricted to EXT1 mutations. Age of onset and bone deformation

differed not significantly from EXT2-linked families.

P196

Investigation of a potential splice mutation in the VMD2 gene in a patient with autosomal dominant vitreoretinochoroidopathy (ADVIRC)

Fiebig B.(1), Kellner U.(2), Weber B.H.F.(1)
1) Institut für Humangenetik, Regensburg, Germany
2) Augenzentrum, Siegburg, Germany

ADVIRC with nanophthalmos is a rare autosomal dominant condition with characteristic retinal and vitreous findings caused by mutations in the VMD2 gene (Yardley et al. IOVS 45:3683-9, 2004). A strong genotype-phenotype correlation for ADVIRC-associated sequence alterations was suggested: mutations that result in missense substitutions also appear to cause exon skipping thus likely abolishing partial or complete protein function. The manifestation of nanophthalmos suggests that VMD2 may also play an important role in development of the eye. Here we report on a female patient with the clinical features of ADVIRC including a positive family history but without nanophthalmos. Sequence analysis of the coding exons of VMD has revealed a c256G>A mutation in exon 4. This mutation has also been reported in a family with ADVIRC and nanophthalmos. The splicing behaviour of the mutated pre-mRNA will be analyzed in a plasmid minigene-construct. In addition, lymphoblast cDNA of the patient is available and has been shown to express the VMD gene. Together, our data will clarify the consequences of the c256G>A mutation on the protein sequence and will help to further delineate functional domains of bestrophin.

P197

Rare and unknown globin gene mutations identified in a population of hemoglobinopathy patients in Switzerland

Hergersberg M.(1), Rüegg C.(1), Bernasconi L.(1), Herklotz R.(1), Ohanessian A.(1), Benedek E.(1), Huber A.(1)
1) Zentrum für Labormedizin, Aarau, Switzerland

Introduction: The inherited hemoglobinopathies result from mutations in the genes encoding the α- and β-globin chains. Diagnosis of anemia in general and thalassemias/hemoglobinopathies in particular relies since many years on molecular biological analysis of patient samples. For about 500 blood samples annually, DNA analysis has been used as a diagnostic tool. Beside screening for common mutations, detailed mutation analysis is in many cases necessary to elucidate the molecular cause of the hemoglobinopathy.
Material and methods: Mutation identification is performed using polymerase chain reaction (PCR), denaturing HPLC (DHPLC), and DNA sequencing. Depending on the results of the hematologic analysis, the α-globin gene locus and/or the β-globin gene locus are analysed using the methods mentioned above.
Results and conclusions: An algorithm for the identification of rare globin gene mutations by DHPLC and DNA sequencing has been established. 4 overlapping regions covering the β-globin gene and 4 spanning over of the α2-globin gene are amplified and subsequently analyzed by DHPLC. PCR products with an abnormal DHPLC profile were sequenced. In a patient with increased HbA2 we identified a mutation at base 108 in the first intron of the β-globin gene. In the α2-globin gene three different unknown mutations were found in three persons with hypochromia. One mutation results in an amino acid exchange of amino acid 116. Two other mutations result from a complex sequence rearrangement. Identification of the mutations in probands with thalassemia on the DNA level is the prerequisite for genetic counselling and prenatal diagnosis.

P198

AmpliChip CYP450 – ein Schritt in Richtung individualisierte Arzneimitteltherapie

Radelof U.(1), Wagner F.(1), Drescher B.(1)
1) RZPD Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin, Germany

Menschen verstoffwechseln Wirkstoffe unterschiedlich. Die individuelle genetische Ausstattung spielt hierbei eine sehr wichtige Rolle. Eine auf diese Ausstattung abgestimmte Medikation ermöglicht eine wirksamere Behandlung von Patienten und vermeidet den Einsatz ungeeigneter Medikamente und falscher Dosierungen, die zu unerwünschten Arzneimittelwirkungen führen können. Schätzungen zufolge ereignen sich in Deutschland jährlich rund 16.000 Todesfälle durch unerwünschte Arzneimittelwirkungen und 120.000 schwere Arzneimittel bedingte Zwischenfälle [1].
Die Verstoffwechselung von ca. 25% aller verschreibungspflichtigen Medikamente erfolgt durch die Leberenzyme CYP2D6 und CYP2C19. Mit Hilfe des auf der DNA-Chip-Technologie von Affymetrix basierenden AmpliChips CYP450 von Roche Diagnostics werden die 33 wichtigsten Mutationen in den Genen dieser Enzyme gleichzeitig in einem einzigen Experiment analysiert. Als Ergebnis wird ermittelt, welchem Stoffwechseltyp ein Patient angehört, d.h. ob er ein Medikament ultraschnell (UM), schnell (EM), mittel (IM) oder langsam/gar nicht (PM) metabolisieren kann. Dieses Resultat gestattet dem Arzt eine wirkungsvollere Wahl und Dosierung von Medikamenten [2].
Der AmpliChip CYP450 ist der erste DNA-Chip mit CE-IVD-Kennzeichnung für die Routinediagnostik. Seit Dezember 2004 steht er in Deutschland für den klinischen Einsatz zur Verfügung; Anfang Januar 2005 wurde er von der FDA für die Diagnostik in den USA zugelassen. Derzeit findet die CYP450-Analyse in ausgewählten Servicezentren, zu denen auch das RZPD Deutsches Ressourcenzentrum für Genomforschung gehört, statt.
[1] Schönhöfer P, Arzneimitteltherapie, Vol. 17: 83-86, 1999
[2] Roots et al., Drug Metab. Rev., Vol. 36, pp. 617-638, 2004

P199

Parkinson's disease: Hot spot mutation screening of the LRRK2 gene in patients from Germany

Schlitter A.M.(1), Woitalla D.(2), Mueller T.(2), Epplen J.T.(1), Dekomien G.(1)
1) Ruhr Universität Bochum, Humangenetik, Bochum, Germany
2) Ruhr Universität Bochum, St. Josef-Hospital, Neurologie, Bochum, Germany

Background: Recently, mutations within the LRRK2 gene were shown to cause autosomal dominantly transmitted Parkinson's disease (PD). Especially the common mutation G2019S accounts for ~1-2% of typical sporadic PD and 3-6% of cases with a positive family history. Several putatively pathogenic mutations were identified spread across 9 different exons.
Methods: The 9 exons representing hot spot regions within the LRRK2 gene were screened in a cohort of 120 patients recruited in Germany suffering from the early- as well as the lateonset forms of PD. 25.8% of the patients presented a family history of PD.
Results: Several common exonic (G1624G, K1637K, S1647T) and intronic single nucleotide polymorphisms (SNPs; Ivs33-31T>C, Ivs34+32A>G, Ivs34-51A>T, Ivs35+23T>A, Ivs38+35G>A) were identified. Three patients (2.5%) carried mutations in heterozygous state, two harbor the common mutation G2019S (age of onset 30 and 44 years), one patient exhibited a novel mutation A1151T (age of onset 55 years). No mutation was identified in healthy controls from Germany. In our study, 6.7% of the patients presenting a positive family history for PD and 1.1% of patients with sporadic PD are mutation carriers. 20% of the patients suffering from early-onset PD harbor a mutation, yet only 0.9% of the patients with late-onset PD.
Discussion: Mutations in the LRRK2 gene, especially the frequent mutation G2019S are a comparatively common cause of PD in Germany among patients with a positive family history and early-onset form of PD, respectively.

P200

Mutations of the calcium-sensing receptor in patients with familial hypocalciuric hypercalcemia and autosomal dominant hypocalcemia

Haag C.(1), Hentze S.(1), Schulze E.(1), Lorenz A.(1), Frank-Raue K.(1), Raue F.(1)
1) Endokrinologisch-Humangenetische Gemeinschaftspraxis, Heidelberg, Germany

The calcium-sensing receptor (CaSR) is a member of the G-protein-coupled receptor family. It regulates the secretion of parathyroid hormone (PTH) in response to changes in extracellular calcium concentrations as well as the reabsorption of urinary calcium. Heterozygous gain-of-function mutations cause autosomal dominant hypocalcemia (ADH) whereas loss-of-function mutations cause familial hypocalciuric hypercalcemia (FHH).
The clinical differentiation between ADH and idiopathic hypoparathyroidism (IHP) as well as between FHH and primary hyperparathyroidism (pHPT) is not always possible. Therefore we screened 8 patients with hypocalcemia and 140 patients with hypercalcemia for mutations of the CaSR gene. Mutations of the CaSR were detected after specific amplification of the six coding

exons by PCR and direct sequencing of the PCR products.
Within the group of patients with hypocalcemia we identified one novel (A844T) and three already described (Q245R, T151R, P221L) mutations. The serum calcium concentrations of the patients with mutations were 1.91 – 1.98 mmol/l and the PTH levels were slightly reduced or within the normal range.
We also detected six novel mutations of the CaSR (W530G, W718X, M734R, L849P, Q926R and D1005N) and two already described mutations (E250K and P55L) in the patients with hypercalcemia. The patients with mutations of the CaSR had serum calcium concentrations of 2.68 – 3.05 mmol/l. The differentiation between FHH and primary hyperparathyroidism is important to avoid unnecessary parathyroidectomy in patients with FHH. As hypocalciuria is not always detectable, the molecular genetic analysis of the CaSR is a useful tool to differentiate FHH from pHPT in patients with mild hypercalciuria and slightly elevated PTH. It is also important to distinguish between ADH and idiopathic hypoparathyroidism because of the incidence of renal complications found in ADH patients when treated with high dosis of vitamin D to raise serum calcium concentration.

P201

An X-linked mental retardation resequencing array

Jensen L.(1), Chen W.(1), Amende-Acar M.(1), Lipkowitz B.(1), Moser B.(1), Moraine C.(2), Fryns J.-P.(3), Chelly J.(4), Gecz J.(5), Reinhardt R.(1), Ropers H.-H.(1), Kuss A.(1)
1) Max Planck Institute for Molecular Genetics, Human Molecular Genetics, Berlin, Germany
2) INSERM, Services de Génétique, Tours, France
3) University Hospital Leuven, Centre for Human Genetics, leuven, Belgium
4) Centre National de la Recherche Scientifique, Institut Cochin de Génétique Moléculaire, Paris, France
5) University of Adelaide, Department of Genetic Medicine, Adelaide, Australia

Severe mental retardation (MR) defined as IQ<50 affects 0.5 % of the male population in developed countries and approximately 10-15% of these cases are due to mutations on the X-chromosome. The majority of affected males show non-syndromic X-linked mental retardation (NS-XLMR) in which MR is the only clinically recognizable feature. NS-XLMR is genetically heterogeneous. To date, >20 genes have been implicated in this condition, but most of these genes account for less than 1% of the total number of mutations, which is a major obstacle for the molecular diagnosis of this common disorder. Cumulatively, however, the mutation frequency of these 20 genes is comparable to that of FMR1, the gene involved in fragile X syndrome.
Here we report on a DNA chip, which is based on the Affymetrix 50k platform and designed for re-sequencing known XLMR genes, with a focus on NS-XLMR. It encompasses coding regions and splice sites of the following XLMR genes: ACSL4, ARX, ATRX, DLG3, FTSJ1, GDI1, IL1RAPL1, JARID1C, MECP2, NLGN4, PAK3, PHF6, PHF8, PQBP1, SLC6A8, TM4SF2 and ZNF41. This new tool should facilitate the molecular diagnosis in NS-XLMR families that are too small for linkage mapping, and ruling out mutations in known genes should greatly facilitate the search for novel ones.

P202

Phosphorylation on Ser106 modulates the cellular functions of the SHOX homeodomain protein

Marchini A.(1), Daeffler L.(2), Marttila T.(1), Schneider K.U.(1), Blaschke R.J.(1), Schnölzer M.(3), Rommelaere J.(2), Rappold G.(1)
1) University of Heidelberg, Institute of Human Genetics, Heidelberg, Germany
2) German Cancer Research Center (DKFZ) and Inserm U375, Applied Tumour Virology Program, Heidelberg, Germany
3) German Cancer Research Center (DKFZ), Protein Analysis Facility, Heidelberg, Germany

Mutations within the homeobox SHOX gene have been associated with short stature and the skeletal deformities found in Léri-Weill, Turner and Langer syndromes implying an involvement of SHOX in growth and bone formation. Despite its clinical significance, the precise role of SHOX and the mechanisms which modulate its functions remain unknown. We have previously reported that SHOX is a nuclear protein that specifically binds DNA and acts as a transcriptional activator. We have also shown that ectopic expression of SHOX leads to cell cycle arrest and apoptosis in osteosarcoma and primary cells. To further characterize SHOX, we investigated whether the protein could be a target for phosphorylation. Here we report that SHOX is phosphorylated exclusively on serine residues in vivo. Two dimensional phospho-peptide mapping showed that SHOX is phosphorylated to various extents on multiple sites. Site-directed mutagenesis demonstrated that Serine 106 is the major SHOX phosphorylation site. We also show that casein kinase II efficiently phosphorylates SHOX on Serine 106 in vitro and specific casein kinase II inhibitors strongly reduce SHOX phosphorylation in vivo. Finally, we provide evidence that phosphorylation may play an important role in modulating SHOX biological activities, since a S106A SHOX mutant, defective in phosphorylation, does not activate transcription and fails to induce cell cycle arrest and apoptosis.

P203

Identification of lineage specific and polymorphic copy number variations in the human genome by inter- and intra-species array comparative genomic hybridization (aCGH)

Goidts V.(1), Schempp W.(2), Armengol L.(3), Conroy J.(4), Hameister H.(1), Kehrer-Sawatzki H.(1)
1) University of Ulm, Human Genetics, Ulm, Germany
2) University of Freiburg, Anthropology and Human Genetics, Freiburg, Germany
3) Center for Genomic Regulation, Program in Genes and Disease, Barcelona, Spain
4) Roswell Park Cancer Institute, Cancer Genetics, Buffalo, United States of America

Comparative analyses of human and great ape genomes expose the full spectrum of genomic changes that accompanied human evolution. In particular the concomitant evaluation of divergence and diversity has the power to identify those genes or genomic regions that evolved under selective constraints during human evolution and might be associated with human specialization. Recent analyses showed that copy number variations (CNVs) of a few kb up to several 100 kb have contributed significantly to the genomic divergence between humans and other primates. Since most of these CNVs contain complete or partial genes, lineage specific gene losses and gains are the consequences of these variations. In order to identify CNVs that occurred specifically in the human lineage, we performed interspecies array CGH including five great ape species. The human BAC array used in an initial step of this project covered 30% of the human genome. A total of 14 sites of putative human specific CNVs were discovered. Thirteen of them overlap with segmental duplications (SDs). In order to identify the human specific duplicons among these highly homologous SDs, we designed a custom made array including all SDs under suspicion to contain human copy number gains. In a second step of our analyses, this region specific SD-array was used for comparative hybridizations using a pool of human genomic DNA as reference together with pooled DNA from gorilla and in a further experiment from orangutan. These experiments were performed to assess the precise sites of human lineage specific gains. To identify absence-presence polymorphisms of these variant sites in the human genome, we performed comparative genomic hybridization with 10 human DNA samples from ancient African human populations. The characterization of polymorphic human specific CNVs enables not only to reproduce the evolution of the human genome but also has the potential to highlight putative disease associated genotypes.

P204

Candidate gene testing for Emery-Dreifuss muscular dystrophy

Bethmann C.(1), Wasner C.(1), Wehnert M.(1)
1) Institut für Humangenetik, Molekulare Humangenetik, Greifswald, Germany

Until now two genes, STA and LMNA, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Further genes are likely to be involved. Forced by the lack of families suitable for positional cloning we started a functional candidate gene approach. We considered such genes as candidates for EDMD that encode (1) functionally related proteins to emerin and lamin A/C including LMNB1+2, LBR, LAP1+2, NRM and MAN1 or (2) proteins interacting with emerin and/or lamin A/C including Narf, Zmpste24, BAF, PSME3, SREBF1, YT521B. A third group of candidates were those, which are expressed specifically in heart and skeletal muscle – the preferentially affected tissues in EDMD (FLNC, SMPX, POP1-3, AKAP 7, mAKAP, Nesprin1+2). Additionally, we tested two genes, which are associated or candidates for LGMD (FKRP, Connexin40). Until now we have studied 24 genes in 110 patients from Germany. We identified five unique variations in Nesprin1α (c.-48A>G, p.R257H, p.V572L, p.E646K, c.2172G>A), one in Nesprin2α (c.-366G>A), one in Nesprin1β (c.3537A>C),one in SrebF1 (p.R812Q), one in YT521B (c.1977*633G>A), one in LAP2 (p.E384K), one in NRM (715C>A), one in FLNC (6508C>T), four in Zmpste24 (c.-156TYC, c.159G>A, p.I351T, p.R369Q), one in Connex-

in40 (c.1197*479G>A) and six in mAKAP (p.R1134H, c.6962*6A>C, c.6960*1209A>C, c.6960*1384T>C, c.6960*1902G>T, c.6960*2494 A>T), which were not detectable in a reference population. None of the DNA variants have so far been associated to EDMD. Interestingly, a Nesprin2α c.-366G>A variation was also found in the patient's affected son, but not in the healthy son and partly affected daughter and the Nesprin1α p.R257H variation was not found in the patient´s healthy relatives. So these variations seem to segregate with EDMD. Our results might be the first hint that Nesprins are involved in the pathogenesis of EDMD. But to validate such an effect, in vitro mutagenesis and transfection experiments are required.

P205

Positional cloning of a candidate gene for Congenital Fiber Type Disproportion (CFTD)

Kestner D.(1), Bache I.(2), Botzenhart E.(1), Hansen C.(2), Tümer Z.(2), Belloso Sanchez J.M.(2), Clarke N.(3), Northe K.(3), Ferreiro A.(4), Jiménez-Mallebrera C.(5), Nishino I.(6), Muntoni F.(5), Rasmussen M.(7), Tommerup N.(2), Scherer G.(1)

1) Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg i. Br., Germany
2) Wilhelm Johannsen Centre for Functional Genome Research, Department of Medical Biochemistry and Genetics, University of Copenhagen, Copenhagen, Denmark
3) Neurogenetics Research Unit, Children´s Hospital at Westmead, Sydney, Australia
4) Institut de Myologie, Groupe Hospitalier Pitie-Salpetriere, Paris, France
5) The Dubowitz Neuromuscular Centre, Imperial College School of Medicine, London, United Kingdom
6) Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
7) Department of Pediatrics, Rikshospitalet, Oslo, Norway

CFTD (OMIM 255310) is a congenital myopathy, characterized histologically by the different size of type I (slow twitching) compared to type II (fast twitching) muscle fibers, type I fibers being at least 12% smaller than type II fibers. Clinical features seen in CFTD include muscle hypotonia, generalized weakness, scoliosis, multiple joint contractures, congenital hip dislocation and a high-arched palate. The course is very variable, ranging from mild to severe, even lethal forms, with lethality usually due to respiratory complications. The causes for this disorder are still unknown. Both autosomal recessive and dominant modes of inheritance have been suggested.

In diseases with unknown pathogenesis, regions for candidate genes can be identified via translocation breakpoints found in patients. Here we define the breakpoints of a CFTD patient with a balanced translocation t(10;17)(p11.2;q25) (Gerdes *et al.*, 1994). Starting with FISH analysis, the breakpoints were initially mapped to overlapping/flanking BACs. For fine-mapping of the breakpoints, we separated the chromosomes of interest in rodent-somatic cell hybrids. By PCR analysis of hybrids containing the der(10) or der(17) chromosomes, the breakpoints could be mapped to intervals of a few hundred base pairs. Sequence analysis of a breakpoint-spanning PCR product defined the der(17) breakpoint down to two bases. Southern blot analysis using DNA from the patient and probes from the breakpoint regions were in complete agreement with the results from the somatic cell hybrids.

Whereas the breakpoint on chr10 lies within an intergenic region, it disrupts a 20 exon gene of unknown function (*LOC284001*) on chr17, representing a candidate gene for CFTD. We started to screen a cohort of 65 patients for mutations in this gene, using dHPLC and DNA sequencing. Within the 14 exons analysed so far, we found an amino acid substitution in one patient, which is no described polymorphism.

P206

Glycogen storage disease IV due to a homozygous large deletion in the GBE1 gene

Siebers-Renelt U.(1), Janecke A.(2), Mosel A.(3), Kennerknecht I.(1), Utermann G.(2), Horst J.(1)

1) Institut für Humangenetik der Universität, Münster, Germany
2) Genetische Beratungs- und Untersuchungsstelle der Universität, Innsbruck, Austria
3) Klinikum Osnabrück, Bereich für Pränatale Diagnostik, Osnabrück, Germany

Glycogen storage disease (GSD) IV is a rare metabolic disorder due to mutations in GBE1 encoding glycogen branching enzyme. We report on a German healthy couple without known consanguinity with 3 children presumably affected with fatal GSD IV and the difficulties in the diagnostic work-up. In the 3rd pregnancy polyhydramnios occurred in the 23rd week followed by fetal loss in the 32nd week. Pathological examination did not reveal any specific findings. In the 4th pregnancy a girl was delivered preterm (29th week) because of massive polyhydramnios since the 24th week. She was severely hypotonic, did not breath spontaneously, developed cardiomyopathy and hepatomegaly and deceased at the age of 4 months. Post mortem analysis showed polyglucosan deposits in the muscle compatible with GSD IV. Enzymatic diagnosis could not be performed. In the sixth pregnancy, the couple was referred for genetic counselling. We tried to verify the diagnosis of GSD IV by GBE1 mutation analysis of genomic DNA from the healthy parents. Using DHPLC and direct sequencing of the coding region of the GBE1 gene no pathogenic mutations were identified. PCR amplification of paraffin-extracted DNA from the affected child was unsuccessful. Leucocyte cDNA analysis of GBE1 of both parents using published protocols revealed normal results. Pregnancy went on regularly and no signs of cardiomyopathy or hepatomegaly nor polyhydramnios were detectable by ultrasound until the 33th week. In the 35th week polyhydramnios developed and cesarian sectio became necessary. The child had respiratory insufficiency, cardiomyopathy and severe muscular hypotonia. PCR amplification of the entire GBE1 gene showed a homozygous 25 kb-deletion of exon 4 to 7. This deletion was also detected at the cDNA level in both parents in heterozygosity using a novel protocol. Although up to now published only once, large deletions in GBE1 must be considered in GSD IV requiring new diagnostic protocols.

P207

High frequency and variable penetrance of WDR36 mutations in glaucoma patients

Pasutto F.(1), Mardin C.Y.(2), Sticht H.(3), Michels-Rautenstrauss K.(1), Chavarria-Soley G.(1), Rautenstrauss B.(1), Kruse F.(2), Reis A.(1)

1) Human Genetics, FAU Erlangen-Nuremberg, Erlangen, Germany
2) Department of Ophtalmology, FAU Erlangen-Nuremberg, Erlangen, Germany
3) Biochemistry, FAU Erlangen-Nuremberg, Erlangen, Germany

Mutations in WDR36 have recently been identified in adult-onset primary-open angle glaucoma (POAG) patients (Monemi et al., 2005). We now explored a study group of 309 unrelated glaucoma patients with diverse age of onset and intraocular pressure levels for WDR36 mutations. We screened the entire coding sequence through direct sequencing in all patients and identified 11 non-synonymous alterations, of which 6 are novel (P31T, D33E, Y97C, H212P, T403A, and H411L). We investigated all variants in a group of 94 healthy individuals of comparable age, who had repeated normal ophthalmologic examinations. Five of these 11 variants (D33E, Y97C, H212P, H411L and A449T) were regarded as full mutations, while three appear to have reduced penetrance (L25P, A163V and D658G) and were also found in controls at reduced frequency. The significance of two rare variants (P31T and T403A) remains still unclear, while one (I264V) represents a frequent polymorphism. These 10 variations were found in a total of 32 patients (10.3 %). The here presented structure analysis of the WDR36 protein based on homology modelling revealed a probable structure of 14 WD-repeats. 7 variants were predicted to affect the structural integrity of WD-repeats. Mutations were associated with open angle glaucoma with high and low intraocular pressure and with a broad range of age of onset. We hypothesize that variable penetrance of mutations may explain the wide range of clinical manifestation and age of onset up to subclinical presentation in controls. Our findings extend the evidence that WDR36 plays an important role in the aetiology of glaucoma

P208

First case of oculocutaneous albinism type 3 in a European patient

Müsebeck J.(1), Rooryck-Thambo C.(2), Roudaut C.(2), Robine E.(2), Lohner R.(1), Arveiler B.(2)

1) Centre for Human Genetics, University of Bremen, Bremen, Germany
2) Department of Medical Genetics, University Hospital Pellegrin-Enfants, Bordeaux, France

Oculocutaneous albinism (OCA) is a rare genetic disorder of pigmentation characterised by reduced or absent melanin production in the melanocytes of the skin, hair follicle, and eye. It is clinically and genetically heterogeneous and occurs as isolated disease or in association with other abnormalities as part of a syndrome. The isolated forms are inherited in an autosomal recessive fashion and were formerly differentiated into tyrosinase negative and positive albinism. Recently, four types (OCA1-4) are subdivided. This classification follows the distinct genes mutated: Oculocutaneous albinism type 1 (OCA1)

and type 2 (OCA2) are most common and caused by mutations in the TYR (tyrosinase) and P (OCA2) genes, respectively. OCA3 corresponds to rufous or brown albinism, with TYRP1 (tyrosinase-related protein 1) mutations identified so far in black African populations only with the exception of a Pakistani family. OCA4 is associated with mutations in the MATP (membrane-associated transporter protein) gene and has already been described in German patients. – We investigated a German patient of Caucasian origin with consanguineous parents (second cousins), with a light-yellow skin, yellow-gold hair with orange highlights, fair eyelashes, several pigmented naevi, and no tendency to tan. Eye-colour is blue-green with substance defects of the iris. After no mutation had been detected by molecular analysis of the TYR and P genes, two different mutations (c.1066G>A and c.106delT) were found in the TYRP1 gene. The missense mutation was inherited from the mother, whereas the single-base deletion was de novo. To our knowledge, this is the first case of TYRP1 mutations in a European patient. This finding raises the question whether mutation screening should be extended to the TYRP1 gene in patients from all ethnic origins in cases where no mutations have been identified in the other genes.

P209

NOTCH2 as a second gene in addition to JAG1 involved in Alagille syndrome

Pabst C.(1), Gläser C.(1), Hansmann I.(1)
1) Institut für Humangenetik und Medizinische Biologie, Halle/Saale, Germany

Alagille syndrome (AGS) is an autosomal dominant disorder with chronic cholestasis, congenital heart anomaly, skeletal defects, eye and kidney abnormalities and a characteristic face. Mutations within Jagged 1 (JAG1), a ligand of the NOTCH signalling pathway are being found only in ~70-80% of patients with AGS. The failure to identify JAG1 mutations in the remaining cases might be due to methodological shortcoming or due to genetic heterogeneity. Only double heterozygous Jag1/Notch2 but not Jag1/+ mutated mice resemble the clinical picture of AGS with cardiac, liver, renal and eye defects (McCright B et al. 2002). This indicates that in addition to the ligand JAG1 the human NOTCH2 receptor is a candidate gene causing or even modifying the expression of AGS. As a first step we investigated whether NOTCH2 mutations are present in such AGS patients which were found to have no JAG1 mutation. For identifying genomic variants of NOTCH2 in 38 patients SSCP analysis for all 34 exons was performed and amplicons with band shifts were sequenced. In 7 of 38 such AGS patients 3 heterozygous sequence variants were found. In 1 patient a transition G>A at position 1109 of the translated sequence (c.1109G>A) was detected in exon 7. This missense mutation results in the substitution of glycine by aspartic acid (codon 370, p.Gly370Asp) within the 9th EGF-like domain. A substitution C>T 17 base pairs upstream of the acceptor splice site of exon 10 (IVS9-17C>T) was found within intron 9 in 4 patients. The third variant was identified in 2 patients and is located within intron 17, 44 base pairs upstream of the acceptor splice site of exon 18 (IVS17-44C>T, rs2493420). None of these 3 sequence variants were observed in the sample of 153 controls (306 chromosomes) indicating that NOTCH2 appears to be involved in Alagille syndrome. Experiments are underway to investigate the functional consequences of these sequence variants.

P210

Analysis of expression and function of stomach-specific gene SX

Oidovsambuu O.(1), Göring W.(1), Zoll B.(1), Engel W.(1), Adham I.M.(1)
1) Institut für Humangenetik, Göttingen, Germany

The gastrointestinal tract develops from the embryonic gut, which is composed of endodermally derived epithelium surrounded by cells of mesodermal origin. Cell signaling between these two tissue layers appear to play a critical role in coordinating patterning and organogenesis of the gut and its derivatives. Using PCR select cDNA subtraction method, we have isolated a cDNA fragment which is predominantly expressed in stomach. Analysis of deduced amino acid sequence revealed that SX belongs to immunoglobulin like adhesion protein superfamily. Northern blot and immunohistological analyses revealed that SX gene is expressed during prenatal and postnatal stages of stomach development. The protein is restricted to basolateral plasma membran of epithelial cells. Immunohistological analysis of stomach from E16.5 revealed that the SX protein is restricted to the glandular gastric epithelium. Western Blot analysis suggests that the SX protein is highly glycosylated. To elucidate the function of the SX gene in stomach development, generation of SX deficient mice is underway.

P211

Mutational analysis in restrictive dermopathy

Nguyen Thuy D.(1), Bethmann C.(1), Hoeltzenbein M.(1), Hausser I.(2), Albrecht B.(3), Wehnert M.(1)
1) Institut für Humangenetik, Moleculare Humangenetik, Greifswald, Germany
2) Dermatologische Klinik, Heidelberg, Germany
3) Institut für Humangenetik, Essen, Germany

Restrictive dermopathy (RD) is a lethal human genetic disorder characterized by intrauterine growth retardation, tight and rigid skin with erosions, prominent superficial vasculature, epidermal hyperkeratosis, bone mineralization defects, arthrogryposis, preterm delivery and early neonatal death. This disease was recently reported to be associated with a single heterozygous mutation in ZMPSTE24 or LMNA. So RD was supposed to be a digenic disorder (Navarro et al. 2004). ZMPSTE24 encodes a zinc metalloprotease, which is involved in the post-translational processing of prelamin A – a gene product of LMNA – to mature lamin A, an intermediate filament component of the nuclear envelope. We have investigated LMNA and ZMPSTE24 in five unrelated patients with symptoms of RD. No mutation was found in the LMNA gene, whereas four patients were found to be either homozygous for a common ZMPSTE24 mutation c.1085-1086insT or compound heterozygous for the mutation c.1085-1086insT combined with a novel mutation c.50delA. Additionally, we found a novel homozygous ZMPSTE24 mutation c.209-210delAT in a consanguineous family associated to RD. All the mutations lead to a functional loss of zinc metalloprotease ZMPSTE24 resulting in an unprocessed prelamin A. Thus our study confirmed RD to be an autosomal recessive disorder preferentially caused by homozygous or compound heterozygous ZMPSTE24 mutations. Moreover, the present study confirmed that the c.1085-1086insT mutation represents a mutational hotspot within exon 9 as shown previously by Navarro et al. (2005) and Casey et al. (2005). This mutation occurs in approximately 80% of the RD patients.

P212

Structural and functional analysis of a member of the heat shock protein 110 family

Held T.(1), Paprotta I.(1), Janchif K.(1), Engel W.(1), Adham I.M.(1)
1) Institute for Humangenetics, Göttingen, Germany

Heat shock proteins (Hsps) have a highly conserved amino-terminal ATPase domain and a conserved carboxy-terminal peptide-binding domain, which is divided into a substrate-binding domain and a C-terminal domain. Hsps act as molecular chaperones that protect the cells against environmental changes and apoptosis. We have isolated and characterized the expression of Apg1, a member of the heat shock protein 110 family. The Apg1 gene transcribes two RNA isoforms, which are different in the length of the 3'-untranslated region. Apg1 is expressed ubiquitously in pre- and postnatal murine tissues. A high level of Apg1 mRNA is found in testis. Using Northern blot and immunohistological analyses, we have determined the expression of Apg1 during the postnatal development of testis. Northern blot analysis revealed that Apg1 is highly expressed in pachytene spermatocytes. To elucidate the function of Apg1 in vivo, the murine gene was inactivated by deletion of exon 1 containing the ATG codon. Both Apg1+/- and Apg1-/- mice are viable and show no malformations. However, 60 % of homozygous males produce no offspring. Histological analysis of testis revealed that the seminiferous tubules of Apg1-/- mice contain all different types of germ cells, but there is a significant increase of apoptotic germ cells. Analyses of different sperm parameters revealed that the number and motility of Apg1-deficient sperm are significantly reduced. These results suggest that Apg-1 plays important roles in male germ cell development.

P213

Functional analysis of Pelota during the cell cycle

Buyandelger B.(1), Burnicka O.(1), Hartung M.(1), Ebermann L.(1), Engel W.(1), Adham I.M.(1)
1) Institute of Human Genetics, Goettingen, Germany

Mutations in either the Drosophila Pelota (Pelo) or the S. cerevisiae homologue dom34 cause defects of spermatogenesis and oogenesis in Drosophila and delay of growth and failure of sporulation in yeast. Both phenotypes suggest

a requirement of Pelo for normal progression of the mitotic and meiotic cell cycle. To explore the function of Pelo in mammals, we have disrupted the mouse Pelo gene and shown that the gene is essential for normal mouse embryonic development. Development of homozygous embryos arrests about 6.5-7.5 days after conception. The failure of mitotic active inner cell mass (ICM) of the Pelo-/- blastocysts to expand in growth after 4 days in culture and survival of mitotic inactive trophoblast indicate that the lethality of Pelo null embryos is due to defect in cell proliferation. Increase of percentage of cells exhibiting polyploidy at E7.5 can be directly responsible for the arrested development and suggests that the Pelo is required for the maintenance of the genomic stability. Approaches to establish Pelo-/- cells by culture of Pelo+/- ES cells in medium containing high concentration of G418 failed to detect Pelo deficient cells. These results suggest that Pelo is essential for cell viability and cellular proliferation. Using Pelo- specific antibody, we found that Pelo is associated with cytoskeleton. Western blot analysis revealed the presence of Pelo in the cytoskeleton and membrane-fractions but not in nuclear and cytoplasmic fractions. These results suggest a possible role of Pelo in cytoskeleton organization and cell motility. Using yeast two hybrid system, we isolated several putative interaction partners of Pelota, which are associated with the cytoskeleton. To overcome the early embryonic lethality of Pelo deficient mice, generation of conditional knockout mice is underway.

P214

Truncating mutations of EFNB1 cause transcript depletion in skin fibroblasts of CFNS patients

Wieland I.(1), Reardon W.(2), Goldenberg A.(3), Volleth M.(1), Tinschert S.(4), Wieacker P.(1)
1) Institut für Humangenetik, Universitätsklinikum, Otto-von-Guericke Universität, Magdeburg, Germany
2) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Dublin, Ireland
3) Centre Hospitalier Universitaire de Rouen, Unité de Génetique Clinique, Hôpital Charles Nicolle, Rouen Cedex, France
4) Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus,TU Dresden, Dresden, Germany

Mutations in EFNB1 (MIM 300035) are responsible for familial and sporadic craniofrontonasal syndrome (CFNS [MIM 304110]) in the vast majority (92%) of patients. CFNS is characterized by craniofrontonasal dysplasia and extracranial manifestations in heterozygous females. The EFNB1 gene is located in Xq12 and it is subjected to random X inactivation. Accordingly, the typical craniofacial manifestations in heterozygous females have been attributed to cellular interference of divergent migrating cells, particularly of those derived from the neural crest. Missense mutations constitute 42 % of EFNB1 mutations in CFNS. Nonsense, frameshift and splice site mutations leading to premature termination codons (PTCs) account for 55% of EFNB1 mutations. To assess the effects of various mutations on EFNB1 RNA levels, expression of EFNB1 transcripts was studied by RT-PCR in primary fibroblast cultures established from biopsies of CFNS patients. Missense mutations p.P54L and p.T137A showed about equal amounts of transcript compared to the wt-allele in primary cell cultures exhibiting a random or nearly random X-inactivation. In contrast, severe depletion of transcripts was observed for the alleles harboring either splice site mutation c.407-2A>T at the exon 2/3 boundary or frameshift mutation c.377-384del. Both mutations are predestined to produce internal PTCs. Although splice site mutations at the exon 2/3 junction could cause skipping of exon 3 without changing the reading frame, our results support retention of intron 2. Both of the truncating EFNB1 mutations investigated here satisfy the requirements to trigger decay of the mutant transcripts by the nonsense-mediated mRNA decay pathway. However, stabilization of nonsense mRNAs in the presence of cycloheximide was not observed. This may suggest additional mechanisms such as inefficient splicing and nonsense-mediated transcriptional silencing to be responsible for depletion of PTC containing EFNB1 transcripts.

P215

UBC9-induced SUMOylation of TRPS1 promotes HDAC1-mediated repression

Kaiser F.J.(1), Lüdecke H.-J.(1), Weger S.(2)
1) Universitätsklinikum, Humangenetik, Essen, Germany
2) Freie Universität Berlin, Institut für Virologie, Berlin, Germany

The activity of many transcriptional repressors can be regulated by SUMOylation. We found an interaction of TRPS1, a transcriptional repressor involved in the tricho-rhino-phalangeal-syndromes, with the SUMO-conjugating enzyme UBC9 in yeast two-hybrid- and immunoprecipitation-assays (IPs). In line with these findings, UBC9 induces the modification of TRPS1 with multiple SUMO-1 residues both *in vitro* and *in vivo*. Of the five predicted SUMO-target sites within TRPS1, two are located within the C-terminal repression domain (RD) at amino acids 1191 to 1194 (termed S1) and amino acids 1200 to 1203 (S2), respectively. Point mutation of S2, but not of S1, resulted in the loss of one SUMO-attachment site. Co-expression of wildtype UBC9 increased the repressional activity of TRPS1, whereas addition of the SUMOylation-deficient UBC9 point mutant C93S, which is still able to interact with TRPS1, had no influence on TRPS1 activity. Furthermore, TRPS1 constructs harbouring a point mutation in the S2 SUMOylation-site showed a clearly reduced repression activity.
Based on recent findings, one possible mechanism for SUMOylation mediated transcriptional repression is the recruitment of histone deacetylase (HDAC) activity. An interaction of TRPS1 with HDAC1 could be detected in IPs. Using a luciferase reporter, driven by a synthetic promoter harbouring several Gal4 binding sites, we found a strong enhancement of the repressional activity of the isolated TRPS1 RD fused to a GAL4 DNA-binding domain after HDAC1 overexpression. This effect was abolished when the two SUMOylation sites in the TRPS1 repression domain were mutated. In summary, our data provide insights into the molecular mechanisms of TRPS1-mediated gene repression, which seems to involve the SUMOylation dependent recruitment of factors promoting transcriptional repression.

P216

MODY (Maturity Onset Diabetes of the Young): Novel mutations in the glucokinase-, hepatic nuclear factor 1α- and hepatic nuclear factor 4α-gene

Clausmeyer S.(1), Schwab K.O.(2), Krause A.(2), Rondot S.(1), Hentze S.(1), Schulze E.(1), Frank-Raue K.(1), Raue F.(1)
1) Endokrinologisch-Humangenetische Gemeinschaftspraxis, Heidelberg, Germany
2) Zentrum für Kinderheilkunde und Jugendmedizin des Universitätsklinikums, Freiburg, Germany

In Europe and the United States the different forms of MODY account for 2-5% of non insulin dependent diabetes. The most common forms are MODY 2 and MODY 3, caused by mutations in the glycolytic enzyme glucokinase (GCK) and the transcription factor hepatic nuclear factor 1α (HNF1α), respectively. Mutations in four other transcription factor genes have been identified so far, resulting in clinically heterogenous phenotypes: hepatic nuclear factor 4α (HNF4α, MODY 1), insulin promoter factor-1 (IPF-1, MODY 4), HNF1ß (MODY 5) and NeuroD1/ß2 (MODY 6). MODY is characterized by an autosomal dominant mode of inheritance, early onset usually before the age of 25 years, and the absence of obesity.
254 patients were screened for mutations in the GCK (MODY 2) and HNF1α (MODY 3) gene by direct sequencing of PCR amplified genomic DNA. In 78 (30.4%) of these patients 50 different mutations associated with either MODY 2 or MODY 3 were detected, 24 of which were novel. In 57 (73.1%) of these 78 individuals a mutation in the GCK gene was found and in 19 (24.4%) patients a mutation in the HNF1α gene was detected. In addition, two patients were identified carrying novel mutations in the HNF4α gene. Therefore, our results confirm the reported high frequency of novel mutations in the MODY genes. The mutations found include missense, nonsense, splice site and frameshift mutations. Analysis of the pedigrees with novel mutations revealed a cosegregation of the respective mutation with the diabetic phenotype in two or more consecutive generations.
In conclusion, molecular genetic testing for discrimination between MODY and other forms of type 2 diabetes is of great importance, since the diagnosis of MODY has implications for the clinical management of the index patient and affected family members. The detection of mutations in about 30% of our patients suggests a high prevalence of MODY which may be an underestimated disorder.

P217

Familial hypercholesterolemia due to mutations of the LDLR gene

Haag C.(1), Bahr M.(2), Clausmeyer S.(1), Hentze S.(1), Schulze E.(1), Raue F.(1)
1) Endokrinologisch-Humangenetische Gemeinschaftspraxis, Heidelberg, Germany
2) Medizinische Hochschule Hannover, Abt. Gastroenterologie, Hepatologie und Endokrinologie, Hannover, Germany

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in the low-density lipoprotein receptor gene (LDLR), affecting approximately 1 in 500 individuals in western countries. FH is characterized by high concentrations of LDL and patients have a

100-fold increased risk to die from coronary artery disease (CAD) before the age of 40 years. Rare homozygous (1:1.000.000) or compound heterozygous patients present manifestations of CAD before the age of 20 years. So far, more than 900 mutations have been reported for the LDLR gene.
We screened seven unrelated patients at the age of 19 to 44 years with a clinical diagnosis of FH for mutations in the LDL-receptor gene. Genomic DNA was extracted from peripheral blood lymphocytes and the promoter and the 18 exons of the LDL-receptor gene were amplified by PCR. PCR products were sequenced directly. Large rearrangements like deletions or insertions of various sizes were analyzed by MLPA. By sequence analysis of the LDL-receptor gene we identified three patients with a heterozygous point mutation (IVS7 -2 A>G, codon 146 TGC>TGA, IVS14 +5 G>A) and one patient with three combined point mutations (codon 29 GCT>TCT, codon 461 GAC>AAC, codon 740 ACG>ATG). MLPA analysis revealed two patients with a deletion of a single exon (exon 1 and exon 16, respectively) and one patient with a duplication of the exons 4-8. Familial hypercholesterolemia is one of the most common single gene determinants of severe cardiovascular disease. Early prevention and treatment can significantly reduce cardiovascular morbidity and mortality. However, there is a large number of undiagnosed FH patients. The detection of mutations in index patients is prerequisite for the identification and treatment of family members.

P218

Mutational analysis of the PHEX gene in familial and sporadic cases of X-linked hypophosphatemic rickets

Clausmeyer S.(1), Clemens P.C.(2), Hentze S.(1), Schulze E.(1), Raue F.(1)
1) Endokrinologisch-Humangenetische Gemeinschaftspraxis, Heidelberg, Germany
2) Helios Klinik, Kinderklinik, Schwerin, Germany

X-linked hypophosphatemia (XLH) is a dominant inherited bone disorder, characterized by renal phosphate wasting, inappropriately normal to low vitamin D serum levels and severe skeletal and dental defects from early childhood. Inactivating mutations in the PHEX gene (phosphate regulating gene with homologies to endopeptidases on the X-chromosome) have been identified as the underlying cause, although the pathomechanism is unknown. The PHEX gene encodes a membrane-bound metalloprotease that is expressed mainly in bone and teeth, but not in the kidney. We screened 31 patients with suspected XLH by direct sequencing of PCR amplified genomic DNA. In 17 patients (55%) 14 different inactivating mutations in the PHEX gene were identified. Several types of mutations were detected, including missense, nonsense, splice site and frameshift mutations. In one family a deletion of exon 22 was found. Twelve of these mutations are novel mutations. Among our patients there were four families with hypophosphatemia with pedigrees of two or more consecutive generations. Most cases, however, are sporadic. In one of the families, hypophosphatemic rickets was found in four generations. The two year old index patient showed deformities of the lower extremities and low serum phosphate levels which normalized under therapy (phosphate substitution, Vitamin D). Her mother underwent surgical corrections of the lower extremities at the age of 13, while the grandmother showed a less severe phenotype. From the grandmother's father severe deformities with a complete loss of mobility are reported. Molecular genetic testing revealed two novel missense mutations in the index patient: the substitution of cysteine 59 by serine in exon 2 and the replacement of alanine 363 by valine in exon 10. In conclusion, early confirmation of the diagnosis of XLH by molecular genetic testing has considerable implications for the early onset of therapy of the patient and affected family members.

P219

Multiple endocrine neoplasia type 2 (MEN 2) caused by germline mutations in the RET-protooncogene

Rondot S.(1), Frank-Raue K.(1), Hentze S.(1), Schulze E.(1), Raue F.(1)
1) Endokrinologisch-Humangenetische Gemeinschaftspraxis, Heidelberg, Germany

Multiple endocrine neoplasia type 2A and 2B (MEN 2A, MEN 2B) and familial medullary thyroid carcinoma (FMTC) are autosomal dominant inherited diseases, that are caused by mutations in the RET-protooncogene. Mutations in exons 10 and 11 lead mainly to the classical MEN 2A phenotype, mutations in exon 16 to MEN 2B, whereas mutations in exons 13 to 15 are typical for FMTC. The MTC in the different cancer syndromes shows variability regarding age of manifestation, development and agressiveness.
Methods: The exons 8, 10, 11, and 13-16 of the RET-protooncogene were sequenced after amplification of genomic DNA by PCR.
Results: In 162 patients mutations in the RET-protooncogene were detected. The most frequent mutation, codon 11, exon 634, was identified in 66 patients (40.7%). In 62 (38.3%) patients mutations in exons 13-15 were identified: exon 13 (codon 768, n=2; codon 790, n=7; codon 791, n=10), exon 14 (codon 804, n=28) und exon 15 (codon 891, n=9). Additionally we found mutations whose influence on the development of a MTC is suspected. A mutation of codon 649 was identified in two families. In both two generation families (5 and 3 patients) the index patient displayed a MTC, one family member had pathologic calcitonin levels. One patient with MTC displayed two different mutations (codons 649 and 804). A mutation in exon 11, codon 632, was documented in a single patient. There was one patient carrying a mutation in both the MEN-1-gene and in the RET-protooncogene (codon 791).
Conclusion: In our patients we found a striking accumulation of mutations in exons 13 to 15. This may be due the analysis of all patients of our practice with MTC for mutations in the RET-protooncogene, also patients with clinical not hereditary MTC. Therefore it is recommend to sequence the RET-protooncogene in all patients with a MTC (exons 8, 10, 11 and 13-16) even when no familial involvement is known.

P220

Towards understanding the pathomechanism of Stüve-Wiedemann syndrome

Bliedtner A.(1), Spranger S.(2), Tinschert S.(1), Rump A.(1)
1) Institut für Klinische Genetik, TU Dresden, Dresden, Germany
2) Praxis für Humangenetik, Bremen, Germany

Stüve-Wiedemann syndrome (SWS) is a severe autosomal recessive condition (OMIM 601559) characterized by bowing of the long bones, camptodactyly, respiratory distress, feeding difficulties and hyperthermic episodes responsible for early lethality. SWS is caused by null mutations in the leukemia inhibitory factor receptor (LIFR) gene, which maps to chromosome 5p13.1 and consists of 19 coding exons. The LIFR protein belongs to the family of hematopoietic cytokine receptors.
SWS is a very rare disorder. Thus, information concerning the pathomechanism in this disease is marginal and many open questions still remain:
(1) How do the null-mutations, observed on gene level, affect transcription and translation?
(2) Is the aberrant mRNA produced at all?
(3) Does it lead to truncated versions of the receptor, as predicted from the genomic mutation?
And most important:
(4) Why do some individuals with verified mutations in both LIFR-alleles survive whereas most patients die within the first two years of life?
In order to elucidate at least some of these questions, we studied LIFR-expression in cultivated fibroblasts and lymphoblastoid cells from a 12-year-old girl, who is one of the very rare survivors of SWS. The girl has non-consanguineous Caucasian parents and we have identified two different frame-shift-mutations in her LIFR-alleles. The maternal allele carries a deletion of 4 bp, which eliminates two coding nucleotides as well as the adjacent splice donor at the 5'-end of exon four. The paternal allele carries a 7 bp-deletion within exon 17, which is also likely to produce a frameshift during translation. Our data on cDNA level indicate that the patient is able to activate cryptic intra-exonic splice sites in such a way that the maternal frame shift mutation is spliced out and the resulting mRNA carries an in-frame deletion, which may be able to encode a functional or semi-functional LIFR-protein that lacks 31 amino acids within a region of yet unclear function.

P221

A mutation creating a functional upstream initiation codon in the *SOX9* 5´ UTR causes campomelic dysplasia

von Bohlen A.E.(1), Böhm J.(1), Johnson D.(2), Tolmie J.(2), Kohlhase J.(1), Morris-Rosendahl D.(1), Scherer G.(1)
1) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
2) Institute of Medical Genetics, Yorkhill NHS Trust, Glasgow, United Kingdom

Campomelic dysplasia (CD) is a semilethal skeletal malformation syndrome that results from *de novo* heterozygous mutations in *SOX9*. These mutations are distributed over the entire coding region and cause loss-of-function of the protein, resulting in haploinsufficiency. We report on a 4

year-old girl with a 46,XX karyotype who has clinical and radiological features of surviving CD, including micrognathia, tracheomalacia, small scapulae, 11 pairs of ribs and short ischia. Sequence analysis of the *SOX9* coding region failed to reveal a mutation. However, a heterozygous mutation G>A at position 188 in the 5´ UTR was found. The patient´s mother, father and healthy brother were homozygous G/G at this position, as were 100 control chromosomes. Paternity was confirmed by microsatellite marker analysis. The G>A mutation creates an upstream translation start codon (GUG>AUG) with a much better fit of its flanking sequence to the Kozak consensus than that at the wildtype AUG start codon (4/6 vs. 1/6). The reading frame starting at the upstream AUG terminates just after the wildtype AUG start codon and codes for a short peptide of 62 amino acids. That the upstream AUG functions as an efficient translation initiation codon that drastically decreases translation from the wildtype AUG was shown by both a coupled *in vitro* transcription / translation system and by transient transfection of expression constructs in COS-7 cells followed by immunoblotting. Reduced amounts of wildtype SOX9 protein from the mutant mRNA are compatible with the milder phenotype of the patient. Sequence analysis of the 5´ UTR in 30 CD or CD-like cases without a *SOX9* coding region mutation failed to uncover another case with a mutant upstream AUG. Although described only rarely in human genetic disease and for the first time here for CD, mutations creating upstream AUG codons may be more common than generally assumed.

P222

Specific promoters drive sense/antisense transcription of variant 3 of DYT3/TAF1, the transcription unit altered in XDP.

Herzfeld T.(1), Kreß J.(1), Nolte D.(1), Müller U.(1)

1) Institut für Humangenetik, Gießen, Germany

X-linked dystonia-parkinsonism (XDP) is associated with disease-specific sequence changes (DSCs) within the transcript system DYT3/TAF1. There are four major alternative transcripts of DYT3/TAF1. Two include portions of TAF1 (variants 1,2), and two utilize exons of an entirely novel coding unit (variants 3,4). Exon 4 is utilized by all four major variants of DYT3/TAF1. We investigated regulation of expression of variant 3 that is composed of exons 2,3,4. We show that variant 3 is a sense/antisense transcription unit (TU). In silico analysis of the 5´region of exon 2 (the first exon of variant 3) revealed multiple putative sites for human transcription factors including Sp1, HIF-1, MEF-2, Ikaros, and c-Ets-2. Cloning of various fragments upstream of exon 2 of 1644bp, 639bp, 253bp, and 137bp, resp. in pGL3/luciferase and expression in U87 and NT2/D1 cells demonstrated activation of luciferase using the 1644bp and 639 bp constructs. The smaller fragments did not result in luciferase activation.

Coexpression of ikaros isoform 2 in cells containing either the 1644bp or the 639bp insert demonstrated promoter suppression. Other tested elements such as HIF-1 and MEF-2c did not affect this promoter. The antisense LTR promoter (3´of exon 4) is a member of the HERV9 family and is included in a 521bp fragment that was functional in the luciferase assay. We are currently exploring the possibility of reciprocal regulation of both promoters.

P223

Molecular dissection of inherited pterygium corneae in a Costa Rican family

Chavarría G.(1), Bergua A.(2), Rautenstrauss B.(1)

1) Institute of Human Genetics, Erlangen, Germany

2) Friedrich-Alexander University, Ophthalmologic Department, Erlangen, Germany

The pterygium corneae is a potentially vision-threatening lesion of unknown etiology. It has been related to unprotected exposure to sunlight, especially ultraviolet rays. Hereditary forms follow an autosomal dominant inheritance with estimated 70% penetrance. Increased matrix metalloproteinase 1 and vascular endothelial growth factor, as well as decreased pigment epithelium-derived factor have been associated with pterygium corneae. Furthermore mutations in Ki-ras and accumulation of P53 have been found in pterygium-derived cDNA samples. Association studies revealed an increased risk for carriers of a glutathione S-transferase M1 null type polymorphism. We performed a genome-wide linkage analysis (Affymetrix 10K SNP chip) for a Costa Rican family. The family has 12 affected individuals in two generations with an autosomal dominant pattern of inheritance. Most of the affected have indoor-professions without extended solar exposure. We obtained a LOD score suggestive of linkage at 2,36 (NPL score 9,18) on chromosome 9p21.1-22.3. Segregation analysis defined an interval of approximately 11,7Mb. In concordance with incomplete penetrance 4 family members with the candidate affected haplotype did not yet develop a pterygium corneae. One phenocopy due to a childhood accident is probably present in the family. The majority of genes in the critical interval belongs to inflammatory pathways. 12 genes (LEDGF, RPS6, SH3GL2, MLLT3, ELAVL2, ADAMTSL1, ADFP, CDKN2a, SLC24A2, SNAPC3, ZDHHC21, RRAGA) from a total of 78 in the candidate interval were selected for sequence analysis based on expression in the cornea. The analysis resulted in multiple variations with benign nature. Additionally the GSTM1 null type polymorphism was homozygous in 4 patients. Currently a synergistic effect of several variations cannot be ruled out, a potential association with each variant has to be verified in a large cohort of patients.

P224

Getting the proto-Pax by the tail

Vorobyov E.(1), Horst J.(1)

1) Universitätsklinikum Münster, Institut für Humangenetik, Münster, Germany

Pax genes encode transcription factors governing the determination of different cell types and even organs in the development of multicellular animals. Pax proteins are characterized by the presence of three evolutionarily conserved elements: two DNA-binding domains, the paired domain (PD) and paired-type homeodomain (PtHD), and the short octopeptide sequence (OP) located between PD and PtHD. PD is the defining feature of this class of genes, while OP and/or PtHD may be divergent or absent in some members of the family. Phylogenetic analyses of the PD and PtHD sequences do not distinguish which particular type of the extant Pax genes more resembles the ancestral type. Using experimental and computational approaches we analysed complete repertoire of the paired-type homeodomain genes in genomes of the nematode, fruit fly, tunicate, fish, mouse and human. We present evidence for the existence of a fourth evolutionarily conserved domain in the Pax proteins, the Paired-type Homeodomain Tail (PHT). Based on structural considerations, we propose a scenario for the evolutionary emergence of the proto-Pax gene. Our data imply that the hypothetical proto-Pax protein most probably exhibited a complex structure, PD-OP-PtHD-PHT, which has been retained in the extant proteins Pax3/7 of the ascidia and lancelet, and Pax7 of the vertebrates.

P225

Htra2-ß1 – a splicing modulator of SMN2 transcripts: functional studies in transgenic and knock out mice

Mende Y.(1), Hofmann Y.(2), Anadol E.(2), Jablonka S.(3), Sendtner M.(3), Wirth B.(1)

1) Institute of Human Genetics and Center of Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

2) Institute of Human Genetics, Bonn, Germany

3) Institute for Clinical Neurobiology, University of Würzburg, Würzburg, Germany

Spinal muscular atrophy is an autosomal recessive disorder caused by homozygous loss of the survival motor neuron gene 1 (SMN1). The loss of the gene leads to degeneration of α- motor neurons in the spinal cord resulting in a progressive weakness of limbs and trunk. Patients retain at least one of a nearly identical copy of the gene – SMN2 – which differs to SMN1 only in 5 bp. Due to alternative splicing most of the transcripts generated from SMN2 lack exon 7, resulting in SMN protein deficiency. The remaining correctly spliced transcripts are not successful to compensate the loss of SMN1 in patients. We have already shown that the SR-like splicing factor Htra2-ß1 restores the correct splicing of SMN2 exon 7 in vitro. The protein is therefore a promising candidate for an in vivo modulation of SMN RNA processing to serve as a therapeutic tool to prevent SMA. Compared to humans, mice possess only one Smn gene whose loss is embryonic lethal. Transgenic Smn-/- mice carrying the human SMN2 show an SMA-like phenotype, whereas the phenotypic severity like in human is correlating with the SMN2 copy number. We have generated mice overexpressing the Htra2-ß1 neuronal specific. Overexpression of Htra2-ß1 was confirmed via immunostaining of cultured motor neuron cells.The transgenic Htra2-ß1 mice are phenotypically normal. Our final goal is to create Smn-/-, SMN2+, Htra2-ß1+ animals to investigate if Htra2-ß1 shows a rescue effect on the SMA phenotype in vivo. Further we have developed a knock-out strategy for the murine homologue Sig41 via the Cre/loxP system to investigate the function of the Tra2-ß1 in mammalians. By using PCR-strategy we have previously excluded splice variants of the mSig41 in all tissues of interest. We have already succeeded in ES cell transfection and generation of chimeric mice. Since reduced SMN protein level also leads to reduced Htra2-ß1 protein

level, the construction of Tra2-ß1 knock-out mice is of particular interest.

P226

Secondary effects of fibrillin-1 fragments and fragmentation of the aortic wall in Marfan syndrome

Robinson P.N.(1), Booms P.(1), Guo G.(1), Ney A.(1), Halushka M.(2), Dietz H.C.(3), Mundlos S.(1)
1) Universitätsklinikum Charite, Institut für Medizinische Genetik, Berlin, Germany
2) Johns Hopkins University, Department of Pathology, Baltimore, United States of America
3) Johns Hopkins University, Institute of Genetic Medicine, Baltimore, United States of America

Mutations in the gene for fibrillin-1 cause the Marfan syndrome (MFS). Proteolysis, increased concentrations of matrix metalloproteinases (MMP), and tissue fragmentation are thought to contribute to the complex pathogenesis of MFS. In the present work, we have followed two strategies to investigate a possible role of fibrillin-1 fragments and fragmentation of the aortic wall in the pathogenesis of MFS.

Fragments of ECM proteins such as fibronectin can increase the expression and production of certain MMPs. Therefore, we constructed a recombinant fibrillin-1 fragment that contains the single RGD motif of fibrillin-1, and incubated dermal fibroblasts for 48 h with the fragment. Quantitative RT-PCR and Western blotting demonstrated a statistically significant increase in expression and production of MMP-1 and MMP-3.

Tropoelastin contains multiple motifs that conform to the consensus GxxPG and can influence the expression of certain MMPs and act as a chemotactic stimulus by binding to the elastin-binding protein (EBP). Fibrillin-1 contains three such GxxPG sequences. Using experiments similar to above and a recombinant fibrillin fragment containing one of the GxxPG sequences and a similar , we showed a statistically significant increase in MMP-1. A mutation in the GxxPG sequence abolished the effect.

To investigate the role of tissue fragmentation, we analyzed the effects of aortic samples from the mgR mouse model for MFS on the chemotactic activity of macrophages using a modified Boyden chamber. mgR/mgR extracts and the GxxPG fibrillin fragment both significantly increased chemotaxis. Additionally, aortic surgical samples of 28 MFS patients were investigated with CD68 immune staining and showed a significant increase in macrophage infiltration.

Our results suggest that secondary effects following tissue fragmentation such as inflammation and increased MMP activity could be a component in the complex pathogenesis of MFS.

P227

Recombinant expression of vitamin K-dependent coagulation-factors

Fregin A.(1), Rost S.(1), Oldenburg J.(2), Müller C.R.(1)
1) Institut für Humangenetik, Molekulargenetik, Universität Wuerzburg, Germany
2) Institut für experimentelle Haematologie und Transfusionsmedizin, Universität Bonn, Germany

Vitamin K serves as a cofactor for the post-translational modification by gamma-carboxylation of several proteins with regulatory function, the group of Gla-proteins. Vitamin K-dependent proteins include the coagulation factors II, VII, IX, and X and proteins S, C and Z, illustrating the therapeutic importance of vitamin K-metabolism. Inhibitors of the coumarin-type like warfarin reduce coagulation activity by interfering with the vitamin K 2,3-epoxide reductase enzyme complex (VKOR). Mutations in the VKORC1 (VKOR-component 1) -gene lead to combined deficiency of vitamin-K-dependent clotting factors type 2 (VKCFD2; OMIM: 607473) or warfarin resistance. The production of vitamin K-dependent coagulation factors like factor IX which is being produced for treating Haemophilia B is challenging, because a significant amount of the recombinantly expressed protein is undercarboxylated. The supernatant of HEK-293-Cells which recombinantly express hFIX-cDNA driven by a CMV-promotor has an FIX-activity of 28% compared to normal blood-plasma. Interestingly when comparing the antigen concentration by Western blot-analysis the supernatant shows a much stronger signal than plasma. These data indicate that the recombinant FIX is insufficiently gamma-carboxylated. Since VKORC1 is the essential key-protein for gamma-carboxylation, we are trying to coexpress the coagulation factors II, VII, IX or X in combination with VKORC1 to improve carboxylation and thereby enhance the functionality of the expressed proteins.

P228

Human fragile X chromosomes in murine STO cells: Partial demethylation of previously fully methylated full mutation alleles.

Steinbach D.(1), Lindenthal H.(1), Patzke A.(1), Steinbach P.(1)
1) University of Ulm, Department of Human Genetics, Ulm, Germany

Full expansion of the CGG repeat in the 5'-NTR of the FMR1 gene is the major genetic cause of fragile X syndrome. By yet unknown mechanisms, full expansion leads to hypermethylation and mitotic stabilization, as well as chromatin remodelling and transcriptional suppression. Such full mutation alleles become demethylated, mitotically destabilized, and transcriptionally reactivated when introduced into murine embryonic carcinoma (EC) cells (Woehrle et al. 2002). EC hybrids gave valuable insights into the behaviours of full mutations in undifferentiated embryonic cells but were difficult to handle because of karyotype instability. Murine STO cells derived from the SIM line of embryonic fibroblasts are used as feeder cells in embryonic stem cell cultures. Fibroblasts of a fragile X patient (ML) harbouring a fully methylated full mutation allele without somatic or methylation mosaicism were fused to HPRT-deficient STO cells (ATCC CRL-1503). Hybrid clones were isolated after dilution plating in selection medium (HAT) and analysed on EcoRI plus EagI Southern blots. Thereby we identified exceptional clones, some with a small proportion of the full expansions demethylated, and others harbouring mitotically destabilized full mutations. The latter clones underwent two rounds of subcloning to test for the maintenance of the unstable phenotype, which apparently was not the case. However, subcloning of previously unstable alleles resulted for the first time in new cell lines with fully demethylated full mutation alleles of fragile X syndrome isolated in a murine background with a stable karyotype. Demethylation, therefore, only occurred in the background of the murine STO cells. The methylation status of the CpG dinucleotides in the promoter of the human FMR1 gene and its transcriptional activity is currently investigated in the new cell lines that should become valuable tools to explore reactivation and the fate of the fully expanded CGG repeat in the FMR1 mRNA.

P229

Molecular diagnostics in CHARGE syndrome

Pauli S.(1), Zoll B.(1), Wehner L.-E.(1)
1) Institut für Humangenetik, Göttingen, Germany

CHARGE syndrome is an autosomal dominant disorder consisting of coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, ear anomalies and deafness often in combination with hypoplasia of the semicircular canal. The clinical spectrum shows a high variability. Cleft lip/palate and tracheoesophageal fistula are further commonly associated anomalies. Since the discovery of CHD7 mutations as the underlying cause of the CHARGE syndrome, a molecular analysis in these patients is possible. In our study we describe the genetic background in three patients with clinical features of the CHARGE syndrome. In patient one, who presents with atresia of the choanae, deafness and genital hypoplasia, we detected the new missense mutation R1620G. In patient two we found the known stop mutation R157X and in patient 3 we detected the new stop mutation R947X. Patients 2 and 3 show a severe phenotype. These cases lead to the hypothesis that patients with stop mutations show a more severe phenotype than those with missense mutations. Further studies are needed to confirm this hypothesis. In summary, genotyping of individuals with suspected CHARGE syndrome, especially patients with atypical features, might broaden the clinical spectrum. So far, a genotype-phenotype correlation could not be detected, but there might be evidence that less severe mutations lead to a less severe phenotype.

P230

SALL1 mutations in sporadic Townes-Brocks syndrome are of predominantly paternal orgin without obvious paternal age effect

Böhm J.(1), Munk-Schulenburg S.(1), Felscher S.(1), Kohlhase J.(1)
1) Institute of Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany

Autosomal dominant Townes-Brocks syndrome (TBS) is characterized by imperforate anus, triphalangeal and supernumerary thumbs, dysplastic ears and sensorineural hearing loss, but may also involve other organ systems. Strong inter- and intrafamiliar variability is known. Approximately 50 % of TBS cases are sporadic and due to de novo mutations in the SALL1 gene. SALL1 encodes a zinc finger protein operating as a transcriptional repressor and localising to pericentromeric heterochromatin. We traced the parental origin of SALL1 mutations in sporadic TBS by analysis of linkage between SALL1 mutations and exonic or intronic polymorphisms in 16 families with 9 different mutations. Mutations were of paternal origin in 14 of 16 cases (88 %). Paternal origin was independent of the mutation type, nonsense mutation or short deletions/ insertions. The mean paternal age at conception was 30.1 years. We conclude that de novo mutations in SALL1 mostly occur on the paternally derived chromosome 16 without an obvious age effect.

P231

Townes-Brocks syndrome: 25 novel mutations in sporadic and familial cases and update on the mutational spectrum

Botzenhart E.(1), Rösler B.(1), Kohlhase J.(1)
1) Institut für Humangenetik, Freiburg, Germany

Townes-Brocks syndrome (TBS; OMIM#104780) is a rare autosomal dominantly inherited malformation syndrome due to mutations in SALL1, a human gene related to the developmental regulator SAL of Drosophila melanogaster. The clinical presentation of TBS is highly variable. It is predominantly characterized by renal, anal, ear and limb anomalies. Different types of hearing loss, urogenital malformations cardiac malformations, and mental retardation may additionally occur. The SALL1 gene is coding for a zinc finger protein thought to act as a transcriptional repressor. It is composed of four highly conserved evenly distributed double zinc finger domains and a C2HC motif at the amino terminus. A single C2H2 motif is attached to the second double zinc finger domain. Initially SALL1 mutations have been postulated to cause TBS by haploinsufficiency. A null allele of mouse SALL1 however does not mimick the human syndrome. Another mouse model, mimicking a typical TBS causing mutation showed that TBS mutations lead to the formation of truncated proteins with dominant negative effect in mice thereby causing the disease. Further research into the molecular mechanisms of these mutations will however be necessary to demonstrate the functional consequences of SALL1 mutations in humans. To date 23 SALL1 mutations leading to TBS have been described. Nearly all of these with the exception of R276X occur only in single patients or families thereby preventing phenotype-genotype correlations. Here we present 25 novel mutations associated with TBS in 26 unrelated families increasing the number of SALL1 mutations to 48.

P232

Role of (epi)mutations in 11p15 in Silver-Russell syndrome-like patients and related growth retardation phenotypes

Schönherr N.(1), Meyer E.(1), Eggermann K.(1), Wollmann H.A.(2), Eggermann T.(1)
1) University Hospital, Institute of Human Genetics, Aachen, Germany
2) University Children´s Hospital, Tübingen, Germany

(Epi)mutations affecting chromosome 11p15 are well known to be associated with growth disturbances. The finding of 11p15 mutations in the overgrowth disease Beckwith-Wiedemann syndrome (BWS) lead to the identification of paternally expressed growth-promoting genes and of maternally expressed growth-suppressing genes. Recently, the opposite (epi)mutations of the same region resulting in growth retardation have been reported: hypomethylation of the telomeric 11p15 imprinting domain (ICR1) as well as maternal duplications of 11p15 could be identified in more than 30% of patients with Silver-Russell syndrome (SRS). SRS is a heterogenous and mostly sporadic syndrome characterised by intrauterine and postnatal growth retardation (IUGR/PNGR). Further major features include a triangular face, clinodactyly V, relative macrocephaly and asymmetry. Apart from the aforementioned 11p15 disturbances, a maternal uniparental disomy 7 can be detected in 7-10% of patients. In general, SRS and BWS may be regarded as opposite genetic disturbances of the same chromosomal region.
Here we further elucidated the significance of mutations in 11p15 for SRS and other phenotypes associated with growth retardation. We screened 125 growth retarded patients for duplications and telomeric epimutations in 11p15. These patients were classified into three groups a) patients with SRS-like features b) patients with growth retardation, congenital anomalies and further dysmorphic features not-consistent with SRS, and c) probands with isolated growth retardation. While in none of these patients duplications of 11p15 material could be observed, epimutations in the telomeric 11p15 imprinting region were detected only in 20% of the SRS-like patients. At the moment, the diagnostic analysis of duplications and epimutations in 11p15 should therefore be restricted to patients with SRS-like features.

P233

The mutation rate but not the mutation pattern changes at a GC-content transition in the primate NF1 gene region: Implications for the evolution of the mammalian genome structure.

Schmegner C.(1), Hoegel J.(1), Pietsch B.(1), Schmoll B.(1), Wieland-Lange S.(1), Vogel W.(1), Assum G.(1)
1) Universität Ulm, Humangenetik, Ulm, Germany

A structural characteristic of the mammalian genome is its composition of sequence stretches with widely differing GC contents, called isochores or GC content domains. The pattern of these domains is associated with a number of functional features and was conserved throughout mammalian evolution. Three mechanisms, regional variation of the mutation pattern, biased gene conversion and selection on the GC content are discussed as possible forces responsible for the evolution and the maintenance of the regional GC-content differences. To further elucidate the influence of the mutation pattern and of the two forces acting postmutationally we analysed the mutation frequency, the mutation pattern and the pattern of interspecies divergences at the primate NF1 gene locus, a region where two different GC-content domains face each other, with a sharp boundary between them. The results demonstrated significant differences between the mutation rates of the GC-rich and the GC-poor sequences and a precise colocalizition of the boundaries between the GC-content domains and the domains with high and low mutation rates. The mutation pattern was found to be the same in both domains, with a higher probability of a GC pair to mutate into an AT pair than vice versa. In consequence the absolute numbers of mutations replacing a GC by an AT pair (GC>AT mutations) and mutations replacing an AT by a GC pair (AT>GC mutations) were almost equal in the GC-poor domain but a significant excess of GC>AT mutations was found in the GC-rich domain. The numbers of GC>AT and AT>GC substitutions observed in interspecies comparisons were compatible with the assumption of equality not only for the GC-poor but also for the GC-rich domain. These results led to the conclusion that the GC content of the GC-poor domain can directly be explained by the mutation pattern. The GC content of the GC-rich domain, however, is the result of the action of either biased gene conversion or selection.

P234

Haplotyping in two Silver-Russell syndrome families consistent with the inheritance of mutations in 11p15

Meyer E.(1), Schönherr N.(1), Korsch E.(2), Wollmann H.A.(3), Eggermann T.(1)
1) University Hospital, Institute of Human Genetics, Aachen, Germany
2) Children´s Hospital, Cologne, Germany
3) University Children´s Hospital, Tübingen, Germany

Silver-Russell syndrome (SRS) is a clinical and genetic heterogeneous growth disorder. Main features are intrauterine and postnatal growth retardation, a relative macrocephaly and a small triangular face. Further characteristic but non-obligatory signs include clinodactyly V, down-turned corners of the mouth and asymmetry of head, limbs and trunk. The majority of SRS cases are sporadic, occasionally a familial occurrence with autosomal dominant, autosomal recessive or X-linked inheritance is reported. Different studies indicate the involvement of chromosome 7 and 11 in the aetiology of SRS: In 7-10% of the patients maternal uniparental disomy of chromosome 7 can be observed. In single cases aberrations of different chromosomes have been described, many rearrangements affect chromosome 7. For chromosome 11 maternal duplications of 11p15 have been discovered in growth retarded children with SRS-like features. In recent examinations an epimutation in the telomeric imprinting region (ICR1) of 11p15 has been detected in at least 35% of SRS patients. We investigated three families with two or three affected individuals for common haplotypes for chromosome 11p15. The families were ascertained as part of our ongoing studies on genetic causes of SRS. Short tandem repeat typing revealed that in two families the affected

members carry the same maternal or paternal chromosome, a finding consistent with inheritance of epimutations such as imprinting switches as proposed for familial cases of Beckwith-Wiedemann syndrome (Bliek et al., 2001). However, an epimutation in ICR1 as well as point mutations in IGF2, H19 and CDKN1C in 11p15 in the two families were excluded. Nevertheless, the finding of same haplotypes in affected individuals in the two families makes the role of mutations or epimutations in 11p15 in the aetiology of SRS conceivable.

P235

Novel *OCRL1* mutations associated with a Dent's-like phenotype

Utsch B.(1), Benz M.(2), Dötsch J.(1), Fründ S.(3), Karle S.(1), Kuwertz-Bröking E.(3), Neb M.(1), Rascher W.(1), Tasic V.(4), Ludwig M.(5)
1) University of Erlangen/Nuremberg, Children's Hospital, Erlangen, Germany
2) Dr. von Haunersches Kinderspital, Kindernephrologie, Munich, Germany
3) KfH, Nierenzentrum für Kinder und Jugendliche, Münster, Germany
4) University Children's Hospital, Dept. of Pediatric Nephrology, Skopje, Macedonia
5) University of Bonn, Department of Clinical Biochemistry, Bonn, Germany

Background: Dent's disease is an X-linked tubulopathy that can be caused by mutations affecting the voltage-gated chloride channel and chloride/proton antiporter, ClC-5. Interestingly, recent studies showed that defects in *OCRL1*, a gene encoding a phosphatidylinositol 4,5-bisphosphate 5-phosphatase and usually found mutated in patients with Lowe syndrome, can also lead to a Dent's-like phenotype.

Methods: 18 male patients from 16 families with a phenotype resembling Dent's disease and without *CLCN5* mutation, were analyzed for defects in the *OCRL1* gene. Dent's disease was assumed according to the presence of LMWP and at least one additional feature (either hypercalciuria, or nephrocalcinosis/nephrolithiasis, or renal insufficiency).

Results: In three families we detected novel *OCRL1* mutations and the mother turned out to be a carrier in two of these cases (T121Nfs122, R476W). The third mutation, I257T, was shown to be arisen de novo. Interestingly, two of these mutants are associated with an increase in serum creatine kinase. Mental retardation was absent in any of these patients and careful ophthalmologic examination did not reveal cataracts, a classical hallmark of Lowe syndrome.

Conclusion: These findings demonstrate that Dent's disease or a Dent's-like phenotype are not only caused by *CLCN5*/*OCRL1* mutations but, in addition, should be attributable to defects in further, so far unknown genes. Diagnostic criteria and the concept of Dent's disease and Lowe syndrome need to be revised.

P236

Expansion of coding region tandem repeats in developmental genes during vertebrate evolution

Kohn M.(1), Goidts V.(1), Kehrer-Sawatzki H.(1)
1) University of Ulm, Department of Human Genetics, Ulm, Germany

Tandem repeat expansions and contractions in developmental genes that regulate morphogenesis are hypothesised to be a major source of phenotypic variation established during evolution. Alterations in repeat tract length are able to modify transcription rates, mRNA processing, protein translation, folding and stability, as well as protein/protein interactions. Divergence of gene-associated tandem repeats might therefore facilitate evolution by providing abundant variation that could enable rapid morphological adaptation. Strong advertences in favour of this hypothesis are provided by the observation of pronounced selection for divergence at repeats in coding regions of developmental genes among different domestic dog breeds. We have investigated the evolutionary history of the repeats of 14 genes (ALX4, DLX2, HLX1, HOXA2, HOXA7, HOXA10, HOXA11, HOXC13, HOXD11, HOXD13, RUNX2, SIX3, SOX9 and ZIC2) in 12 different species, including great apes, an Old world monkey, mouse, rat, opossum, chicken, frog, pufferfish and zebrafish. Notably most of all tandem repeats investigated are absent from the respective genes in the fish species and slowly develop in vertebrates like frog and chicken. A burst in tandem repeat expansion however is observed in the Eutherians (higher mammals). In the primate lineage significant differences in repeat length were not observed.

Structural analyses to identify the functional relevance of the repeat expansions revealed that they frequently encode poly-Ala, poly-Gly or poly-Pro domains. Obviously the corresponding proteins and in particular the repeat encoded domains seem to develop differently in higher mammals compared to cold-blooded vertebrates like fishes. Although detailed further analyses are needed, our findings suggest that the development and the expansion of tandem repeats in genes regulating morphogenesis occurred specifically in the lineage of higher mammals with an associated higher propensity to generate new allelic variants.

P237

MutYH associated Polyposis (MAP) among patients initially diagnosed as FAP

Sutter C.(1), Jung C.(1), Kadmon M.(2)
1) Institut für Humangenetik Heidelberg, Abteilung Humangenetik, Heidelberg, Germany
2) Chirurgische Universitätsklinik Heidelberg, Abteilung Allgemeinchirurgie, Heidelberg, Germany

Introduction: Patients with biallelic mutations in the MUTYH gene are at risk to develop MUTYH associated Polyposis (MAP) and colorectal cancer. MAP patients have been detected among individuals primarily classified as FAP patients but lacking an APC mutation. We screened 13 patients with a differential diagnosis of familial adenomatous polyposis (FAP) lacking a family history or a vertical pattern of inheritance. None of these cases showed an APC germline mutation.

Methods: Exons 1-16 of the MUTYH gene (GenBank U63329.1) were amplified by PCR-Primers covering exons and flanking introns. Sequencing products were separated on an ABI 3100 automated sequencer.

Results: Biallelic MUTYH mutations were detected in 8/13 of the patients (62%). The mutational spectrum comprised homozygosity for the variant Y165C, and compound heterozygosity for the variants Y165C/G382D, Y165C/c.1105 delC, c.1105delC/S69X, Y165C/R227W, G382D/IVS10+3A>C, P391L/P281L. S69X (located in exon 3) is a novel truncating mutation while P281L (exon 10) represents a novel MUTYH unclassified variant.

Conclusion: We have detected MUTYH-carriers in 60% of the patients clinically diagnosed as "FAP" cases. MUTYH-positive patients showed a variety of homozygous or compound heterozygous mutations. Therefore, complete sequence analysis appears mandatory to detect MUTYH variants. Polyp numbers in MUTYH mutation carriers varied from 35 to multiple polyps. Therefore, if APC mutations and vertical pattern of inheritance are absent in patients initially diagnosed as FAP, MAP has to be taken into account.

P238

A novel locus for early-onset dilated cardiomyopathy, diffuse myocardial fibrosis and sudden death on chromosome 10q25-26

Probst S.(1), Sasse-Klaassen S.(1), Ellinor P.(2), Gerull B.(1), Krings G.(3), Hennies H.-C.(1), MacRae C.A.(2), Thierfelder L.(1)
1) Max-Delbrueck Center for Molecular Medicine, Berlin, Germany
2) Massachusetts General Hospital, Boston, MA, United States of America
3) Pediatric Cardiology, Charité, Berlin, Germany

Genetic studies have mapped multiple loci for inherited dilated cardiomyopathy (DCM). However only a limited number of the causal genes have been identified. Family studies have shown clinical overlap between DCM and other inherited cardiomyopathies.

We report on two large families, CM-50 and CM-100, in which early-onset DCM segregates as a simple autosomal dominant trait. This unusal form of DCM is characterized by left ventricular dilatation, prominent diffuse interstitial myocardial fibrosis and sudden death.

Genome-wide linkage analysis in these two families identified a novel locus on chromosome 10q25.3-q26.13. Peak two point LOD scores of 3.39 and 3.16 were obtained with marker D10S1773 in CM-50 and with marker D10S1483 in CM-100, respectively. Haplotype analyses in both families identified a shared critical interval of 14 centimorgans between D10S1237 and D10S1723 corresponding to a physical distance of 9.5 megabases. Subsequent multipoint linkage analyses confirmed this interval and generated a peak LOD score of 8.2. The novel locus for DCM contains 76 known or predicted genes. Some candidate genes encode for proteins involved in cellular signaling.

Conclusions: Our studies confirm the genetic heterogeneity of DCM and describe a specific phenotype of DCM with early onset. The identification of the causative gene in this interval will be an important step in understanding the fundamental mechanisms of heart failure and sudden death.

P239

A novel monoallelically expressed non-coding transcript in the Prader-Willi syndrome region

Buiting K.(1), Nazlican H.(1), Galetzka D.(2), Groß S.(1), Horsthemke B.(1)
1) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany
2) Johann-Gutenberg Universität Mainz, Institut für Humangenetik, Mainz, Germany

Prader-Willi syndrome is a neurogenetic disorder which results from the loss of paternal contribution for a 1.5 Mb imprinted region on the proximal long arm of chromosome 15. This region contains several protein coding genes that are transcribed from the paternal chromosome only (*MKRN3*, *MAGEL2*, *NDN* and *SNURF-SNRPN*). The *SNURF-SNRPN* gene also serves as a host for several snoRNA genes. We have previously identified a testis specific gene, *C15orf2*, which is located between *NDN* and *SNURF-SNRPN* and biallelically expressed in adult testis. Here we report the identification of two novel transcripts, which are located between *NDN* and *C15orf2*. Both transcripts have no coding potential and are subject to alternative splicing. By data base search we found at least five partially duplicated copies in a 700 kb region upstream of *C15orf2*. Similar to *C15orf2*, one of these transcripts is exclusively expressed in human testis. Analysis of an expressed single nucleotide polymorphism (SNP) revealed biallelic expression in this tissue. The second transcript is most abundant in testis, but also expressed at a low level in several other tissues including fetal brain. SNP analysis revealed biallelic expression of this transcript in human testis and kidney, whereas in two independent human fetal brain RNA samples we detected monoallelic expression of three different isoforms, two of which occur in fetal brain only. Methylation analysis of a CpG island located 15 kb upstream of exon 1 showed complete absence of methylation in DNA from human spermatozoa, but the presence of methylated and unmethylated alleles in DNA from human fetal brain. Data base searches revealed that both transcript sequences are conserved in chimpanzee but not in mice. We are currently investigating whether these transcripts harbour small RNA genes.

P240

Novel *CCM1* mutations in familial and sporadic CCM with multiple cavernous angiomas

Gätzner S.(1), Stahl S.(1), Sure U.(2), Bertalanffy H.(2), Kunze E.(3), Felbor U.(1)
1) Department of Human Genetics, University of Würzburg, Germany
2) Department of Neurosurgery, University of Marburg, Germany
3) Department of Neurosurgery, University of Würzburg, Germany

Cerebral cavernous malformations (CCM), also called cavernous angiomas or cavernomas, are biologically active vascular abnormalities. CCMs usually cause recurrent headaches, seizures, and focal neurological deficits during the second to fourth decade of life. The frequency in the general population has been estimated to be 0.5%. 10% to 20% of European cases are hereditary and follow an autosomal dominant mode of inheritance.

DNA sequence analysis of the *CCM1*, *CCM2*, and *CCM3* genes revealed four novel *CCM1* mutations: A mutation in exon 17 was found in a German CCM family (IFCAS-102). In addition to a 4 bp deletion, patients carry the largest thus far published *CCM1* insertion: c.1780-1783delGCACinsTACCTGTTACCAAA (p.A594fsX607). A 4 bp deletion in exon 13 (c.1246_1249delAACA; p.K417fsX435) was detected in a CCM family with three affected siblings (17-25 years) and three asymptomatic children (11-15 years). A three year-old Bosnian girl with a large symptomatic brainstem and multiple supratentorial cavernomas carried a 1 bp insertion in exon 16 of *CCM1* (c.1683_1684insA; p.V562fsX567). MR imaging of her asymptomatic parents revealed multiple lesions in her 27-year-old mother who proved to be a mutation carrier. In a further seemingly sporadic case, a 15 year old girl was found to have a de novo *CCM1* splice site mutation (c.2143-2A>G).
Our results demonstrate that genetic counseling and testing is indicated for both patients with a positive family history and sporadic cases with multiple lesions. The identification of a mutation enables precise genetic testing for relatives. The benefits of genetic testing are twofold: a positive test result in a presymptomatic carrier permits close neuroradiological surveillance and timely neurosurgical intervention; a negative test result relieves the proband of unwarranted anxiety and unnecessary medical supervision.

P241

Origin and evolution of genomic imprinting: molecular studies in monotremes

Waldschmidt M.(1), Grützner F.(2), Tsend-Ayush E.(2), Haaf T.(1), Zechner U.(1)
1) Universitätsklinikum Mainz, Institut für Humangenetik, Mainz, Germany
2) University of Adelaide, School of Molecular and Biomedical Science, Adelaide, Australia

Imprinted genes are expressed from only one allele in a parent-of-origin-specific manner. Current evidence based on the analysis of two genes (*IGF2*, *M6P/IGF2R*) suggests that imprinting evolved in a common ancestor of viviparous mammals after divergence from the the egg-laying monotremes. Up to now, nothing is known about imprinting of other genes in monotremes. To gain new insights into the evolution of imprinting, we isolate monotreme orthologues of representative human and mouse imprinted genes and determine their imprinting status in two monotreme species, Ornithorhynchus anatinus (duckbill platypus) and Tachyglossus aculeatus (echidna). Using cross-species RT-PCRs and RACE-PCRs from platypus and echidna cell lines and tissues, we cloned partial cDNAs, which are are highly homologous to the human and mouse *COPG2* and *PEG1* genes. To analyze the imprinting status of the monotreme orthologues, we measure allelic expression of the two genes by analyzing transcribed SNPs in RT-PCR products from several tissues of heterozygous platypus animals using pyrosequencing. Screening of a platypus BAC library with platypus cDNA probes identified BAC clones containing the platypus *IGF2*, *M6P/IGF2R*, *COPG2* and *PEG1* genes. To test whether asynchronous replication, a hallmark of imprinted genes, is conserved in monotremes, we perform interphase FISH dot assays with these BACs on platypus fibroblast cultures. Up to now, hemizygous replication patterns consistent with asynchronous replication of the two parental alleles during S phase were detected for platypus *PEG1* and *COPG2*. Our studies will contribute to a better understanding of the molecular evolution, mechanisms and function of genomic imprinting and of the phylogenetic distribution of imprinted genes.

P242

Recent advances in genetic testing for phenotypically defined subgroups of arthrogryposis multiplex congenita (AMC)

Heller R.(1), Becker J.(1), Zimmermann K.(1), Fahrig M.(1), Bolz H.(1), Wirth B.(1)
1) Institut für Humangenetik, Klinikum der Universität zu Köln, Köln, Germany

Introduction: Arthrogryposis multiplex congenita (AMC) is a heterogeneous group of congenital neuromuscular diseases that present at birth with multiple flexion and / or extension contractures. Phenotypic heterogeneity is manifest in the distribution of affected joints and presence or absence of associated findings such as facial dysmorphism. Exclusion of known exogenic causes and a positive family history are the strongest indicators of a genetic cause in affected patients.
Method: We focussed our diagnostic efforts on sporadic and autosomal dominant distal arthrogryposis types 1 (DA1), 2a (DA2a, Freeman-Sheldon syndrome) and 2b (DA2b). In a first step, information on phenotype and pedigree was collected by a questionnaire. Where possible, haplotype analysis for the two known loci on chromosome 9p13.1 and 11p15.5 as well as for a recently described third locus on 17p13.1 (communciation at the ASHG 2005 meeting) was performed to guide subsequent mutation analysis.
Results: We present data on mutation analysis and intrafamilial variable expressivity in 7 families with autosomal-dominant distal arthrogryposes. In view of the phenotypic variability for DA1 and DA2b, we conclude that haplotype analysis prior to direct mutation analysis is recommended whenever possible. In contrast to DA1 and DA2b, the phenotype in DA2a is sufficiently specific in order to justify direct mutation analysis in sporadic cases.

P243

Paramyotonia congenita: Clinical and genetic characterisation of a Chilean family

Stiller B.(1), Coria C.(2), Cornejo I.(3), Fuentealba A.(2), Heimbach A.(1), Cid L.P.(3), Ramirez A.(1)
1) University of Cologne, Institute of Human Genetics, Cologne, Germany
2) Herminda Martin de Chillan Hospital, Neurology Department, Chillan, Chile
3) Center of Scientifical Studies (CECS), Valdivia, Chile

Paramyotonia Congenita (PMC, OMIM 168300) is a skeletal muscle disease characterised by a myotonia, increased by cold and paradoxically by excersices. The PMC is inherited as an autosomal dominant trait and is caused by mutations in the gene coding for the alpha subunit of the muscular sodium channel SCN4A.
In an extended Chilean pedigree, affected with PMC, linkage analysis was performed in the lo-

cus harbouring the complete SCN4A gene. In this regard, a 4,5 Mb region between the markers D17S1855 and D17S1874 on chromosome 17q23 was defined. A maximum two-point LOD score of 5,3 was found at è=0 with marker D17S948. The complete genomic structure of SCN4A was delineated and a PCR-based testing strategy on genomic DNA was established. All coding exons and their corresponding intron/exon boundaries were amplified from the index patient. Mutation analysis was made by direct sequencing of each amplicon. Thus, two heterozygous coding polymorphisms were identified in the same allele. The first one is located in exon 11 and the second in exon 22. A perfect cosegregation was found in the family. We could not identify both changes in 300 control chromosomes of Chilean and German origin. The analysis at the protein level showed that both changes produced an aminoacid exchange. A search in the public domain databases revealed that one of them was already described as a causative mutation for a form of PMC called Paramyotonia Permanens. This missense mutation showed in the electrophysiology an effect in the channel inactivation. The second change was not associated with any known form of PMC. Interestingly, the phenotype observed in this family showed some clinical aspects not described in this severe form of PMC, such as malignant hyperthermia due to anaesthetics. This raises the question whether this second polymorphisms/mutation could modify the already described phenotype. Currently, functional studies are undergoing to analyse the effect of this second polymorphism.

P244

Towards characterizing *COH1*: Transcriptional and functional analysis, and mutational spectrum in Cohen syndrome

Seifert W.(1), Holder-Espinasse M.(2), Kühnisch J.(3), Horn D.(3), Hennies H.C.(4)
1) Universität zu Köln, Cologne Center for Genomics, Köln, Germany
2) Hôpital Jeanne de Flandre, Génétique médicale, CHRU, Lille, France
3) Institute of Medical Genetics, Charité, University Medicine of Berlin, Berlin, Germany
4) Cologne Center for Genomics, Universität zu Köln, Köln, Germany

Cohen syndrome is a rare autosomal recessive disorder, clinically highly variable and mainly characterized by mental retardation, postnatal microcephaly, facial dysmorphism, pigmentary retinopathy, myopia, and intermittent neutropenia. The Cohen syndrome phenotype was found to be associated with mutations in the gene COH1 (VPS13B). COH1 maps to chromosome 8q22, and codes for various splice forms. It comprises up to 62 exons, and the longest transcript is 14,093 nt in length with an open reading frame of 4,022 codons. Here we describe molecular and clinical findings in 24 patients with Cohen syndrome, descending from 16 families originating from France, Germany, Poland, Turkey, Scottland, Canadia, and the U.K. We detected 25 different COH1 mutations, 19 of these were novel, including nine nonsense mutations, eight frameshift mutations, four verified splice site mutations, three larger in-frame deletions, and one missense mutation. A consistent genotype/phenotype correlation, however, has not been established so far. Overall, 73 different COH1 mutations have now been reported in association with Cohen syndrome. Most of them are terminating mutations and predicted to result in a null allele, while missense mutations and larger deletions are much less common. Eight coding SNPs found indicate that the COH1 product appears to have some tolerance to such variations. The function of the protein COH1, which is supposed to be involved in vesicle-mediated protein transport, is still unclear. We have therefore embarked on a detailed analysis of COH1 transcript variants by RT-PCR and Northern blot hybridization. Furthermore, we are studying the expression and localization of COH1 in humans with respect to the different splice forms using various antibodies. To further analyse the potential tissue specificity of COH1 and its variants, particularly in different parts of the brain, we are additionally investigating the mouse orthologue, COH1.

P245

Screening for genomic rearrangments in NF1 patients from Germany

Preisler-Adams S.(1), Dworniczak B.(1), Hitschfeld B.(1), von Rüden S.(1), Horst J.(1)
1) Westfälische-Wilhelms-Universität, Institut für Humangenetik, Münster, Germany

Neurofibromatosis type 1 (NF1) is the most common autosomal dominant tumor-predisposing disease, affecting approximately one in 3500 individuals. Clinical features comprise café au lait spots, neurofibromas, plexiform neurofibromas, iris haematomas (Lisch nodules) and optic or chiasma glioma. NF1 is fully penetrant but shows wide intra- and interfamiliar phenotypic variability. Furthermore, clinical diagnosis is hampered by the fact that not all patients – especially at younger age – fulfill the diagnostic critera. Genetic testing is therfore a critical component of diagnosis and has significant impact on clinical management and counselling. Using conventional PCR-based techniqes mutation detction in the NF1 gene does not exceed 60% to 70%. The spectrum of herewith identified pathogenic mutations includes small deletions/insertions and nonsense mutations together with splice defects. Whole gene deletions are assumed to account for 5% of NF1 defect alleles, but a considerable proportion of cases remains unexplained. Using sequence analysis of all coding exons including flanking intron regions we screened 40 patients for mutations in the NF1 gene and identified 23 pathogenic mutations, 12 nonsense, 6 frameshift and 5 splice mutations. Due to the presence of polymorphisms in heterozygous state, whole gene deletions could be excluded in most of the negatively tested patients. Since sequence analysis is not able to detect gross genomic rearrangements, we performed multiplex ligation-dependent probe amplification (MLPA) in order to investigate the proportion of such mutations in the remaining cases. Screening of 17 patients did not reveal any further NF1 defect alleles, suggesting that large deletions or duplications do not constitute a substantial fraction in the mutational spectrum of NF1. However, one has to consider the small number of analyzed patients and – since most of them were children – probably the difficulty of clinical diagnosis in early childhood.

P246

Temporal and spatial variation of mutation rates in the human genome

Sluka S.(1), Schmegner C.(1), Högel J.(1), Vogel W.(1), Assum G.(1)
1) Universität Ulm, Humangenetik, Ulm, Germany

Mutation and recombination are two basic processes bringing about the genetic variability observed between members of a population. Recent experiments showed that mutation and recombination do not occur uniformly within the genome. Especially recombinations seem to be concentrated in hotspots, regions of several kb in length with very high recombination frequencies. Moreover, mutation and recombination do not occur independently from each other and it was postulated that the recombination process itself may be mutagenic. In order to verify this correlation we estimated the mutation rates of two recombination hotspots. One located in the MHC (TAP2) and the other in the betaglobin gene region (HDB). The mutation rates were estimated using the interspecies divergence between human and chimpanzee and by analysing the SNP densities in the human population. For both hotspots the interspecies divergence was found not to be higher than the typical values for the respective regions, demonstrating that the mutation rates in the hotspots were not elevated for a longer period of time. The SNP density in the TAP2 hotspot, however, was found to be very high and could not be explained by the mutation rate typical for the region. For HDB no elevated SNP density was detected. These results demonstrate that at least the TAP2 recombination hotspot is also a hotspot of mutation. The elevation of the SNP density, but not of the interspecies divergence show that, in parallel to the recombination rate, the mutation rate was raised relatively late during human evolution. The normal SNP density in the HDB region point to an even younger age of this hotspot. In this case the elevation of the mutation rate may not yet have let to an elevation of the SNP density detectable in a limited sample of probands. In summary the results show that not only the recombination rate, but also the mutation rate may fluctuate in a regional and temporal differenciated manner in the human genome.

P247

Differences in the variability pattern of two genomic regions result from a bottleneck in the history of the European population.

Assum G.(1), Högel J.(1), Vogel W.(1), Schmegner C.(1)
1) Universität Ulm, Humangenetik, Ulm, Germany

The inference of the demographic history of populations from genetic variability data is not only of academic interest. It also provides background information for the identification of genes which have been under selection and may have played a role in human evolution or in the etiology of human disease. To obtain a clear picture of this background it is necessary to search for common patterns in data obtained from a number of genomic loci. Therefore we analysed two genomic regions, NF1 on chromome 17 and MN1/PITPNB on chromosome 22, which, due to their very low recombination rates, can be regarded as further suitable loci. A combined resequencing and SNP typing project in an Euro-

pean population revealed striking differences in the variability patterns of the two regions, although no signs of selection were present at either locus. At the NF1 gene locus two well separated subgroups of sequences were present with a TMRCA (time to the most recent common ancestor) of 700,000 years for the whole sample and no signs of population growth. Regarding the two subgroups separately the data indicated TMRCAs of 150,000 years and a subsequent population growth. In contrast, at the MN1/PITPNB locus the whole sample presented itself more homogeneous with a TMRCA of 260,000 years and strong signs of a population expansion. The differences between the two groups of sequences established from two genomic loci of the same sample of German probands may be explained by a population bottleneck which occured during the emigration of humans out of Africa. In the case of NF1, sequences belonging to two ancient lineages went through this bottleneck, whereas all MN1/PITPNB sequences represent only one ancient lineage. Taken together our results demonstrate the range of possible outcomes of studies of genetic variability in European populations due only to the effects of genetic drift in the founder population.

P248

High degree of heterozygosity and 14 new alleles in the MBL2 gene

Boldt A.B.W.(1), Tsuneto L.T.(2), Souza I.R.(2), Culpi L.(2), Seibold F.(3), Lang F.(4), Luty A.(1), Grobusch M.P.(1), Kombila M.(5), Dzeing A.(5), Kremsner P.G.(1), Kun J.F.J.(1), Petzl-Erler M.L.(2)

1) Institute of Tropical Medicine, Tübingen, Germany
2) Federal University of Paraná, Curitiba, Brazil
3) University of Bern, Bern, Switzerland
4) Institute of Phisiology I, Tübingen, Germany
5) University of Libreville, Libreville, Gabon

The MBL2 gene codes for the mannose binding lectin, a protein capable of activating the complement system and opsonizing pathogenic microorganisms. Known variants are frequent in various populations, although some were found associated with the susceptibility or increased severity of a number of diseases. The promoter and exon 1 of MBL2 were sequenced in 44 Kaingang, 59 Guarani, 7 Oriental-Brazilians, 105 Germans and 373 Gabonese Africans. The segments were amplified with sequence-specific primers (SSP-PCR) to identify the phase between the variants. 27 single nucleotide substitutions and two deletions were observed. Thirteen from these 28 variants (46%) are novel. Three (10.7%) occur in the coding sequence (two of them in the same codon, in absolute linkage disequilibrium), generating L12G and C25S. Sixteen (51.7%) of the variants are polymorph in at least one of the populations. All genotypic distributions were found to be in Hardy-Weinberg equilibrium. The number of kb/polymorphic site was significantly lower than expected for the average of the chromosome 10, where the MBL2 gene resides (0.07 compared to 2.09, respectively; $P < 0.0001$). The opposite occurs with the nucleotide diversity (0.81% compared to the expected of 0.08%). The observed heterozygosity was also very high in Europeans (75.2%) and Africans (81.9%). The existence of strong linkage disequilibrium among the variants allowed us to group them into 26 alleles ("haplotypes"), 14 previously unknown. There was no observed reduction on MBL concentration in the serum of homozygote and heterozygote individuals for the "new" alleles, excepting the rare allele with the variant C25S. The hypothesis of selective neutrality could be rejected based on the results of Fu & Li's D test for the German population ($D = -1.92$, $p = 0.04$). The action of balancing selection in the past was also evident in the phylogenetic analysis by the presence of two allelic lineages separated by several successive mutational events.

P249

Regulation of corepressor expression by thyroid hormone: Implication for the RTH-syndrome

Heller A.(1), Tenbaum S.(2), Munoz A.(2), Baniahmad A.(1)

1) Institute of Human Genetics and Anthropology, Friedrich-Schiller-University, Jena, Germany
2) Instituto Investigaciones Biomedicas, Medical Faculty, Madrid, Spain

Thyroid hormone is essential for normal neuronal differentiation. Hypothyroidism leads to various degrees of mental retardation and to cretinism. In line with this the syndrome of Resistance to Thyroid Hormone (RTH) is associated with various degrees of mental retardation, attention deficit, deafness and goiter. The molecular basis of the RTH-Syndrome is mostly due to just point mutations that lead to amino acid exchanges of the thyroid hormone receptor β located on chromosome 17 (17q21.3). However, the exact role of the thyroid hormone receptors and the influence of a particular mutation in neuronal differentiation is not fully understood. Interestingly, neuroblastoma cancer cells reduce proliferation and undergo differentiation upon treatment with thyroid hormone. The thyroid hormone receptor associated with corepressors to repress its target genes. Here, we show the role of co-repressors for the expression of neuronal specific genes and their ligand-controlled recruitment to chromatin at thyroid hormone response elements. Interestingly mutant thyroid hormone receptors from patients with RTH-syndrome lack or have reduced ligand-controlled corepressor binding. Furthermore, we show that the expression of the corepressor Alien is regulated by thyroid hormone in brain and implies a negative feed-back mechanism in vivo to attenuate the potent repressive function mediated by thyroid hormone receptors.

P250

Molecular cloning and characterization of a novel member of the LIM domain gene family

Chung B.-D.(1), Chen Y.(2), Kubisch C.(1), Ramirez A.(1), Netzer C.(1)

1) Institut für Humangenetik, Klinikum der Universität zu Köln, Köln, Germany
2) Department of Medicine III, University of Munich, and GSF, Clinical Cooperative Group ´Leukemia´, München, Germany

In the context of a positional cloning project we have cloned and characterized a gene on chromosome 6p21.1 with the provisional annotation C6orf49. Since the gene annotation was based solely on in silico analyses with gene prediction programs, we (1) analyzed the genomic structure and expression pattern of the gene, thereby verifying its existence, (2) cloned the largest open reading frame (ORF) into an eukaryotic expression vector, (3) analyzed the respective protein by western-blotting, (4) determined the intracellular localization by indirect immunofluorescence microscopy, and (5) checked the ability of the protein to affect transcription in a reporter gene assay.

We could demonstrate that the gene comprises five coding exons. Dot Blot and Northern Blot analysis revealed an ubiquitous expression pattern in humans. Strongest signals were detected in pancreas, heart, liver and kidney, which may indicate an important role in these tissues. The protein-coding region of the gene consists of 1038 bp and encodes a 346-amino-acid polypeptide with a predicted and subsequently in our study experimentally confirmed molecular weight of 38 kDa. In silico analysis showed that the C6orf49 protein contains a single PET and two zinc-binding LIM domains. The latter, which are defined by conserved cystein-rich sequences, are modular protein-binding interfaces that are found in numerous eukaryotic proteins. LIM proteins function in diverse biological processes, the unifying themes of which seem to be nuclear control of gene expression and cytoskeletal function. Consistent with these facts, we observed that C6orf49 is predominantly localized in the nucleus and partly in the cytoplasm of transiently transfected COS-7 cells. Furthermore we could show that the protein acts as a transcriptional repressor in a luciferase reporter gene assay.

Our results suggest that C6orf49 shuttles between the cytoplasm and the nucleus and may modulate the activity of DNA-binding transcription factors.

P251

Regulation by cytokines of GDDR (down-regulated in gastric cancer) gene expression

Lubka M.(1), Baus Loncar M.(2), Blin N.(3)

1) Human Genetics, Molecular Genetics, Tübingen, Germany
2) School of Medicine, J.J. Strossmayer University, Department of Medical Biology, Osijek, Croatia
3) Humangenetik, Univ. Tübingen, Molekulare Genetik, Tübingen, Germany

Introduction: Proper function of the gastrointestinal (GI) tract relies on its surface integrity maintained by various factors. Trefoil factor family (TFF) proteins (TFF1, 2 & 3) play a multifunctional role in protecting GI mucosa. The mechanism of TFFs' action as secretory proteins depends on their interaction with cell surface membrane proteins. Recently, a novel TFF1-interacting protein has been identified as a BRICHOS domain containing protein, at first described as GDDR (down regulated in gastric cancer) and later as TFIZ1 (trefoil factor interaction). BRICHOS domain has been reported in several previously unrelated proteins that are linked with major diseases as familial dementia, chondrosarcoma, stomach cancer and respiratory distress syndrome, pointing to the importance of this domain. The aim of our study was to see how the GDDR gene is regulated by different cytokines.

Method: The GDDR promoter (position –1906 to -20) was cloned into the pGL3 basic vector (Promega). Following TNF-α, IL1-β, IL-6 and TGF-β stimulation expression of GDDR gene was monitored in gastrointestinal cell lines HT-29 and KATO III by luciferase reporter gene assay. Down-regulation of GDDR transcription was observed when applying TNF-α, IL1-β and IL-6, while TGF-β caused an up-regulation of this gene. Such a regulatory pattern is also observed for TFF1 where various cytokines and transcription factors cause down- or up-regulation.
Conclusion: GDDR gene expression is regulated by various cytokines in parallel to TFF1 thus allowing an interaction of both proteins. This cytokine-dependent mechanism is suggesting a cross-talk of GDDR/TFF with or in response to the immune system.

P252

Analysis of transmembrane domains in bestrophin and its family members crucial for oligomerisation
Rauscher F.R.(1), Milenkovic V.(2), Lindner E.(3), Langosch D.(3), Weber B.H.F.(1)
1) Institute of Human Genetics, University of Regensburg, Regensburg, Germany
2) Institute of Human Genetics, University of Wuerzburg, Wuerzburg, Germany
3) Biopolymer Chemistry, Technical University of Munich, Freising, Germany
Purpose: The VMD2 gene is mutated in Best vitelliform macular dystrophy (BMD), an early-onset autosomal dominant retinal disorder. VMD2, a member of the TM-RFP protein family, encodes bestrophin and has been suggested to form a Ca2+-dependent chloride channel. Thus far, little is known about subunit assembly although the high sequence identity in the N-terminal halves of the TM-RFP proteins suggests similar functional properties of all family members. With this study, we aim to identify the essential structural properties for channel formation of the TM-RFP proteins and to investigate the pathomechanism of VMD2 mutations associated with BMD.
Methods: The potential for homo-dimerisation of the human VMD2 transmembrane domains (TMD) was tested by the ToxR-two hybrid system (Langosch et al. J Mol Biol. 8;263(4):525-30, 1996). Hypothetical TMD1 to TMD6 were cloned into plasmid ToxR-(TMDX)-MalE, followed by transformation in the E.coli FHK12 reporter strain. Upon dimerisation ToxRactiv binds to the ctx promotor (DNA binding domain from V.cholerae) initiation lacZ transcription. Subsequently, monitoring of quantitative homophilic interactions was achieved by measuring beta-galactosidase activity. In addition to the regular TMD sequences, mutations within the respective TMDs were investigated.
Results: Applying the ToxR-assay, 3 of 6 TMDs of bestrophin were observed to contribute to oligomerisation. These TMDs with high dimerisation potential were further analysed by introducing patient related mutations. In several instances, significant differences between wild type and mutated TMDs were observed.
Conclusions: We have identified TMDs of bestrophin with high dimerisation potential. In addition, selected TMD bestrophin mutations known to occur in BMD reveal significant differences to the normal TMD sequences. This strongly suggests that an impaired oligomerisation of bestrophin may be part of the pathomechanism in this disorder.

P253

Binding properties of retinoschisin, a secreted protein defective in X-linked juvenile retinoschisis (RS)
Loenhardt T.(1), Gehrig A.(2), Weber B.H.F.(1)
1) Humangenetik, Regensburg, Germany
2) Humangenetik, Wuerzburg, Germany
Purpose: X-linked juvenile retinoschisis (RS) is caused by mutations in the RS1 gene which encodes a protein termed retinoschisin. Retinoschisin is a 24 kDa protein that is secreted from photoreceptor and bipolar cells as a large disulfide-linked multisubunit complex. The monomer consists of a leader sequence with a putative signal peptidase cleavage site and a discoidin domain spanning over 80% of the protein. Functional properties of the discoidin domain are not well understood although in some proteins the discoidin domain has been implicated in cell-adhesion and cell signalling processes through protein-protein, protein-carbohydrate, or protein-lipid interactions. In RS, deficiency of retinoschisin has been established as the underlying disease mechanism, however little is known about the molecular events leading to the degenerative manifestation in the neural retina. In this study, we focused on the binding mechanism of retinoschisin to the outer membrane surface.
Methods: Tissues including lung, brain, liver, heart, kidney and retina were isolated from 3-week old Rs1h-deficient mice, homogenised and tested for binding to recombinant RS1. Soluble and membrane bound fractions were assessed by SDS-PAGE separation and Western blot analysis. The binding of recombinant RS1 to artificial phospholipid membranes (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine) and different types of Collagen (I-IV) was analysed by ELISA.
Results: Recombinant RS1 binds strongly to retinal membranes but not to membrane fractions of other tissues indicating that membrane binding of retinoschisin is tissue-specific. Furthermore, our data show that recombinant RS1 fails to bind to artificial phospholipid membranes of various compositions. Finally, no binding of recombinant RS1 to the collagens tested was evident.
Conclusion: Our data suggest that RS1 membrane binding is mediated by a thus far unknown binding partner specific to the retinal outer membrane surface.

P254

Congenital insensitivity to pain with anhidrosis (CIPA) caused by a novel mutation in the NGF receptor NTRK1
Huehne K.(1), Raab K.(2), Zweier C.(1), Rautenstrauss B.(1)
1) Institute of Human Genetics, Erlangen, Germany
2) Cnopf'sche Kinderklinik, Nuremberg, Germany
The hereditary sensory and autonomous neuropathy type IV (HSAN IV, CIPA) is a rare autosomal recessive disorder characterized by absent pain sensitivity, recurrent episodes of high fever and disability of sweating resulting in thermo regulation problems. Further symptoms are mental retardation, hypotonia, inflammatory processes and immune deficiency. CIPA is caused by mutations in the neurotrophic tyrosine receptor kinase 1 (NTRK1) gene which codes for the nerve growth factor (NGF) receptor. Here we report a two year old girl of Turkish origin. Her two brothers, dizygotic twins and diagnosed as HSAN IV, died at the age of 11 and 18 month. Their diagnosis resulted from clinical course and evaluation of N. suralis biopsy of one of the boys. The girl showed developmental psychomotoric delay, selfmutilation, insensitivity to pain and absence of sweating. Sequence analysis of the NTRK1 gene revealed a homozygous mutation c.1715T>G (I572S) in a highly conserved region of the tyrosine kinase domain. The parents are heterozygous carriers of this variation. The exchange from isoleucine to serine replaces an aliphatic hydrophobic residue with a hydrophilic one. The amino acid isoleucine in position 572 is highly evolutionary conserved in paralogous and orthologous genes from various growth factor receptors in human, mouse and chicken. In functionally related proteins isoleucine is sometimes replaced by valine which is also hydrophobic and has a comparable molecular size as isoleucin. Peripheral nerve tissue involvement in CIPA/HSAN IV patients might reflect NGF-mediated dysregulation of control mechanisms leading to a chronic inflammatory disease status and immunological dysfuntion.

P255

Conserved non-coding sequence elements control spatiotemporal expression of GLI3
Paparidis Z.(1), Malik S.(1), Abbasi A.A.(1), deGraaff E.(2), Elgar G.(3), Grzeschik K.-H.(1)
1) Zentrum fuer Humangenetik, Philipps-Universitaet, Marburg, Germany
2) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands
3) MRC Rosalind Franklin Centre for Genomics Research, Genome Campus, Hinxton, Cambridgeshire CB SB, United Kingdom
During development, the products of the GLI gene family translate signals of the hedgehog proteins (HH) into specific patterns of gene expression. Their co-ordinated function appears to determine a GLI-code, which, e.g. in the limb, governs pattern formation in anterior-posterior direction and which is similarly employed to pattern a great variety of other tissues. Malfunction of the signaling is associated with tumourigenesis. Factors controlling the localized and timely expression of GLI genes and their targets are unknown.
We report the identification among highly conserved non-coding sequence blocks of cis-regulatory elements controlling expression of GLI3 and describe their functional analysis.
The predicted transcriptional start of human GLI3 was corrected experimentally. Within the genomic sequence upstream of exon 1 a minimal promoter we identified by deletion analysis in a 300 bp element located 70 bp upstream of the transcription initiation site a minimal promoter region with a high capacity for transcriptional activation of a luciferase reporter gene in cell culture. To assay the involvement of trans-active factors, predicted binding sites within this region were eliminated by mutagenesis. Comparison of the human, mouse and fugu genomic GLI3 se-

quences showed regions of very high conservancy residing in intronic regions. These segments were tested for their potential to regulate luciferase expression in cell culture. Three segments differing in these properties were further analyzed for their ability to control time and localization of beta-galactosidase reporter gene expression in transgenic mouse embryos. Localization and time course of the reporter gene expression recapitulates part of the established GLI3 expression pattern. The detection of sequence elements controlling in cis the expression of GLI3 contributes to the understanding of the pattern formation and addresses the question of highly conserved noncoding DNA sequences in vertebrate genomes.

P256

Production of transgenic pigs expressing α1,2-fucosyltransferase suitable for xenotransplantation

Lipinski D.(1), Jura J.(2), Kalak R.(3), Zeyland J.(3), Michalak E.(3), Kalak R.(3), Plawski A.(1), Smorag Z.(2), Slomski R.(3)
1) Institute of Human Genetics, Polish Academy of Science, Poznan, Poland
2) National Research Institute of Animal Production, Department of Animal Reproduction Biotechnology, Immuno- and Cytogenetics, Balice, Poland
3) Agricultural University, Department of Biochemistry and Biotechnology, Poznan, Poland

The use of animals as a source of organs and tissues for xenotransplantation can overcome the growing shortage of human organ donors. However, the presence of xenoreactive antibodies in humans directed against swine Gal antigen present on the surface of xenograft donor cells leads to the complement activation and immediate xenograft rejection as a consequence of hyperacute immunological reaction. To prevent hyperacute rejection it is possible to modify swine genome by human gene modifying the set of donor's cell surface proteins. For this purpose genetic construct containing human gene encoding α1,2-fucosyltransferase enzyme (HT, H transferase) under the human cytomegalovirus (CMV-IE) immediate early promoter was prepared. The gene construct was introduced by microinjection into the male pronucleus of fertilized oocyte. As a result of this experiment, the founder male pig was obtained with the transgene mapped to chromosome 14q28. Approximately 35% of the F1 generation demonstrated presence of transgene. The RT-PCR analysis revealed expression of HT gene driven by CMV promoter. The founder and his offspring showed no changes in phenotype and behavior.

P257

Spontaneous testicular neoplasm in TSPY transgenic mice with testicular feminization

Schubert S.(1), Kamino K.(2), Gonzales-Fassrainer D.(1), Schmidtke J.(1)
1) Hannover Medical School, Institute of Human Genetics, Hannover, Germany
2) Hannover Medical School, Institute of Cell and Molecular Pathology, Hannover, Germany

The TSPY gene is conserved in placental mammals and encodes the testis-specific protein, Y encoded. Within the testis, TSPY expression is restricted to premeiotic germ cells and round spermatids, and the topology and timing of TSPY expression indicate that TSPY plays a role in the proliferation of germ cells. TSPY is strongly expressed in gonadal tumour cells of testicular carcinoma in situ, seminoma and gonadoblastoma, and is regarded as a candidate for the gonadoblastoma locus on the Y chromosome, which predisposes dysgenetic gonads of 46,XY sex-reversed females (e.g. males with 46,XY testicular feminization, Tfm) to develop gonadoblastoma. In contrast to the situation in cattle and primates, where TSPY is organized in a moderately repetitive cluster, the situation in rodents is diverse. While tspy is functional in the rat, Mus musculus derived laboratory mice possess a single-copy pseudogene that is unable to generate a functional transcript. We generated a TSPY transgenic mouse line that carries a human TSPY gene in approximately 50 copies on the mouse Y chromosome. In order to elucidate TSPY expression under complete androgen insensitivity and to investigate the role of TSPY in gonadoblastoma, we crossed TSPY transgenic males with Tfm-females heterozygous for the X-linked testicular feminization mutation (Tfm) and thus generated sex-reversed TSPY transgenic Tfm hemizygous males and sex reversed non transgenic Tfm hemizygous mice. Three out of twenty nine TSPY transgenic Tfm males (10,3%) developed testicular Leydig cell tumours, whereas no testicular neoplasms were seen in controls.

P258

Significant differences in the IGVH and BCL6 mutation status in aggressive B-cell lymphomas with and without MYC breakpoints

Pott C.(1), Trautmann H.(1), Harder L.(2), Kneba M.(1), Siebert R.(2), on behalf of the MMML-Network (3)
1) II. Med. Klinik, Universitätsklinikum Schleswig-Holstein, Kiel, Germany
2) Institut für Humangenetik, Universitätsklinikum Schleswig-Holstein, Kiel, Germany
3) Deutsche Krebshilfe e.V., Verbundprojekt ´Molekulare Mechanismen maligner Lymphome´, Studienzentrale, Göttingen, Germany

Somatically mutated IGVH regions are a hallmark of germinal center (GC) B-cells. Moreover, aberrant somatic hypermutation (SHM) of oncogenes, like changes in the 5´ non-coding region of the BCL6 gene occurring in 75% of DLBCL, are a mechanism of oncogene activation independently of chromosomal translocations.
Large scale mutational screening using DHPLC and direct sequencing was applied to determine the somatic hypermutation status of clonal IGVH as well as several oncogenes like BCL6 and MYC in a series of more than 250 aggressive B-cell lymphomas included in the Deutsche Krebshilfe funded network „Molecular Mechanisms in Malignant Lymphoma". Mutation patterns were correlated with the results of molecular cytogenetic analyses. MYC breakpoints detected by FISH clearly differentiated two groups of aggressive B-NHL with significantly different VH mutation status. MYC-positive lymphomas by FISH carried VH genes with a mutation frequency significantly lower compared to aggressive lymphomas lacking these features (median 4.8% vs.11.,0%, p<0.0001). Similarly, the mutation frequency of BCL6 was significantly lower in MYC-positive than in MYC-negative lymphomas (median 0.13% vs 0.25% p=0,020). We observed a bias in VH gene usage in both groups with an overrepresentation of VH4 (40% in both groups) and VH3 gene (24% in MYC-positive, 40% in MYC-negative). The group of MYC-positive lymphomas was heterogeneous with regard to the pattern of chromosomal aberrations. (IGH, MYC, BCL2, BCL6, MALT1 and REL loci). Lymphomas with IG-MYC fusion lacking breakpoints in BCL2, BCL6, MALT1 or REL loci as well as non-MYC associated IGH translocations displayed a median IGVH mutation frequency of 4,8% vs. 12,2% in those cases with additional chromosomal translocations (p=0.0060).
Molecular classification of aggressive B-cell lymphomas according to MYC breakpoints distinguishes subgroups with significant differences in the VH and BCL6 mutation frequencies.

P259

Molecular characterization of a pericentric inversion of chromosome 3 by Fluorescence- *in situ* -hybridization

Dutta U.(1), Matthes F.(1), Peisker K.(2), Hansmann I.(1), Schlote D.(1)
1) Institut für Humangenetik und Medizinische Biologie, Halle (Saale), Germany
2) Institut für Biochemie und Molekularbiologie, Freiburg, Germany

Chromosomal abnormalities and gene mutations are a significant cause of human disorders. The mutations can be very small affecting individual nucleotides or can be big involving many genes or even whole chromosomes. Here we report a case of short stature in a girl with a karyotype of 46,XX,inv(3)(p24.2q26.1). Cytogenetic analysis had revealed a familial inversion 3, being heterozygous in the proband, her mother and grandmother. In order to characterize the breakpoints FISH (Fluorescence- *in situ* –hybridization) experiments were performed. Initially YAC (Yeast Artificial Chromosome) clones were selected by *in silico* analysis using the respective human genome database. From the five p-specific YACs selected, YAC clone CEPHy904H0787 (1090 kb) gave a split signal on the metaphase chromosomes of the proband indicating that the target sequence carries the inversion breakpoint on 3p24.2. YAC insert end sequencing was also done which lead to the identification of BACs (Bacterial Artificial Chromosome) for further FISH analysis. Out of the 10 YACs selected on the q arm, YAC CEPHy904G07889 (1610 kb) showed a split signal assigning the breakpoint to chromosomal band 3q26.1. To further narrow down the breakpoint region ten BACs were selected and BAC RP11-12N13 gave a split signal. This narrowed down the breakpoint region to 100 kb. We have now identified several ESTs (Expressed sequence tags) specific for the area of interest. RFLP analysis and detailed FISH experiments using isolated subfragments of the BAC with the split signal as probes will further narrow down the breakpoint region. This helps us in correlating the inversion and the phenotype of the proband.

P260

Functional analysis of PKHD1 splice mutations in autosomal recessive polycystic kidney disease (ARPKD)

Frank V.(1), Küpper F.(1), Schmidt C.(1), Senderek J.(1), Zerres K.(1), Bergmann C.(1)
1) Department of Human Genetics, Aachen University, Aachen, Germany

Autosomal recessive polycystic kidney disease (ARPKD) is an important cause of renal- and liver-related morbidity and mortality in children and adolescents with variable disease expression. While most cases manifest peri-/neonatally with a high mortality rate in the first month of life others survive to adulthood. ARPKD is caused by mutations in the PKHD1 (Polycystic Kidney and Hepatic Disease 1) gene on chromosome 6p12. PKHD1 is an exceptionally large gene with a longest open reading frame transcript of 67 exons predicted to encode a 4,074 aa multidomain integral membrane protein (polyductin/fibrocystin). Various alternatively spliced transcripts may result in different isoproteins. The large size of PKHD1, its complicated pattern of splicing, multiple allelism and lack of knowledge of the encoded protein's functions pose significant challenges to DNA-based diagnostic testing. Nucleotide substitutions, particularly if residing in regulatory elements or introns outside the splice consensus sites, are often difficult to assess without further functional analyses. Investigations on the transcript level are hampered as PKHD1 is not widely expressed in blood lymphocytes. We thus determined the functional significance of three novel splice mutations by minigene assays. While the mutations c.53-3C>A and c.1512+1G>A are most likely pathogenic and revealed different mechanisms of alternative splicing (exon skipping and intron retention), a biologic relevance of the third change c.1234-5C>T could not be corroborated given an identical splicing pattern in the WT and mutant constructs. To the best of our knowledge, this is the first study that defines the consequences of PKHD1 splice mutations and underlines the relevance of functional analyses in determining the pathogenicity of changes of unknown significance.

P261

ED1 mutation analysis in patients with hypohydrotic ectodermal dysplasia

Hehr A.(1), Bohring A.(2), Gross C.(1), Kohlhase J.(3), Horn D.(4), Muschke P.(5), Küpferling P.(6), Roth H.(7), Horst J.(2), Hehr U.(1)
1) Zentrum für Humangenetik, Regensburg, Germany
2) Universitätsklinikum Münster, Institut für Humangenetik, Münster, Germany
3) Albert-Ludwigs-Universität Freiburg, Institut für Humangenetik und Anthropologie, Freiburg, Germany
4) Universitätsklinikum Charité Berlin, Institut für Medizinische Genetik, Berlin, Germany
5) Otto-v-Guericke-Universität Magdeburg, Institut für Humangenetik, Magdeburg, Germany
6) Praxis und Labor für Humangenetik, Cottbus, Germany
7) Gemeinschafspraxis Dres. Mai, Schmitt, Mulfinger, Würzburg, Germany

Mutations in the ectodysplasin-A gene (ED1; Xq12-q13.1) are the most frequent cause of hypohidrotic ectodermal dysplasia and can be identified in about 95% of X-linked families (OMIM 305100). Affected patients present with sparse scalp and body hair (hypotrichosis), congenital absence of teeth (hypodontia) and reduced ability to sweat (hypohidrosis) leading to heat intolerance and episodes of hyperthermia. Female carriers of X-linked hypohidrotic ectodermal dysplasia may present with usually mild manifestations of one or more of the characteristic clinical features. In addition, deficient milk production during nursing has been reported. The identified ED1 mutations are distributed throughout the ED1 coding region without any obvious genotype-phenotype correlation.
In addition, a rare autosomal-rezessive (OMIM 224900) and a milder autosomal dominant form of the disorder (OMIM 129490) have been associated with mutations in the gene encoding the ectodysplasin anhidrotic receptor gene (EDAR).
Here we report the clinical and molecular data of 12 probands (7 males, 5 females; age range 1 – 59 years) from 8 independent families with hypohidrotic ectodermal dysplasia resulting from previously unreported ED1 mutations.
The male patients usually presented with the classical phenotype of hypohydrotic ectodermal dysplasia. As expected, a wide clinical variability was observed in the female carrier relatives. In two of the families with identified ED1 mutations suspected sudden infant death syndrome (SIDS) was reported for a close male relative. Interestingly, a 12 bp in frame deletion was identified in a 5 year old severely affected female index proband with scanty eyebrows, fine and scanty hair, periorbital wrinkles, dry skin, hypohidrosis, diffuse hyperpigmentation at the upper limbs, supernumerary nipple on the right, and oligodontia with taurodontism and normal intellectual development.

P262

Wide phenotypic variability of Treacher Collins syndrome in a cohort of 28 probands with identified TCOF1 mutation

Gross C.(1), Wieczorek D.(2), Spranger S.(3), Strobl-Wildemann G.(4), Prager B.(5), Kobelt A.(6), Zenker M.(7), Mitulla B.(8), Strenge S.(9), Hameister H.(10), König R.(11), Toral F.(12), Gillessen-Kaesbach G.(2), Hehr U.(1)
1) Zentrum für Humangenetik, Regensburg, Germany
2) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany
3) Praxis für Humangenetik, Bremen, Germany
4) Medizinisch Genetisches Zentrum München, München, Germany
5) Gemeinschaftspraxis für Humangenetik, Dresden, Germany
6) Klinikum Chemnitz, Institut für Medizinische Genetik, Chemnitz, Germany
7) Universität Erlangen-Nürnberg, Institut für Humangenetik, Erlangen, Germany
8) SRH-Zentralklinikum Suhl, Genetische Beratungsstelle, Suhl, Germany
9) Medizinische Fakultät Universität Leipzig, Institut für Humangenetik, Leipzig, Germany
10) Universitätsklinikum Ulm, Abteilung Humangenetik, Ulm, Germany
11) Johann Wolfgang von Goethe Universität Frankfurt, Institut für Humangenetik, Frankfurt, Germany
12) Hospital Universitario de Asturias, Seccion de Genetica Pediatrica, Oviedo, Spain

Treacher Collins syndrome (TCS; syn. Franceschetti Klein syndrome, OMIM 154500) is an autosomal dominant disorder, characterized by a striking facial appearance with downward slanting palpebral fissures, bilateral zygomatic hypoplasia, malformed ears and micrognathia. High arched or cleft palate, macrostomia as well as coloboma of the lower eyelid may be present. Frequently, absence or malformation of the external ear canal is observed, resulting in conductive hearing loss.
More than 90% of typical TCS patients carry heterozygous mutations in the TCOF1 gene, located in 5q32-q33. The mutations are distributed throughout the TCOF1 gene coding region with a cluster of mutations in exons 23-24, almost all of them resulting in premature truncation of the gene product treacle. There is no obvious genotype-phenotype-correlation. A wide clinical variability has been observed, both between family members and different families with identical TCOF1 mutation, pointing to additional genetic and/or external modifying factors.
Here we report the clinical and molecular data of 28 patients from 18 families with the clinical diagnosis of Treacher Collins syndrome and TCOF1 mutation, identified by sequencing of the entire coding region (12 frameshift, 2 missense, 2 in frame deletions, 2 nonsense). 8 of the identified mutations were located in exons 23-24. In 13 families previously unreported TCOF1 mutations were observed. For 5 sporadic cases DNA of both parents was available and testing confirmed a de novo mutation in the index proband. Interestingly, in 5 of the TCS families at least one close relative, reported to be very mild affected or unaffected, was identified as mutation carrier (mutation spectrum: 3 frameshift, 1 in frame deletion, 1 missense). Our data confirm the previously reported wide clinical variability in TCS families and indicate that the rate of seemingly unaffected mutation carriers might be higher than initially anticipated.

P263

Inhibition of prostate cancer cell growth by corepressors

Reeb C.(1), Papaioannou M.(1), Moehren U.(2), Baniahmad A.(1)
1) Institute for Human Genetics and Anthropology, Molecular Genetics, Jena, Germany
2) Faculty of Medicine, Division of Biochemistry, Leuven, Belgium

The androgen receptor gene, located on chromosome Xq11.2-q12, encodes for a nuclear hormone receptor and plays an important role in male development and prostate cell proliferation. The androgen receptor (AR) also controls the growth and proliferation of prostate cancer. Many mutations of the AR gene are associated with the progression of prostate cancer. Antihormones (androgen antagonists), used in prostate cancer therapy, inhibit the transcriptional activity of the AR through the recruitment of corepressors, which bind to the receptor. However mutations in the AR ligand binding domain (eg. T877A or H874Y) turn certain antihormones into strong agonistic ligands. The aim of our study is the analysis of the involvement of the corepressor Alien in both antihormone mediated AR ac-

tion and inhibition of prostate cancer cell proliferation.
For this study we have used the modified mammalian two-hybrid assay, co-immunoprecipitation (CoIP), chromatin-immunoprecipitation (ChIP) and colony formation assays.
Our findings demonstrate the interaction of the corepressor Alien with AR in an antihormone specific manner. The CPA mediated interaction of Alien with AR was confirmed both with Co-IP and in the context of chromatin (ChIP) in vivo. Interestingly, mutation of the sumoylation sites of AR abrogates Alien binding to AR suggesting that sumoylation regulates AR activity through the recruitment of corepressors. Furthermore, the expression of Alien leads to an antihormone dependent inhibition of cell proliferation. This inhibition of proliferation occurs also when the T877A mutation is present in the human AR. Taken together these observations indicate, that corepressors play an important role in the antihormone mediated prostate cancer therapy.

P264

Nijmegen breakage syndrome due to maternal isodisomy of chromosome 8

Varon R.(1), Müer A.(1), Wagner K.(2), Zierler H.(2), Sodia S.(2), Rauter L.(3), Petek E.(2), Tönnies H.(1), Neitzel H.(1), Sperling K.(1), Kroisel P.M.(4)
1) Institut für Humangenetik, Berlin, Germany
2) Institut für Medizinische Biologie und Humangenetik, Graz, Austria
3) Abteilung für Kinder- und Jugendheilkunde, Leoben, Austria
4) Institut für Humangenetik, Greifswald, Germany

The inheritance of two copies of a chromosome from only one parent is termed as uniparental disomy (UPD). Here we report on a patient with congenital microcephaly and facial dysmorphism. Chromosome analysis identified constitutional mosaicism with partial trisomy 8 and chromosomal instability in the patient's cells. The clinical and cytogenetic findings suggested the diagnosis Nijmegen breakage syndrome (NBS). Mutation analysis of the NBS1 gene showed that the patient is homozygous for the founder mutation 657del5. The mother was carrier of the mutation, whereas the father was homozygous for the wild type allele. Microsatellite analyses with markers spanning the whole of chromosome 8 revealed that the patient had inherited two identical chromosomes 8 from his mother carrying the 657del5 mutation and that the chromosome 8 fragment is of paternal origin. The most likely UPD mechanism is partial monosomic rescue due to mitotic non-disjunction. This is the first report of UPD as a cause of NBS.

P265

A proteomic approach to identify MPP4-associated proteins in the retina

Förster J.R.(1), Lochnit G.(2), Stöhr H.(1)
1) Institut für Humangenetik, Würzburg, Germany
2) Institut für Biochemie, Giessen, Germany

Objectives: Membrane-associated guanylate kinases (MAGUKs) are typically characterized by several conserved protein domains which mediate the assembly and maintenance of large multiprotein complexes at specialized cellular compartments. We have recently identified MPP4 as a retina-specific MAGUK abundantly present in the photoreceptor ribbon synapse. In addition, MPP4 is a component of the non-motile primary cilium of rods and cones. To further elucidate the diverse functions of this molecule we aim at the identification of interacting proteins.
Methods: Bovine retinal extracts were subjected to immunoaffinity chromatography using a specific monoclonal MPP4 antibody covalently linked to sepharose beads. Bound proteins were eluted from the column at low pH and separated by 1D- and 2D-gel-electrophoresis. Individual components were identified by mass spectrometry and Western blotting. Putative binding partners were further analyzed by immunoprecipitation and GST-pull down assays.
Results: Several proteins were co-purified with MPP4 from bovine retina. This includes cytoskeleton proteins, proteins involved in the phototransduction cascade and PSD95, another MAGUK, which co-localizes with MPP4 at the photoreceptor synaptic terminals.
Conclusions: Using a proteomic approach we have identified several interesting candidates which directly or indirectly associate with MPP4. This indicates an important role of MPP4 as an adaptor in the retina. Dysfunction in the interplay of the molecules within the MPP4 protein complex may be an important mechanism underlying human retinal disease.

P266

Expression of myosin-7A nonsense mutations: Nonsense-associated altered splicing (NAS) is a rare event in Usher syndrome type IB

Ebermann I.(1), Lopez I.(2), Koenekoop R.(2), Bolz H.(1)
1) Institut für Humangenetik, Klinikum der Universität zu Köln, Köln, Germany
2) Montreal Children's Hospital, Dpt. of Ophthalmology, Montreal, Canada

Usher syndrome (USH) designates a group of clinically (USH1-3) and genetically heterogeneous disorders, characterized by sensorineural hearing loss and retinitis pigmentosa. USH is the most common form of genetic deaf-blindness. Twelve loci have been identified, and eight causative genes have been cloned. USH1 is the most severe clinical subtype. Both, truncating and missense mutations in the motor protein myosin-7A (MYO7A) underlie USH1B, accounting for 40 – 60% of USH1 cases. Premature termination codons (PTCs) in mRNAs can have different consequences: Nonsense-mediated decay (NMD) describes a pathway that degrades PTC-containing transcripts.
Alternatively, and particularly if exonic splice enhancer motifs (ESEs) are affected, nonsense-associated altered splicing (NAS) can be observed. ESEs serve as binding sites for serine/arginine-rich splicing factors. NAS includes exon skipping, choice of alternative splice sites, and intron inclusion. We have systematically studied the impact of all PTCs that have been described to date or identified by us in USH1 patients (n=16). First, wildtype and mutant sequences were analysed in silico for a possible impact on different types of ESEs (ESE finder web resource). Fourteen mutations were found to affect the scores for one or several ESE motifs (loss, creation, decrease or increase of motif score). We created 27 genomic minigene-constructs (for both, wildtypes and corresponding mutants). These constructs were subsequently cloned into a splicing vector (pSPL3) and expressed in COS1-cells. RT-PCR products were TA-cloned into p2.1TOPO to allow detection of all splice products. Our results obtained so far (11 PTC-carrying constructs in comparison to wildtype) show that only one mutation, p.R666X, leads to partial exon skipping. Constitutive in-frame exon skipping, eventually resulting in functional isoforms, was observed in two cases. Hence, NMD seems far more common than NAS in known MYO7A PTCs.

P267

Analysis of G2/M checkpoint control in cell lines from MCPH1 patients

Gavvovidis I.(1), Trimborn M.(2), Pöhlmann C.(2), Neitzel H.(2), Schindler D.(1)
1) Department of Human Genetics, University of Würzburg, Wuerzburg, Germany
2) Department of Human Genetics, Charité, Berlin, Germany

Primary microcephaly (MCPH) is a heterogeneous autosomal recessive disorder characterized by a pronounced reduction of the occipitofrontal head circumference and variable mental retardation without additional neurological abnormalities. A hallmark of patients with mutations in the gene MCPH1 is a cellular phenotype of premature chromosome condensation in the G2 phase of the cell cycle and a delayed decondensation in G1 phase post mitosis. MCPH1 is located on human chromosome 8p23.1 and encodes the BRCT (BRCA1 C-terminus)-domain containing protein microcephalin. The presence of BRCT domains in the protein suggests a function of microcephalin in cell cycle control and / or DNA-repair. To analyse the role of microcephalin in G2/M-checkpoint control we exposed lymphoblastoid cell lines from 3 patients and 5 controls to 4 Gy ionizing irradiation and investigated the cell cycle distribution and number of mitotic cells after 2 hours by high resolution flowcytometry. Significant differences in both parameters were not detected in patient versus control cells. In contrast, when we depleted 3 control fibroblast cell lines of microcephalin by RNA interference, the number of cells in mitosis 2 hours after irradiation was 1,2% compared to 0,04% of the same cells without RNAi. This significant difference indicates a potential impairment of the G2/M-checkpoint in MCPH knock-down cells. Loss of checkpoint control in fibroblasts after microcephalin knockdown by RNAi has also been reported by others (Xu et al., 2004). Studies are underway to investigate whether the discrepancy between the irradiation and knock-down experiments can be explained, for example, by off-target effects of RNAi.

P268

Regulation of the oncogene and developmental regulator EVI1 by all-trans retinoic acid (ATRA)

Bingemann S.(1), Wieser R.(1)
1) Med. Univ. Wien, Abt. für Humangenetik, KIMCL, Wien, Austria

The EVI1 gene codes for a zinc finger protein with important roles in embryonic development

and in myeloid leukemogenesis. It is transcribed into several mRNA species with variable 5´-ends. One of these mRNA variants, MDS1/EVI1, gives rise to an EVI1 protein with an extended N-terminus and with functions partially different from those of the shorter EVI1 protein type. The other EVI1 5´-end variants are most likely translated into the same protein, i.e. the short EVI1 protein variant, but their variable 5´-UTRs can be expected to affect the regulation of protein expression. Despite of its important functions, little is known about the regulation of the EVI1 gene. Using PA1 cells, we have previously shown that ATRA increases both the transcription rate and the stability of the EVI1 mRNA. We now extended these observations to two other human cell lines, the teratocarcinoma cell line NT-2 and the APL cell line NB4, the latter of which carries a PML-RARalpha rearrangement. Time course and dose response analyses showed that ATRA induces the EVI1 mRNA in both cell lines. In NT-2 cells, the response was already seen with as little as 10nM ATRA and affected all EVI1 mRNA variants. It occurred rapidly and reached a maximum after 18 hrs, except for the MDS1/EVI1 mRNA, which was noticably induced only after 48 hrs. EVI1 was also induced early in and throughout the course of a 4 week neuronal differentiation protocol. In NB4 cells, significant EVI1 mRNA induction occurred only after 48 hrs, it was less pronounced than in NT-2 cells, and it did not affect all EVI1 mRNA variants. As in NT-2 cells, MDS1/EVI1 was induced with delayed kinetics compared to the other transcript types. Using reporter gene assays, an ATRA responsive region of ~1kb was identified in the EVI1 promoter. Deletion analysis is underway to narrow down the EVI1 retinoic response element. In addition, experiments are being carried out to further characterize the stabilization of the EVI1 mRNA by ATRA.

P269

A nonsense mutation in the corneodesmosin gene in a Mexican family with hypotrichosis simplex of the scalp

Dávalos N.O.(1), García-Vargas A.(2), Pforr J.(3), Davalos I.P.(4), Picos-Cárdenas V.J.(4), García-Cruz D.(4), Figuera L.E.(4), Nöthen M.M.(5), Betz R.C.(3)

1) Doctorado en Genética Humana, CUCS, Universidad de Guadalajara, Guadalajara, Mexico

2) Instituto Dermatológico de Jalisco "Dr. José Barba Rubio" SSJ, Guadalajara, Mexico

3) Institute of Human Genetics, University of Bonn, Bonn, Germany

4) División de Genética, CIBO-IMSS, Guadalajara, Mexico

5) Life & Brain Center, University of Bonn, Bonn, Germany

Hypotrichosis simplex of the scalp (HSS; MIM 146520) is a rare autosomal dominant form of non-syndromic alopecia that affects men and women equally. Up to now, only a small number of families with HSS have been reported. The affected individuals experience a diffuse progressing hair loss from childhood to adulthood that is confined to the scalp. Recently, HSS has been mapped to the short arm of chromosome 6 (6p21.3), allowing mutations in the corneodesmosin gene (CDSN) to be identified as the cause of the disorder. To date, two stop mutations have been found in three unrelated families with HSS of different ethnic origin. Here, we describe a Mexican family presenting HSS. The pedigree extends over six generations comprising 156 individuals, 42 of whom are affected. By direct sequencing of the two exons of the CDSN gene, a novel nonsense mutation was identified in the index patient in exon 2, resulting in a premature stop codon (Y239X). The mutation cosegregated in the family (28 family members, 9 of whom were affected) and was not found in 300 control chromosomes using a restriction enzyme analysis with PsuI.

Our data provide molecular genetic evidence for a 3rd stop mutation in exon 2 of the CDSN gene being responsible for HSS. All to date known nonsense mutations responsible for HSS are clustered in a region including 40 amino acids of exon 2 in the CDSN gene. Further families should be investigated to elucidate if this region of the CDSN gene might have a special effect on hair growth.

P270

Is the gene responsible for Zimmermann-Laband syndrome in 3p14.3?

Abo-Dalo B.(1), Stefanova M.(2), Kim H.-G.(3), Niedermaier M.(4), Mundlos S.(4), Gusella J.(3), Kutsche K.(1)

1) Institut für Humangenetik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

2) Department of Medical Genetics, Medical University, Plovdiv, Bulgaria

3) Center for Human Genetic Research, Boston, United States of America

4) Max Planck Institute for Molecular Genetics, Berlin, Germany

Zimmermann-Laband syndrome (ZLS) is a rare disorder characterized by coarse facial appearance including bulbous nose, thickened lips, thick and floppy ears, gingival hypertrophy, aplasia or dysplasia of hand- and toenails as well as the terminal phalanges, and hyperextensibility of joints. Autosomal dominant inheritance has been suggested, however, the genetic basis of ZLS is unknown. We investigated two apparently balanced translocations in patients with ZLS: A familial 3;8 translocation in both mother and daughter and a 3;17 translocation in a male patient. Delineation of both chromosome 3 breakpoints revealed that the CACNA2D3 gene in 3p14.3 was disrupted by one breakpoint whereas the other one mapped 100 kb downstream of CACNA2D3, strongly suggesting that the ZLS gene is located in this region. Subsequent mutation analysis of CACNA2D3 in 9 sporadic patients with ZLS did not reveal any pathogenic mutation. As we can not yet exclude that both breakpoints cause a position effect by disrupting the disease gene from cis-acting regulatory elements (see below), we analysed 10 genes surrounding both breakpoints for pathogenic mutations in the sporadic patients, however, we could not identify any. Moreover, MLPA analysis of CACNA2D3 and WNT5A, a positional and functional candidate, did not reveal any abnormalities in the copy number of these genes.

WNT5A is expressed at digit tips of mice. It is known that genes involved in early development show a tightly regulated temporal and spatial expression which is controlled by regulatory elements located far away from the gene. By inter species comparative sequence analysis we identified 9 highly conserved non-genic sequences (CNGs) that are located up to 1 Mb distal from the 3' end of WNT5A. Mutation analysis of the CNGs revealed two point mutations at different highly conserved nucleotide positions. Further experiments are ongoing to show whether these sequence variants represent pathogenic mutations or polymorphisms.

P271

Generation and characterization of the *TSPY* transgenic mouse line TgTSPY15

González Fassrainer D.(1), Woog S.(1), Böhm D.(2), Schmidtke J.(1), Schubert S.(1)

1) Hannover Medical School, Institute of Human Genetics, Hannover, Germany

2) University of Göttingen, Institute of Human Genetics, Göttingen, Germany

TSPY (testis-specific protein, Y-encoded) is a product of a Y-chromosome-specific gene family. *TSPY* is testis specifically expressed and expression is restricted to germ cells. The function of TSPY is still enigmatic, but it is assumed to play a role in spermatogonial proliferation and in gonadal tumorigenesis. After being first discovered in man, *TSPY* orthologous gene families have subsequently been characterized in many other placental species along the primate, artiodactyl, perissodactyl and rodent lineages. Because the laboratory mouse carries the *tspy* gene in a naturally silenced state, we decided to restore its activity in *TSPY* transgenic mice. We generated a transgenic mouse line (TgTSPY15) harbouring a complete structural human *TSPY* gene. Genotyping of the offspring revealed an autosomal integration site of the transgene, in contrast to a previous transgenic attempt with Y-chromosomal integration. In the new transgenic line, a single copy of the human *TSPY* construct was introduced into a single integration site of the mouse genome. The transgene is correctly transcribed and spliced according to the human pattern and is almost exclusively expressed in testis. Our findings indicates that the human fragment used for trangenesis contains all cis-acting elements directing organ-specific expression of the TSPY gene. This transgenic mouse line will serve as an in vivo model for further investigations of regulation, expression and function of human *TSPY*.

P272

***CASK*, a candidate gene for X-linked microcephaly and cerebellar hypoplasia**

Ganswindt J.(1), Wimplinger I.(1), Dobyns W.(2), Kutsche K.(1)

1) Institut für Humangenetik, Universitätsklinikum Eppendorf, Hamburg, Germany

2) Department of Human Genetics, Neurology and Pediatrics, University of Chicago, Chicago, United States of America

X-linked inheritance of congenital cerebellar hypoplasia has been described, but only one disease gene, OPHN1, has been identified to date. In a female patient with microcephaly, moderate hypoplasia of the brainstem, severe hypoplasia of the cerebellum, moderate vermis hypoplasia, conductive hearing impairment, and severe psychomotor retardation, molecular characterization of the breakpoints of a paracentric inversion revealed that one breakpoint, in Xp11.4, was located in intron 5 of CASK. CASK encodes a protein with similarity to Ca2+/calmodulin-depend-

ent protein kinase II and membrane-associated guanylate kinase. Transgenic male mice with an insertion in Cask show craniofacial abnormalities. In addition, CASK acts as coactivator of the transcription factor Tbr-1 to induce transcription of T-element containing genes, including reelin, a gene involved in cerebrocortical development. These data suggest that CASK is an attractive candidate gene for human brain malformations. Mutation analysis of the 27 exons of CASK in 23 male patients with microcephaly and cerebellar hypoplasia revealed a single isocoding mutation. This transition (c.915G>A, p.K305) affects the last nucleotide of exon 9 and has not been found in 514 males. Remarkably, the mutated sequence was not detected as a donor splice site by two computer prediction programs suggesting that this sequence variant affects splicing. Since no RNA was available for the patient with the c.915G>A mutation, we performed exon trapping experiments with a genomic fragment containing part of CASK intron 8, exon 9, and part of intron 9, but we did not detect differences in the resulting splicing products: CASK exon 9 was trapped for the wild-type and the mutated sequence. Although these data do not corroborate our hypothesis that the silent c.915G>A mutation has any effect on splicing, we will perform further exon trapping experiments with an extended genomic context of CASK to further investigate this issue.

P273

Knock-out of the BSCL2 homologue in Drosophila

Schwarzbraun T.(1), Ofner L.(1), Jud D.(1), Windpassinger C.(1), Petek E.(1), Sigrist S.(2), Wagner K.(1)
1) Medizinische Universität Graz, Graz, Austria
2) European Neuroscience Institute, Göttingen, Germany

Mutations in the human gene BSCL2 can cause two distinct phenotypes: autosomal recessive congenital lipodystrophy type 2 (CGL2) and autosomal dominant hereditary neuropathy (SPG17). Although much work has been done in the recent years the molecular function of this gene still remains mysterious. To this end we turned to the model organism Drosophila melanogaster. The homologous gene in Drosophila CG9904 encodes a protein highly similar to the human protein. Using excision mutagenesis we were able to establish CG9904 null mutants. This constituted the basis for the phenotypic characterisation. Since the null mutation in human results in a severe phenotype of the adipose tissue (CGL2) we focused on metabolic assays like hatching rate, starvation and longevity. By this means we hope to reveal the cellular function of this gene and gain insight into the biological scenario underlying the human diseases.

P274

Effects of BSCL2 knockdown in preadipocytes differentiation

Jud D.(1), Schwarzbraun T.(1), Ofner L.(1), Petek E.(1), Wagner K.(1), Windpassinger C.(1)
1) Medizinische Universität Graz, Graz, Austria

Mutations in the human gene BSCL2 cause two distinct phenotypes: dHMN-V / SS [MIM 606158; 270865] and CGL Type 2 [MIM 269700]. Null mutations in BSCL2, encoding the protein Seipin, were previously shown to be associated with autosomal recessive Berardinelli Seip congenital lipodystrophy. Due to the near-complete absence of metabolically active and mechanical adipose tissue in this disorder, a role of Seipin in an early stage of preadipocytes differentiation is assumed. To test this hypothesis we performed a differentiation assay using RNA interference (RNAi) on murine preadipocyte cell cultures. Knockdown of native Seipin expression in 3T3-L1 cells significantly decreased the differentiation of preadipocytes to mature adipocytes in one of three RNAi constructs. We hope that our experiments will contribute to a better understanding of the molecular mechanism underlying the human disorder.

P275

Inhibition of ligand activated androgen receptor by corepressor LCoR

Asim M.(1), Patz M.(1), Baniahmad A.(1)
1) Institute for Human Genetics and Anthropology, Friedrich-Schiller-University, Jena, Germany

The androgen receptor (AR), a transcription factor belonging to the nuclear hormone receptor superfamily, is encoded by a single gene located on chromosome Xq11-12 position. Many clinical conditions are known to be caused by the mutations in the receptor e.g., Androgen Insensitivity Syndrome (AIS) and Spinal and Bulbar Muscular Atrophy (SBMA). In addition, prostate cancer progression has also been linked to the mutations in the receptor. Hormone treatments to block AR mediated activation of the target genes have, to some degree, been proved effective to inhibit the cancer growth.
We have found a transcriptional corepressor LCoR (10q24.1), which interacts and blocks the function of the liganded wild type AR as well as the hot spot mutant T877A AR in vivo. cDNA microarray data reveal LCoR expression in prostate cancer cell line such as LNCaP, the model CaP cells. However transcriptional repression by LCoR cannot be observed in LNCaP cells suggesting lack of AR repression is an important growth promoting strategy by cancer cells. In contrast to its inhibition mechanism for other nuclear hormone receptor, a novel carboxy-terminal region of LCoR mediates the inhibition of AR. We show that LCoR binding to AR is influenced by signal transduction pathways. Blocking specifically some signal transduction pathways allows LCoR binding to AR. Utilising specific signaling inhibitors to repress AR in combination with antihormone therapy can be an important clinical tool to achieve growth inhibition of prostate cancer.

P276

Real-time quantitative PCR-based system for determining zygosity in rats transgenic for Huntington´s disease

Holzmann C.(1), Raber K.(2), Koczan D.(3), Riess O.(4), von Hoersten S.(2)
1) University Rostock, Medical Genetics, Rostock, Germany
2) Medical School Hannover, Anatomy, Hannover, Germany
3) University Rostock, Immunology, Rostock, Germany
4) University Tuebingen, Medical Genetics, Tuebingen, Germany

We have recently described a transgenic rat model of Huntington's Disease (HD) which carries a truncated rat huntingtin fragment of 51 CAG repeats under control of the rat Htt promoter. These animals exhibit a slowly progressive behavioral phenotype with emotional disturbance, motor deficits, and cognitive decline. Determination of the zygosity for the transgene is important for breeding a transgenic colony and for the use of transgenic animals in experimental situations in which simple or double expression levels of the transgene may influence the functional outcome of the experiment. Traditionally, the transgene copy number in both founders and G1 hemizygous transgenic animals has been analyzed by blotting techniques, which are tedious and time-consuming methods that require a large amount of DNA sample for each assay. Real-time PCR is a quantitative and extremely precise method that could be applied to the analysis of animals differing only by a factor of two in the amount of target sequences. We defined the technical conditions of real-time PCR to co-amplify a transgene and a reference gene using two fluorogenic probes and the comparative cycle threshold method. We applied these conditions to the analysis of zygosity in transgenic rats. Real-time PCR allowed clear-cut identification of all transgenic animals analysed (n=64) as homozygous or heterozygous. Southern blot analysis of these animals using an internal quantitative control showed ambiguous results in six of them and was concordant with real-time PCR in the rest. Mating of homozygous and heterozygous animals defined by real-time PCR showed transgene transmission to the offspring following the Mendelian laws. Real-time PCR allows rapid, precise, non-ambiguous and high throughput identification of zygosity in transgenic animals. This technique could be helpful in the establishment of breeding programs for transgenic colonies and in experiments in which gene dosage effects could have a functional impact.

P277

Effect of mutated beta-B2-crystallin on lens, brain and behavior of mice

Ganguly K.(1), Favor J.(2), Beckers J.(3), Hölter-Koch S.(1), Klopstock T.(4), Graw J.(1)
1) GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Entwicklungsgenetik, Neuherberg, Germany
2) GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Humangenetik, Neuherberg, Germany
3) GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Experimentelle Genetik, Neuherberg, Germany

4) LMU München, Abt. für Neurologie, München, Germany

A mouse mutant O377 was identified with dominant, progressive lens opacity among offspring of mice paternally treated by 3 Gy X-ray irradiation. The mutation was mapped to distal chromosome 5 and determined to be an A?T bp substitution in the acceptor splice site of intron 5 of Crybb2 resulting in a 57-bp insertion in the mRNA due to alternative splicing. We determined beta-B2-crystallin expression not only in the lens, but also in the olfactory bulb, pyramidal cells of the cortex, some areas of the hippocampus, and Purkinje and stellate cells of the cerebellum. There are no gross morphological alterations in the heterozygous or the homozygous mutant mice. The number of Purkinje cells of the cerebellum is significantly increased by 11% in homozygous mutants. Expression profiling demonstrated a significant upregulation of a few genes in the brains of homozygous mutants only; the largest deviation from the wild type were for the genes coding for calpain 3 and thymosin-beta-4. Behavioural studies suggested increased forward locomotor activity and group contact frequency only of the homozygous mutants. Moreover, assessment of prepulse inhibition revealed a clear deficit in the homozygous mutants indicating a failure in sensorimotor gating. In humans, prepulse inhibition impairment can be found in patients suffering from schizophrenia, obsessive-compulsive disorder and Huntington´s disease. Prepulse inhibition is considered to have a general high prediction capability in Schizophrenia. Since the lens phenotype is expressed as a semi-dominant trait, but the brain and behavioural phenotypes are expressed in a recessive fashion, it might be speculated that beta-B2-crystallin acts in the brain like an enzyme rather than a structural protein. In particular, the Calcium-binding propeties of beta-B2-crystallin might be involved in these processes.

P278

Benign familial neonatal convulsions: always benign?

Steinlein O.(1), Weidner B.(2), Schmitt B.(3)
1) Klinikum der Universität München, Institut für Humangenetik, München, Germany
2) Klinik für Kinder- und Jugendmedizin, Carl-Thiem-Klinikum, Cottbus, Germany
3) Kinderspital, Universität Zürich, Zürich, Switzerland

Benign familial neonatal convulsions (BFNC) is a rare autosomal dominantly inherited seizure syndrome caused by mutations in the potassium channel genes KCNQ2 and KCNQ3. BFNC is characterized by an age of onset between the first day and fourth month of life. The seizures are generalized or multi-focal and of the tonic and/or clonic type. Later in life seizures can reoccur in up to 15% of the patients, starting mostly at school age or in young adulthood. The course of the disorder is described as benign and self-limiting, and in most patients the seizures remit spontaneously within a few days or weeks. However, within our sample of now 17 families with proven KCNQ2 mutations we identified three families with individuals with a severe course of the disorder. In two families the index patient developed drug-resistant seizures and epileptic encephalopathy shortly after birth. In both patients the outcome was severe psychomotor retardation. In one family the patient carried a de novo KCNQ2 mutation, while in the other the KCNQ2 mutation was inherited from the mother who had benign convulsions as a newborn and was of normal intelligence. In the third family three individuals carried the same KCNQ2 mutation: the index patient had delayed psychomotor development and her maternal aunt had moderate mental retardation, while the mother was intellectually normal. There was no discernable type of mutation in those families: one had an amino acid exchange within the transmembrane domain of KCNQ2, the second family had a frame shifting deletion in the large C-terminal region and the third had a splice site mutation. It remains unclear if patients with a severe course of the disorder have a second, unrecognized condition or if the co-occurrence of certain unknown risk factors in combination with a KCNQ2 mutation are responsible. Nevertheless, the rather high rate of 3/17 families in our sample suggest that, despite its name, BFNC might not always be a benign condition.

P279

Unique chromosomal aberration in a recurrent malignant meningeoma

Pelz A.-F.(1), Klawunde P.(2), Mawrin C.(3), Wieacker P.(4)
1) Institut für Humangenetik, Tumorgenetik, Magdeburg, Germany
2) Klinik für Neurochirurgie, Magdeburg, Germany
3) Institut für Neuropathologie, Magdeburg, Germany
4) Institut für Humangenetik, Magdeburg, Germany

Meningeomas are the second most frequent tumours of the central nervous system in adults, accounting for approximately 20% of all primary intracranial tumours. Most meningeomas are benign, but approximately 20% display aggressive features, and recurrence rates for atypical and anaplastic meningeomas have been reported from 38-78%. However, our knowledge is poor in regard to the genetic events associated with aggressive phenotype in malignant meningeomas. Previous cytogenetic and molecular-cytogenetic studies have detected complex numerical and structural aberrations, e.g. monosomy 22 or deletion of 22q, deletions of 14q, 1p, 10q, loss of a sex chromosome, and tetraploid karyotypes. Here we report on a case of recurrent left parietal anaplastic meningeoma with unique additional chromosomal aberrations. Histopathology revealed a malignant meningeoma with high mitotic activity. For the present investigation, tumor cells derived from fresh surgical specimens were long-term cultured in RPMI medium for conventional chromosome preparation and GTG banding. We detected 3 different kayrotypes by conventional cytogenetic analysis: 1) hypodiploid cells with a loss of the Y chromosome, 2) near-triploid cells with multiple chromosomal aberrations like dic(2),del(3p), add(4q) and add(8q), i(9q) and i(17q) and a various number of marker chromosomes, 3) near-tetraploid cells with add(1q) and add(9p), del(5p) and del(14q), loss of chromosome 2 and a various number of marker chromosomes. The malignant meningeoma investigated in this study displayed karyotype alterations previously reported for malignant meningeomas, including the 14q aberration and a possible tetraploid karyotype. In addition, a near-triploid karyotype and alterations like 5p-, 8q+ are chromosomal aberrations which have not been reported in previous studies. Our findings suggest that complex karyotype alterations may be a characteristic feature in malignant meningeomas.

P280

Expression analysis of LGI gene in rodent brain does not support its putative role as a tumour suppressor gene

Gu W.(1), Jakupoglu C.(2), Dörner K.(1), Finacchiaro G.(3), Brüstle O.(2), Steinlein O.(1)
1) Institut für Humangenetik, München, Germany
2) Institute of Reconstructive Neurobiology, Bonn, Germany
3) Istituto Nazionale Neurologico C. Besta, Milan, Italy

Rearrangements of the LGI1 (Leucine-rich, Glioma Inactivated gene 1) gene were detected in the T98G and A172 glioblastoma (GBM) cell line and in several primary GBM. Also, expression of the LGI1 gene product has been linked to down-regulation of a number of matrix metalloproteinases. Therefore LGI1 was proposed to be a tumour suppressor gene preventing the invasion phenotype of glioma cells. However, mutations in the LGI1 coding region were never found in malignant gliomas and data following gene transfer in cell culture were conflicting. We used adult GFAP-eGFP transgenic mice brains to perform fluorescent immunohistochemistry with LGI1 antibody. Pyramidal cells of the frontal cortex, some thalamic nuclei but not the adult striatum, as well as the red nucleus field in mesencephalon showed LGI1 staining. These LGI1 positive neurons were surrounded by, but did not co-localize with GFAP-GFP positive cells. Cerebellar Purkinje cells were strongly positive for LGI1 in perikarya and dendrites, whereas the Bergmann glia was clearly negative for LGI1. In all analyzed slides of the adult brain no co-localization of GFAP-GFP and LGI1 could be observed. GFP-positive and negative cell fractions were FACS-sorted from juvenile (8-14 days) GFAP-eGFP mice brains. RT-PCR was performed with RNA extracted from both fractions for LGI1 as well as the neuronal marker Tubb3 and glial marker GFAP. 5-fold increased GFAP-levels in the GFP-positive cells and at least 2-fold increased Tubb3 level in GFP-negative cells confirmed the enrichment of neuronal cells in the GFP-negative fraction, where LGI1 expression was found at highest levels. We observed that LGI1 shows a predominant neuronal expression in juvenile and adult rodent brains. This result could suggest that the variable levels of LGI1 expression in gliomas simply reflect the presence of neurons entrapped in the tumour. We therefore conclude that the supposed role of LGI1 as a tumour suppressor gene should be reconsidered.

P281

Transgenic mouse model of primary torsion dystonia

Grundmann K.(1), Reischmann B.(1), Huebener J.(1), Kuhn M.(1), Bonin M.(1), Wilbertz J.(2), Schmidt W.J.(3), Riess O.(1)
1) University of Tuebingen, Medical Genetics, Tuebingen, Germany
2) Karolinska Institute, Stockholm, Sweden

3) University of Tuebingen, Dept. of Neuropharmakology, Tuebingen, Germany
Primary torsion dystonia is an autosomal-dominant movement disorder associated with a deletion (GAG) in the DYT1 gene encoding torsin A. The gene defect has incomplete penetrance, with ~ 30% developing clinically evident dystonia. We generated four independent lines of transgenic mice, two overexpressing human mutant torsin A and two overexpressing human wild-type torsin A using a strong murine prion protein (prp) mouse promoter. All lines demonstrated increased levels of torsin A in the brain by Western blot analysis. Immunohistochemistry revealed a normal distribution of torsin A in brain regions. Perinuclear inclusions that stained positively for torsin A were detected in selected brain stem regions such as the pedunculopontine nucleus in all transgenic, mutant and wild-type torsin A overexpressing lines. We also performed neurochemical analyses of striatal dopamine and metabolites which revealed significant differences between transgenic lines overexpressing mutant torsin A and lines overexpressing wild-type torsin A. Behavioral differences between the different lines have been detected by foot print analysis, rotarod analysis and beam walking test.
This transgenic mouse model displays behavioral and pathologic features resembling to features detected in patients with primary torsion dystonia and will therefore provide a useful model to further investigate the pathology and therapeutic options in primary torsion dystonia.

P282

Effects of Valproic acid on isolated motoneurons from a mouse model for Spinal Muscle Atrophy?

Rak K.(1), Jablonka S.(1), Sendtner M.(1)
1) Universität Würzburg, Institut für Klinische Neurobiologie, Würzburg, Germany
Spinal muscle atrophy (SMA) is among the most common monogenetic disorders leading to death in early infancy. Reduced SMN-Protein levels, due to homozygous loss of the SMN gene, lead to a degeneration of spinal motoneuron with weakness of the proximal muscles. Therefore first attempts to cure SMA aim to increase SMN-protein-level. It has been shown, that Valproic acid (VPA), a potent antiepileptic drug and histone deacetylase inhibitor, stimulates SMN-expression in lymphoblastoid cells derived from SMA-patients. SMN expression levels are low in these cells. Therefore we tested whether upregualtion of SMN expression also occurs in neuronal cells that normally express relatively high levels of this gene. We investigated the effects of VPA in motoneurons from a mouse model for SMA. These mice have severe morphological and functional pathologies in motoneuron because of the loss of the murine smn-Gene. Motoneuron of smn-deficient and control E 14 Embryos were cultured on Laminin-1. Doses VPA from 0.5 to 100 µM were applied. We choose these concentrations of VPA, because they are close to the therapeutic dose found in epilepsy patients in cerebrospinal fluid. Treated cells with concentrations higher than 50 µM showed reduced survival. Axonal length and growthcone size were determined by confocal microscopy. Cells treated with VPA from 0.5-10 µM do not show any alterations in neurite length. VPA concentrations from 50 up to 100µM led to significantly reduced survival and reduced axon length and growthcone size in surviving motoneurons. In order to determine the effects of VPA on SMN protein levels, neuronal stem cells from the SMA mouse model were cultured and treated with 100 µM VPA for 3 days. No differences in SMN levels were observed.
We could not determine any effect of VPA at established therapeutic doses in neuronal cells from a mouse model of spinal muscle atrophy.

P283

Spinocerebellar ataxia type 4: phenotype, neuropathology and delineation from the Japanese 16q22.1-linked ataxia

Hellenbroich Y.(1), Rüb U.(2), Pawlack H.(1), Bernard V.(1), Reusche E.(3), Schwinger E.(1), Zühlke C.(1)
1) Universität zu Lübeck, Institut für Humangenetik, Lübeck, Germany
2) J.W. Goethe Universität, Institut für klinische Neuroanatomie, Frankfurt, Germany
3) Universität zu Lübeck, Institut für Pathologie, Lübeck, Germany
Spinocerebellar ataxia type 4 (SCA4) is an autosomal dominant neurodegenerative disorder linked to chromosome 16q22.1. We describe a 5-generation family from Northern Germany with 16 affected family members available for DNA analysis. Linkage studies in this family could refine the SCA4 candidate region to an 8 Mb genomic interval between D16S3019 and D16S512 comprising 139 known genes and loci. The main clinical features of SCA4 are a cerebellar ataxia in combination with an axonal sensory neuropathy. Neuropathological studies of a brain from our SCA4 family revealed an obvious demyelinisation of cerebellar and brainstem fiber tracts with marked neuronal loss in the Purkinje cell layer of the cerebellum, in the cerebellar fastigial nucleus and in many brainstem nuclei. Immunocytochemical analysis using the antipolyglutamine antibody 1C2 failed to detect any immunoreactivity within the SCA4 brain. Analysis of all CAG repeats in the candidate region could also exclude a CAG repeat expansion as the underlying gene defect. So far, we analysed the coding regions of 34 genes and more than 50 tri-, tetra- and pentanucleotide repeats within the candidate region without detecting a cosegregating mutation or repeat expansion. Recently, in Japanese patients a 16q22.1-linked pure cerebellar ataxia was shown to be associated with a C→T single-nucleotide substitution in the 5'UTR of a novel gene named puratrophin-1. This mutation is not present in our SCA4 family from Northern Germany, nor any other sequence variation within the coding, the 5'- and 3'UTR regions of the puratrophin-1 gene. Furthermore, the Japanese single-nucleotide substitution was not detected in 200 German patients with familial ataxia and 303 cases with sporadic ataxia.
In conclusion, our data underlines that SCA4 and the Japanese 16q-linked ataxia are not allelic and emphasizes the unique phenotype and neuropathology of SCA4.

P284

Microarray analysis combined with microdissection identifies candidate genes for normal and trisomy 21 neocortex development

Chotewutmontri S.(1), Zechner U.(1), Tralau T.(2), Haaf T.(1), Galetzka D.(1)
1) Institute for Human Genetics, Johannes Gutenberg University, Mainz, Germany
2) Department of Pediatrics, Ernst Moritz Arndt University, Greifswald, Germany
Development of the human neocortex depends on spatially and temporally correct expression of numerous genes. Disturbances of this highly coordinated process are an important cause of mental retardation and many other brain disorders. The same set of genes may also contribute to cognitive variation. We have developed a cDNA chip with approximately 600 genes that are known to influence some aspect of cognition in humans, mice and/or Drosophila, along with 95 housekeeping genes. This customised gene chip was used for expression profiling of foetal (weeks 17–23 of gestation) brain samples from trisomy 21 fetuses and normal controls (without chromosomal and morphological abnormalities). The mRNA expression levels were quantified in prefrontal cortex sections (prospective area A10) and in microdissected neuronal layers from neocortex. 231 tested genes displayed (at least twofold) expression differences between trisomy 21 and normal prefrontal cortex. Many of these genes were only temporally up- or downregulated. 60 genes showed expression differences between the microdissected neuronal layers of trisomy 21 fetuses and controls. 30 genes were found to be differentially regulated in both experiments, i.e. in total prefrontal cortex and microdissected neocortex. Although some of these genes were reported previously to be differentially expressed in trisomy 21 brains, most identified genes including genes involved in MAP kinase signaling, purine metabolism, and axonal plasticity have not been associated with trisomy 21 so far. Six genes were validated with realtime PCR. For example, SCN1B which when mutated causes general epilepsy with febrile seizures is up-regulated in fetal trisomy 21 (neo)cortex. Collectively our results will provide new insights into the genetics of normal and pathological human brain development.

P285

Morris water maze training induces time-dependent upregulation of several MAP kinases in mouse hippocampus

Haege S.(1), Schmitt U.(1), Hiemke C.(1), Haaf T.(2), Galetzka D.(2)
1) Department of Psychiatry, University of Mainz, Mainz, Germany
2) Institute of Human Genetics, University of Mainz, Mainz, Germany
The hippocampus is essential for learning and memory in rodents. Because neuronal plasticity is generally associated with de novo gene expression, learning of a specific task can be expected to induce specific changes in gene expression. To test this hypothesis, we trained mice in the Morris Water Maze (MWM) test and used microarray technology, reverse Northern blots, and real-time PCR to study gene expression in the hippocampus at several time points. We constructed a specialized cDNA microarray chip containing genes involved in learning and

memory to quantify mRNA expression in dissected hippocampi. C57Bl/6 x FVB mice were given four consecutive trials in the MWM. Mice were divided into three groups: complete controls (CC, test naive mice), swim learners (SL, animals learning to find a hidden platform), and swim controls (SC, MWM without platform - motion and affective controls). Comparative microarray hybridization of SC versus CC hippocampal RNA samples (SC/CC) reveals stress-related expression differences, whereas the SL/SC comparison detects expression differences due to spatial learning in the MWM task. Hippocampal RNA pools (of at least 5 animals each) of CC, SC, and SL mice were prepared at 1 h, 6 h, and 24 h after training and hybridized to the gene chip. We found that four trials were not sufficient for the induction of learning-related gene expression. No differentially regulated genes were detected in the SL/SC comparison at these time points. However, several MAP kinase genes including Mapk8ip (JIP1), Mapk3 (ERK1), and Mapk14 (p38) were upregulated in the SC/CC experiment. We propose that stress-induced upregulation of these genes is important for possible learning effects at later time points.

P286

The functional relevance of DNA polymorphisms in transmissible spongiform encephalopathies

Kashkevich K.(1), Humeny A.(1), Fischer C.(2), Schiebel K.(1)

1) Institut f. Biochemie, Emil-Fischer-Zentrum, Erlangen, Germany

2) Institut f. Humangenetik, Heidelberg, Germany

TSE are caused by either infections, inheritance or spontaneously occurrence. In human, around 5 % of Creutzfeldt-Jakob diseases (CJD) are due to mutations within the prion protein gene (PRNP). Two other, phenotypically different diseases are caused by mutations within the same gene. In GSS, PRNP mutations differ compared to CJD, fatal familial insomnia, however, is caused by a mutation frequently found in CJD leading to a D179N exchange. Both diseases differ with respect to a single SNP resulting in either a M or V at position 129. This frequent SNP was shown to influence age of onset, duration and susceptibility to prion infection.

The infectious form of TSE is transmitted by a misfolded prion protein which is able to induce a misfolding of the endogenous prion protein resulting in neurodegeneration. Humans homozygous for M129 appear to be more susceptible to infections by meat from BSE affected cows since all humans analyzed so far to suffer from the new variant of CJD, have been genotyped MM. Genotype-dependent susceptibility/resistance to Scrapie is also well known in sheep, where three polymorphic positions in PRNP influence the predisposition to the disease.

As little information on bovine DNA polymorphism influencing BSE susceptibility was available, we analyzed both the coding and promoter region of the bovine PRNP and isolated several SNPs and a 12 bp indel polymorphism creating or deleting a Sp1 binding site. As a strong correlation between PRNP expression and the age of onset of TSE infections was demonstrated in mice, we analyzed different haplotypes of PRNP promoter constructs in luciferase assays demonstrating the influence of varying polymorphisms on the expression. Comparative genotyping of control and BSE positive cows revealed a significantly different allele distribution for the 12 bp indel and an additional SNP in the promoter region. These results indicate that in cows there may be an influence of the genotype on BSE susceptibility as well.

P287

Severe phenotype with compound heterozygous mutations in PMP22

Niedrist D.(1), Joncourt F.(2), Mátyás G.(1), Müller A.(3)

1) Institut für medizinische Genetik der Universität Zürich, Schwerzenbach, Switzerland

2) Medizinische Universitäts-Kinderklinik, Abteilung für Humangenetik, Bern, Switzerland

3) Schulthess Klinik, Neurologie, Zürich, Switzerland

We report on a 20-year-old boy with polyneuropathy, loss of muscle mass, progressive scoliosis, and hammer toes. In early childhood the boy had problems with weight gain and frequent vomiting. Apart from walking late (at the age of 24 months) there was no hint for psychomotor retardation. Since puberty a progressive scoliosis developed. At clinical examination at 20 years of age there were pes cavus deformity, kyphoscoliosis, hammer toes, gait disturbance but no dysmorphic features. On neurological examination there were discrete interosseus atrophy and positive sign of Trendelenburg. Walking on the heels was not possible, Romberg was pathologic. There was atrophy of the lower leg muscles including both extensor digiti brevis muscles and intrinsic plantar feet muscles. He had limited sense for vibration over both the carpal regions and the lower leg. The reflexes of the peripheral muscles were missing.

In electrophysiology motor nerve conduction velocity was not measurable on the lower and upper arm and on the N. Peronaeus. Motor nerve conduction velocity was reduced for N. Tibialis with 43m/s. Sensoric nerve conduction velocity was not measurable for the N. Medianus, N. Ulnaris and N. Suralis. The analysis of the PMP22 gene was performed and the heterozygous mutations c.276delG and p.T118M were found in the patient. The father of the patient was also heterozygous for p.T118M. However, neither the father nor the mother were carrier of the mutation c.276delG.

The p.T118M substitution is known in the literature and controversially discussed, as to whether it is a polymorphism or a pathogenic recessive mutation. The mutation c.276delG has not yet been described in the literature in association with CMT or HNPP. This deletion probably causes an early stop codon and a truncated gene product (p.G94AfsX17). We suppose that the deletion c.276delG in association with the mutation p.T118M in the PMP22 gene are responsible for the severe phenotype in our patient.

P288

The search for point mutations in the dystrophin gene using DGGE

Mannefeld M.(1), Kress W.(1), Meng G.(1), Müller-Reible C.(1)

1) Institut für Humangenetik, Würzburg, Germany

The complex genomic structure of the large dystrophin gene (79 exons, 7 promotors, 2.4 Mb) is still a challenge for efficient point mutation detection strategies. To establish and test the efficiency of denaturing gradient gel electrophoresis (DGGE), we selected 56 patients with a Duchenne muscular dystrophy phenotype who had no dystrophin in immunhistological stainings and in the Western blots on their muscle biopsies. They were pre-screened by multiplex deletion analysis using the primer sets of Beggs (1990) and Chamberlain (1988) et al. consisting of 20 amplicons in the deletion hot spot regions of the dystrophin gene. Next, MLPA (multiplex ligation-dependent probe amplification, MRC-Holland) analysis was applied to exclude rare deletions or duplications which are not detected by multiplex PCR. After MLPA, 2 of the 56 patients with rare deletions and 11 with a duplication were excluded from the study.

Multiplex DGGE analysis of all 79 exons and the muscle promoter was performed in 95 amplicons for the remaining 43 patients (Hofstra et al., Hum Mutat 2004). The sensitivity of DGGE was validated using 10 known point mutations, nine of them were recovered (sensitivity 90 %). While aberrant fragments were observed in all patients in at least one exon, after direct sequencing only 21 patients harboured small ("point") mutations, which abolished protein expression. In the rest of the patients (22) no cause for the total lack of dystrophin protein could be found. Biological reasons for these disappointing result are discussed.

P289

Generation and characterization of SCA3 mutant mice.

Hübener J.(1), Reinbold M.(1), Vauti F.(2), Arnold H.-H.(2), Grundmann K.(1), Metzger S.(1), Schmidt T.(1), Riess O.(1)

1) Department of Medical Genetics, Tübingen, Germany

2) Department of Cell- and Molecular Biology, Braunschweig, Germany

Machado-Joseph disease (MJD) or Spinocerebellar Ataxia Type 3 (SCA3) is the most common type of SCA worldwide. SCA3 is an autosomal dominant neurodegenerative disorder caused by polyglutamine-expanded ataxin-3, whose function is still unknown. To better understand the function of Ataxin-3 in vivo, we analysed Ataxin-3 mutant mice generated by the gene trap (GT) approach. Atx3GT/GT survive embryogenesis and do not show obvious developmental defects. Detailed macroscopic and microscopic analysis of heterozygous and homozygous mutant mice revealed no major histological abnormalities. For screening of neurological symptoms we first performed the modified SHIRPA test in combination with footprint analysis. In a secondary screen we looked for the motor performance on rotarod and beam walking test. After a period of six month, we were not able to detect any behavioral differences between heterozygous and homozygous mutant mice in motor coordination and motor learning on the rotar-

od and beam walking test compared to that of their wild-type littermates. These results demonstrate that ataxin-3, a widely expressed protein, seems not to be essential for developing or during adult survival of Atx3GT/GT mice.

P290

Modulation of axon growth in cultured motoneurons from a mouse model of spinal muscular atrophy

Lechner B.(1), Jablonka S.(1), Sendtner M.(1)
1) Institut für Klinische Neurobiologie / Universität Würzburg, Würzburg, Germany

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by loss of α-motoneurons in the spinal chord due to low levels of survival motor neuron (SMN) protein. The genetic cause is the homozygous loss or mutation of the telomeric SMN1 gene and retention of the centromeric SMN2 gene, whose transcripts consist of about 90% truncated and unstable and only 10% functional protein. Motoneurons of Smn-deficient/SMN2 transgenic mouse embryos, compared to wildtype controls, exhibit shorter axons and smaller growth cones associated with reduced β-actin protein and mRNA in the distal part of their axons. β-actin plays a major role in growth cone motility and transmitter release at the presynapse. In addition, we hypothesize that SMN is part of a complex for transporting β-actin mRNA, which is known to be localized and locally translated in distal axons and growth cones. As described by Majumder et al. (J Biol Chem. 2004), the SMN promotor contains a CREB site, and Zhang et al. (J. Cell Biol. 1999) reported a cAMP dependent increase in β-actin mRNA transport towards distal axons and in local β-actin synthesis. We investigated the effects of elevated cAMP on β-actin accumulation in distal axons and axonal growth parameters in Smn-deficient primary motoneurons. Motoneurons of 14 days old Smn-/-; SMN2 transgenic and wildtype mouse embryos were cultured on Laminin-1 for 7 days with 100µM cAMP and the neurotrophic factors BDNF and CNTF. Fluorescence staining and digital measurements revealed a major effect of cAMP treatment on β-actin distribution and growth cone size. These parameters were restored to normal, whereas neurite length remained unaffected. Western blots with neural stem cells (NSC) and Smn+/-; SMN2 transgenic motoneurons treated with 100µM cAMP showed an upregulation of Smn protein levels which could be due to activation of the CREB site in the Smn/SMN2 promotor.
These data point to a potential role of cAMP as target of SMA drug therapy.

P291

In vitro and ex vivo evaluation of second generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy

Hahnen E.(1), Eyüpoglu I.Y.(2), Brichta L.(1), Haastert K.(3), Tränkle C.(4), Siebzehnrübl F.A.(5), Riessland M.(1), Hölker I.(1), Claus P.(3), Romstöck J.(2), Buslei R.(5), Blümcke I.(5), Wirth B.(1)
1) Institute of Human Genetics, Institute of Genetics, and Center for Molecular Medicine Cologne (CMMC), Cologne, Germany
2) Department of Neurosurgery, Erlangen, Germany
3) Department of Neuroanatomy, Hannover Medical School, and Center for Systems Neuroscience (ZSN), Hannover, Germany
4) Department of Pharmacology & Toxicology, Institute of Pharmacy, Bonn, Germany
5) Department of Neuropathology, Erlangen, Germany

Amongst a panel of histone deacetylase (HDAC) inhibitors investigated, suberoylanilide hydroxamic acid (SAHA) evolved as a potent and non-toxic candidate drug for the treatment of spinal muscular atrophy (SMA), an alpha-motoneuron disorder caused by insufficient survival motor neuron (SMN) protein levels. We show that SAHA elevates SMN levels at low micromolar concentrations in several neuroectodermal tissues, including rat hippocampal brain slices and motoneuron-rich cell fractions, and confirm its therapeutic capacity using a novel human brain slice culture assay. SAHA activates the survival motor neuron gene 2 (SMN2), the target gene for SMA therapy, as shown in fibroblast cell lines from SMA patients. It inhibits histone deacetylases at submicromolar doses, giving evidence that SAHA is more efficient than the HDAC inhibitor valproic acid which is under clinical investigation for SMA treatment. In contrast to SAHA, the compounds CBHA, SBHA and M344 display unfavourable toxicity profiles, while MS-275 fails to increase SMN levels. Clinical trials revealed that SAHA, which is under investigation for cancer treatment, has a good oral bioavailability and is well tolerated, allowing to achieve in vivo concentrations shown to elevate SMN levels. Since SAHA crosses the blood-brain barrier, oral administration may be useful to decelerate progressive alpha-motoneuron degeneration by epigenetic SMN2 gene activation. In vivo validation of SAHA using SMA-like mice (Smn-/-SMN2+) is in progress.

P292

Homozygosity mapping of an unknown syndrome of autosomal-recessive spinocerebellar ataxia, spastic paraplegia and mental retardation to chromosome 19p13.3

Maier C.(1), Landwehrmeyer B.(2), Vogel W.(1), Steinbach P.(1)
1) Universitätsklinikum Ulm, Abt. Humangenetik, Ulm, Germany
2) Universitätsklinikum Ulm, Abteilung Neurologie, Ulm, Germany

A consanguineous family of Italian origin presented with a previously undiagnosed syndrome of autosomal-recessive spinocerebellar ataxia in three sibs, two sisters and a brother. Three further sibs were unaffected. The disease manifested itself in neonatal life and showed a slow progression. Associated features included cerebellar atrophy, spastic paraplegia of lower limbs, and developmental delay. The three affected sibs along with three healthy siblings and their consanguineous parents were genotyped at 400 microsatellite loci included in the ABI MD10 panel. Marker genotypes were checked manually for consistency with disease allele transmission, and the data were further confirmed by a genomewide multipoint linkage analysis using GENEHUNTER 2.0. Among two initially determined regions of interest at chromosomes 4q and 19p, both of which giving initial LOD scores of 2.4, further finemapping pointed to only one putative disease locus at 19p13.3 (LOD = 3.0). The critical region spans 5.8 Mb or 15 cM, and is flanked by informative recombinations at D19S216 and D19S581. The extreme gene density in this particular genomic region provides us with approximately 160 candidate genes. Herefrom, two members of the glial-cell-line-derived neurotrophic factor ligands (GFLs), namely neurturin (NRTN) and persephin (PSPN), have been excluded by mutational screaning using exon sequencing.

P293

The benzamide M344, a novel histone deacetylase inhibitor, significantly increases SMN2 RNA/protein levels in spinal muscular atrophy (SMA) cells

Riessland M.(1), Brichta L.(1), Hahnen E.(1), Wirth B.(1)
1) Institute of Human Genetics, Institute of Genetics, and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Proximal spinal muscular atrophy (SMA) is a common autosomal recessively inherited neuromuscular disorder causing infant death in half of all patients. Homozygous loss of the survival motor neuron 1 (SMN1) gene function is the molecular cause of SMA. SMN2, a highly homologous gene copy in the 5q13 region, produces only too low amounts of a protein identical to SMN1, but represents a promising target for an SMA therapy. Due to a silent mutation within an exonic splicing enhancer, SMN2 transcripts are aberrantly spliced to about 90%, thus lacking exon 7. However, the correct splicing of SMN2 can be efficiently restored by over-expression of the endogenous SR-like splicing factor Htra2-β1 as well as by exogenous factors like drugs that inhibit histone deacetylation.
Here, we show that the novel benzamide M344 which belongs to the class of histone deacetylase (HDAC) inhibitors up-regulates SMN2 protein expression in fibroblast cells derived from SMA patients up to 6-fold after 64h of treatment. This is the strongest effect of a drug on the SMN protein level reported so far. The significant reversion of Δ7-SMN2 into FL-SMN2 transcripts as demonstrated by quantitative RT-PCR is most likely facilitated by significantly elevated levels of the splicing factor Htra2-β1. Investigations of the cytotoxicity of M344 using an MTT assay revealed toxic cell effects only at very high concentrations. In conclusion, M344 can be considered as highly potent HDAC inhibitor which is active at low doses and therefore a promising candidate for a causal therapy of SMA.

P294

KCC3 mutations associated with Andermann Syndrome

Uyanik G.(1), Elcioglu N.(2), Gross C.(3), Penzien J.(4), Yilmaz Y.(5), Ölmez A.(6), Demir E.(7), Wahl D.(8), Winner B.(1), Topaloglu H.(6), Hehr U.(3), Winkler J.(1)
1) Department of Neurology, University of Regensburg, Regensburg, Germany
2) Department of Pediatric Genetics, Maramara University, Istanbul, Turkey
3) Center for Human Genetics, Regensburg, Germany

4) Department of Pediatrics, Klinikum Augsburg, Augsburg, Germany
5) Department of Pediatric Neurology, Maramara University, Istanbul, Turkey
6) Department of Neuropediatrics, Hacettepe University, Ankara, Turkey
7) Department of Child Neurology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey
8) Praxis für Humangenetik, Augsburg, Germany

Andermann Syndrome (OMIM 218000) is an autosomal recessive disorder characterized by developmental as well as neurodegenerative defects. It is also known as hereditary motor-sensory neuropathy associated with agenesis of the corpus callosum (HMSN/ACC). It has a high prevalence in the French-Canadian (FC) population in the Saguenay-Lac-St-Jean region of the province of Quebec due to a founder effect. Clinically, the patients suffer from a severe sensory-motor polyneuropathy with areflexia. A variable degree of agenesis of the corpus callosum (ACC) is detected by neuroimaging, where most of the cases show a complete ACC. Additional dysmorphic features may be present like syndactyly, over riding first toe, high arched palate, hypertelorism, and brachycephaly. The affected children present with hypotonia, amyotrophy, and psychomotor retardation with persistant hallucinosis during adolescence in some cases.
Genetically, truncating mutations of the KCC3 gene, also known as SLC12A6, on 15q13-q14 have been associated with Andermann Syndrome.
Here, we describe three cases of Andermann syndrome from Germany and Turkey. We detected two different truncating mutations in the first patient, a homozygous truncating mutation in the second and a homozygous missense mutation in the third patient. Our study for the first time demonstrates, that not only truncating but also missense mutations of the KCC3 gene cause Andermann Syndrome.

P295

Cryptic complex de novo chromosomal rearrangement characterized by conventional cytogenetics, FISH and array CGH in a patient with schizoid psychosis, borderline personality disorder and a microdeletion of 1p31.3.

Kroisel P.M.(1), Klopocki E.(2), Ullmann R.(3), Kistner G.(4), Trimborn M.(5), Roschack K.(1), Kordaß U.(1), Tönnies H.(5)
1) Institute of Human Genetics, Greifswald, Germany
2) Charité Universitätsmedizin Berlin, Institute of Medical Genetics, Berlin, Germany
3) Max Plank Institute for Molecular Genetics, Molecular Cytogenetics, Berlin, Germany
4) Labor für Humangenetische Diagnostik, Rampe, Germany
5) Institute of Human Genetics, Berlin, Germany

Schizophrenia and borderline personality disorders (BPD) are rather frequent in the human population with up to 1% of individuals affected during lifetime. Linkage studies provide overwhelming evidence that genetic factors are involved but inheritance is complex with just a few genes of presumed relevance and predisposing loci identified yet. Recently, positional cloning efforts from familial balanced chromosomal aberrations in affected patients were successfully applied to unravel underlying molecular mechanisms. Here we report on a 20 year old patient with early onset of schizophrenia and BPD. Her phenotype is essentially normal with just mild dysmorphic facial features and moderate mental retardation. Although she is self sustained despite repeated therapy she shows severe depressions, frequent suicide attempts and cannot take care of her baby. Upon genetic counselling a cytogenetic analysis was performed. A minute insertion of chromosomal material of initially unknown origin into 4p was found in the patient, but not in her parents or her son. High resolution GTG-banding and FISH using wcp probes identified that the inserted segment is derived from 1p. However, the insertion seemed to be smaller in size than the deletion of 1p. High resolution array CGH analysis revealed a more complex chromosome rearrangement (CCR) with an interstitial microdeletion of 700kb at the proximal part of 1p31.3, insertion of 1p32.1-p32.3 into 4p15.2 and insertion of the adjacent distal part of 1p31.3 into 2p31.
This study convincingly shows the power of advanced array CGH techniques to identify cryptic CCRs in complex neurogenetic disorders. Further investigations are required to characterize the five chromosomal breakpoints in detail. It is very intriguing that just now another translocation involving 1p31.2 and a breakpoint affecting the PDE4B gene was shown to be related to the occurrence of schizophrenia by regulation of cAMP signalling pathway (Millar K et al. Science Nov. 2005).

P296

Identification of potential gene modifiers in SMA discordant families

Oprea G.(1), Wirth B.(1)
1) Institut of Human Genetics, Institute of Genetics and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany

Spinal muscular atrophy (SMA) is an autosomal recessive disorder which affects the α-motor neurons of the spinal cord. SMA is caused by deletion and/or mutations in the survival motor neuron gene 1 (SMN1) which is duplicated on chromosome 5q13. The second copy (SMN2) differs in only 5 bp. Most of the SMN2 transcripts undergo alternative splicing of exon 7, which encode a truncated and unstable SMN protein. In rare cases, sibs with identical SMN1 mutations and identical SMN2 copies can show variable phenotypes from unaffected to affected indicating the existence of SMA modifying factor(s). Recently we showed that the modifier is influencing the SMN protein level. Using Affymetrix micro-array analysis, the expression profile of RNA from EBV-transformed cell lines belonging to one discordant family (2 sibs unaffected and 2 sibs affected, all having homozygous absence of SMN1 and identical SMN2 copy number), 2 type I and 2 type III SMA patients revealed up-regulation of 6 genes differentially expressed above 2-fold threshold. All the candidate genes were found to be expressed in the brain, spinal cord and muscle, tissues which are involved in the development and progression of the SMA. After validation on RT-PCR in 4 further discordant families, 2 promising transcripts were identified. So far, no mutation within the coding and promoter region was found. However, an in vivo protein interaction with SMN was shown for one candidate so far. This modifier candidate is localized on the X chromosome which correlates with the striking observation that except for a few cases, all milder or asymptomatic siblings in these families are females. Indeed, only these particular individuals showed expression of this potential modifier at RNA and protein level. These interesting findings open new prospects to better understand the mechanism of pathology and the regulation of the SMN protein, as well as the opportunity to develop additional therapies for SMA.

P297

Hypertension in a mouse model for Andermann syndrome

Rust M.B.(1), Faulhaber J.(2), Budack M.(2), Pfeffer C.(1), Maritzen T.(1), Didie M.(3), Boettger T.(1), Beck F.-X.(4), Schubert R.(5), Ehmke H.(2), Jentsch T.J.(1), Hübner C.A.(6)
1) ZMNH, Universität Hamburg, Hamburg, Germany
2) Institut für Physiologie, Universitätskrankenhaus Hamburg-Eppendorf, Hamburg, Germany
3) Institut für Pharmakologie, Universitätskrankenhaus Hamburg-Eppendorf, Hamburg, Germany
4) Institut für Physiologie München, Ludwig-Maximilians-Universität, München, Germany
5) Institut für Physiologie, Universität Rostock, Rostock, Germany
6) Institut für Humangenetik, Universitätskrankenhaus Hamburg-Eppendorf, Hamburg, Germany

The neurodegenerative disorder Andermann syndrome is characterized by polyneuropathy, variable agenesis/hypoplasia of the corpus callosum and mental retardation. It is caused by mutations of the K-Cl co-transporter KCC3 encoded by SLC12A6. Mice with a targeted disruption of the corresponding gene reproduce neurodegeneration of the peripheral and central nervous system (CNS) and display arterial hypertension. Whether hypertension is a feature of the human disorder is not yet clear. As Kcc3 is expressed in vascular smooth muscle cells, it may influence myogenic tone and hence blood pressure. We measured the chloride concentration in vascular smooth muscle cells of Kcc3-/- mice. It was indeed increased in arteries relevant for blood pressure regulation. Isolated arteries, however, reacted indistinguishably to changes in intravascular pressure, stimulation of a1-adrenoreceptors, exogenous nitric oxide or blockade of calcium-activated chloride channels. Likewise, the responses to a1-adrenergic stimulation or exogenous nitric oxide in vivo were identical in both genotypes. These results argue against a major vascular-intrinsic component of arterial hypertension in Kcc3-/- mice. In contrast, either a1-adrenergic blockade or inhibition of ganglionic transmission abolished hypertension. This indicated a neurogenic origin of this phenotype, which was further supported by an increase of urinary catecholamine excretion in Kcc3-/- mice. Our data indicate that local control of myogenic tone does not require KCC3 and that hypertension in Kcc3-/- mice is a consequence of an elevated sympathetic tone.

P298

Characterization of JJAZ1/SUZ12 mediated type-2 NF1 deletions

Kehrer-Sawatzki H.(1), Kluwe L.(2), Mautner V.-F.(2)
1) University of Ulm, Department of Human Genetics, Ulm, Germany
2) University Hospital Hamburg-Eppendorf, Department of Maxillofacial Surgery, Hamburg, Germany

Heterozygous NF1 deletions that encompass more than 1-Mb are the most common recurrent mutations in Neurofibromatosis type 1. Clinically, NF1 deletions are of interest as they have been reported to be associated with early onset of tumor growth, mental retardation, facial dysmorphism, high risk for malignancy and cardiovascular malformations. About 65% of NF1 deletions span 1.4-Mb and have breakpoints in the NF1-LCRs that flank the NF1 region (type-1 deletions). The second most common type among large NF1 deletions encompasses 1.2-Mb. The breakpoints of these type-2 deletions are localized in the JJAZ1/SUZ12 gene and its pseudogene. Aberrant recombination between both is the mechanism underlying these deletions. We have identified 11 patients with type-2 deletions and 10 of them are mosaics. The high frequency of mosaicism with normal cells implies that type-2 deletions arise somatically. Microsatellite-marker and SNP analyses indicate that type-2 deletions originate from intra-chromosomal aberrant recombination, in contrast to the type-1 deletions that are mostly caused by inter-chromosomal recombination during maternal meiosis. The reasons accountable for this region preference during somatic versus meiotic recombination are unknown. We have identified the breakpoints in 8 of the 11 type-2 deletions and observed considerable breakpoint heterogeneity within the JJAZ1 gene. Since the breakpoints are scattered within 30-kb of the JJAZ1 gene and even cases of non-homologous recombination were identified, breakpoint clustering due to a recombination hotspot as observed in type-1 deletions is rather unlikely to be associated with type-2 NF1 deletions. Currently, we are performing structural and sequence complexity analyses in order to identify sequence motifs or structural variants like polymorphic inversions that predispose to rearrangements underlying type-2 NF1 deletions.

P299

Young-onset restless legs syndrome in a German family: Evidence for genetic heterogeneity

Hedrich K.(1), Neumann A.(2), Muhle H.(2), Lohnau T.(1), Kramer P.L.(3), Klein C.(1), Stephani U.(2)
1) Department of Neurology, Luebeck, Germany
2) Department of Neuropediatrics, Kiel, Germany
3) Department of Neurology, Portland, OR, United States of America

Restless legs syndrome (RLS) is a common sensory-motor disorder with a reported prevalence of up to 15%. It is characterized by paraesthesias and an intense urge to move the legs. The symptoms predominantly occur at rest, in the evening and during the night leading to sleep disturbances. Movements relieve symptoms. The pathophysiology of RLS is poorly understood and may be related to dopamine transmission insufficiency and low iron storage in substantia nigra neurons. RLS is idiopathic or secondary (usually associated with iron deficiency). Although there is a high familial aggregation, no gene mutation has yet been found in idiopathic RLS. Three gene loci for RLS have been reported on chromosomes 12q, 14q, and 9p. RLS is currently underdiagnosed and especially in children probably often misinterpreted as attention deficit hyperactivity syndrome.
We report here an RLS family with 37 members in four generations including 11 definitely affected and seven probably affected (by history) individuals. DNA was available for 12 members including six definitely and two probably affected cases. The mean age at onset in our RLS cases was 8 years (range 2 – 16 years). Clinical findings were compatible with typical RLS including a desire to move the limbs, partially associated with paraesthesias. In the evening, patients showed motor restlessness with worsening at rest. Affected children often could not stay in bed, resulting in sleep disturbance and daytime fatigue. To evaluate the role of the known RLS gene loci in this family, we performed genotype analysis using a total of 27 microsatellite markers on chromosome 12q, 14q, and 9p. This excluded linkage to all three known loci indicating at least a fourth gene for RLS. We will perform a genome-wide linkage analysis to identify the genetic cause of RLS in this family.
In conclusion, our findings further underline the genetic heterogeneity of RLS and demonstrate familial aggregation also in young-onset RLS.

P300

Trinucleotide repeat expansion in spinocerebellar ataxia type 17 (SCA17) alleles of the TATA-box binding protein (TBP) gene: An evolutionary approach

Tomiuk J.(1), Bachmann L.(2), Bauer C.(3), Rolfs A.(4), Schöls L.(5), Roos C.(6), Zischler H.(7), Schuler M.M.(1), Bruntner S.(1), Riess O.(3), Bauer P.(3)
1) Institute of Human Genetics, General Human Genetics, Tübingen, Germany
2) University of Oslo, Department of Zoology, Oslo, Norway
3) Institute of Human Genetics, Medical Genetics, Tübingen, Germany
4) Department of Neurology, Rostock, Germany
5) Hertie-Institute for Clinical Brain Research, Tübingen, Germany
6) German Primate Center, Primate Genetics, Göttingen, Germany
7) Institute of Anthropology, Mainz, Germany

Evolutionary studies of genetic diseases improve our understanding of processes determining their epidemiology in human populations. In this study, we focussed on the variability and mutational changes of the CAG microsatellite region in the TATA-box binding protein gene (TBP). In humans, an increase in CAG/CAA repeat number above a threshold of about 42 repeats may lead to the manifestation of spinocerebellar ataxia 17 (SCA17). Firstly, we studied the length variation of the microsatellite of 1394 probands from central European populations. Secondly, we sequenced the microsatellite region of the TBP gene of 25 unrelated individuals from northern Germany (10 SCA17 patients and 15 control individuals). In addition, the microsatellite was sequenced from individuals of ten northern German families with at least one family member affected by SCA17. To study also evolutionary changes of this CAG/CAA microsatellite in non-human primates, the homologous regions were also analysed from Pan troglodytes, Gorilla gorilla, Pongo pygmaeus, P. abellii, Hylobates lar, Nomascus leucogenys, Symphalangus syndactylus, Macaca mulatta, Papio hamadryas, Colobus polykomos, and Callithrix jacchus.
Three major conclusions were drawn from the results: (1) Patterns of synonymous CAA interruptions in the microsatellite are characteristic and can be explained by selection for stabilizing the length of the repetitive region; (2) Interspecific comparison indicates that expansion of microsatellite region is a human trait. The most common allele in humans (37 repeats) is close to the threshold value upon which neurodegenerative changes can occur and may act as a repository for expanded, pathogenic alleles; (3) The cassette-like structure of five out of 17 expanded alleles can be attributed to unequal crossing over that is here considered the dominant process creating drastic increases of the repeat number. This can explain the observed rare and sporadic founder events through de novo mutations.

P301

Mutation analysis in the Filamin A (FLNA) gene in patients with periventricular nodular heterotopia and the finding of a new stop mutation in exon 10

Mainberger A.(1), Pohl K.(2), de Vries B.(3), Korenke C.(4), Newbury-Ecob R.(5), Kohlhase J.(1), Morris-Rosendahl D.(1)
1) Institute für Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
2) Department of Paediatric Neurology, Guy's Hospital, London, United Kingdom
3) Department of Human Genetics, University Medical Centre Nijmegen, Nijmegen, Netherlands
4) Zentrum für Kinder- und Jugendmedizin, Klinikum Oldenburg, Oldenburg, Germany
5) Children's Centre, Royal United Hospital, Bath, United Kingdom

X-linked bilateral periventricular nodular heterotopia (BPNH MIM#300049) is a human neuronal migration disorder in which many neurons destinated for the cerebral cortex fail to migrate. Consequently, bilateral heterotopic nodules of gray matter accumulate along the lateral ventricles. Mutations in the Filamin A gene, which has 48 exons on Xq28 (FLNA) have been identified in patients with BPNH. Most of the patients are female and a high prenatal lethality in males is assumed. Affected women usually have epilepsy of variable severity and normal intelligence or mild borderline cognitive impairment. In male patients somatic PNH mosaicism has been hypothesized. To date 16 mutations in the FLNA gene causing PNH have been identified. Seven of them are single base pair substitutions, five splice site substitutions and four small deletions. In this study we analysed 8 patients with BPNH from 7 families. In one family we identified a stop mutation in exon 10 in the patient and the affected mother (c.1667G>A,Trp556term). In another patient we identified a synonymous base pair exchange in exon 15 (c. 2313A/G) postulated to change an exon splice enhancer.

Documentation of the diversity of mutations in FLNA will assist genotype-phenotype correlations and may contribute to early diagnosis.

P302

No evidence that SNP rs175174 in ZDHHC8 contributes to the risk for schizophrenia in a German population Analysis of a putative functional intronic marker in case-control and family-based association studies

Gawlik M.(1), Faul T.(1), Pfuhlmann B.(1), Jabs B.(1), Stöber G.(1), Bauer M.(2), Jung S.(2), Knapp M.(3)
1) Department of Psychiatry and Psychotherapy, University of Würzburg, Würzburg, Germany
2) Department of Forensic Medicine, University of Würzburg, Würzburg, Germany
3) Institute of Medical Biometry, Informatics and Epidemiology, University of, Bonn, Germany

Background: In large genome scans there has been consistent evidence that chromosom 22q11 is one of the major candidate loci for susceptibility genes of schizophrenia. ZDHHC8, located on chromosome 22q11, has been recently known as a new candidate gene for schizophrenia. The gene product belongs to a family of palmitoyltransferases involved in brain development and signalling pathways. ZDHHC8 has been proposed as candidate gene by animal models and human association studies. The genetic evidence has mainly been sexual distinctive by significant over-transmission of allele A in female, but not in male subjects.
Methods: Using the TaqMan® technology, we examined rs175174 in 204 German proband-parent triads and in an independent case-control study (schizophrenic cases: n = 433; controls: n = 186).
Results: We found a weak gender specific effect as in triads heterozygous parents transmitted allele G preferentially to females, and allele A to males (heterogeneity $\chi 2 = 4.43$; $p = 0.035$). The case-control sample provided no further evidence for overall or gender-specific effects regarding allele and genotype frequency distributions.
Conclusion: We found no consistent evidence that ZDHHC8 is associated with susceptibility to schizophrenia.

P303

A combined approach of real-time quantitative PCR and DNA sequencing reveals many new mutations in the LIS1 gene and questions previous genotype-phenotype correlations in patients with isolated lissencephaly

Morris-Rosendahl D.(1), Zeschnigk C.(1), Ceranski-Schwerda C.(1), Berman Y.(2), Klapecki J.(3), Mangold E.(4), Borozdin W.(1), Kohlhase J.(1)
1) Institute for Human Genetics and Anthropology, University of Freiburg, Freiburg, Germany
2) Department of Clinical Genetics, The Children's Hospital at Westmead, Sydney, Australia
3) Department of Genetics, National Institute of Mother and Child, Warwaw, Poland
4) Institute for Human Genetics, University of Bonn, Bonn, Germany

Lissencephaly is a term used to describe neuronal migration disorders that lead to absent or reduced gyration and a broadened, but poorly organized cortex. The most common form of lissencephaly is isolated, also referred to as classical or type 1 lissencephaly, and results from mutations in the LIS1 (chromosome 17p13) or DCX (chromosome Xq22) genes. The most frequently found mutation for type I lissencephaly is the complete deletion of the LIS1 gene, detectable by Fluorescence In Situ Hybridization (FISH). In contrast, intragenic mutations in the LIS1 gene are less common. We have previously used DNA sequencing to perform mutation analysis in the LIS1 gene and have detected 8 mutations, 6 of which have not previously been described, including a somatic mosaic mutation in a patient with a mild phenotype. Intragenic deletions have to date only been detected via Southern blotting. Recently we have established quantitative PCR to detect intragenic deletions in the LIS1 gene and have observed deletions in two of 9 patients analysed. One deletion includes exons 6 and 7 of the gene, the other the last two exons (10 and 11) and part of the 3' region. The deletions have been confirmed via long-range PCR or Southern blotting. Previous mutation analysis in the LIS1 gene has emphasized a relatively strong genotype-phenotype correlation. Mutations affecting the last (seventh) WD domain of the protein have not previously been described, however, three of our new mutations involve exon 11 and therefore WD7, and illustrate the importance of this domain. Phenotype-genotype comparison in our patients indicates that other factors may be important in determining the severity of isolated lissencephaly.

P304

Inactivation of the mouse Machado-Joseph-Disease (MJD) gene

Schmitt I.(1), Evert B.O.(1), Wüllner U.(1)
1) University of Bonn, Department of Neurology, Neurobiology, Bonn, Germany

Spinocerebellar ataxia type 3 (or Machado-Joseph-disease; SCA3/MJD), the most common autosomal dominantly inherited ataxia, is a neurodegenerative disease caused by expansion of a polyglutamine domain in the protein Ataxin-3. When the number of glutamines surpasses a pathogenic threshold, expansion of the polyglutamine stretch leads to conformational changes of the protein with subsequent oligomerisation, formation of amyloid-like fibrils and intranuclear protein aggregation (NI). Similar to Huntington's disease (HD), homozygous SCA3 patients with two mutant allels show a more severe disease phenotype than those with a single mutant allel. Thus, in addition to a toxic gain of function, specific protein-protein interactions and possible impaired physiological functions as de-ubiquitinating protease and transcriptional co-repressor appear to contribute to the pathogenesis of SCA3. Interestingly, normal non-expanded ataxin-3 is sequestered into NI in SCA3 and other polyglutamine diseases and experiments in drosophila raised the possibility that the loss of physiological ataxin-3 contributes to the pathology in SCA3 and HD. To gain insight into the function of wild-type ataxin-3 and to verify a protective role of normal ataxin-3, we generated MJD knockout mice by targeted mutagenesis. Heterozygous mice exhibit reduced levels of ataxin-3 in western blot analysis without any phenotype. After mating of heterozygotes, a normal Mendelian ratio is observed except that 40% of the mice lacking MJD are females. Nullizygous mice are viable and fertile and lack ataxin-3 in all tissues tested (brain, heart, lung, liver, kidney, spleen, muscle, tetis). Behavioral and biochemical analyses of putative ataxin-3 related functions will help to unreveal the physiological role of ataxin-3.

P305

Identification of Alu elements mediating a partial PMP22 deletion

Rautenstrauss B.(1), Matejas V.(2), Thiel C.(2), Sommer C.(3), Jakubiczka S.(4), Huehne K.(2)
1) Friedrich-Alexander-Universität, Institut für Humangenetik, Erlangen, Germany
2) Institut für Humangenetik, Erlangen, Germany
3) Neurologische Klinik, Würzburg, Germany
4) Institut für Humangenetik, Magdeburg, Germany

Hereditary neuropathy with liabilty to pressure palsies (HNPP) is most frequently caused by deletion of a 1.4-Mb region in chromosome 17p11.2-12 including the peripheral myelin protein 22 (PMP22) gene. Smaller deletions affecting partially the PMP22 gene are less frequently observed. We identified in a HNPP patient a deletion of the 5´ region of PMP22 including non-coding exon 1, coding exons 2 and 3, whereas exons 4 and 5 were present. This partial deletion was initially identified via Southern-Hybridisation. Subsequent SNP analysis revealed the presence of heterozygosity for PMP22 coding exons 4 and 5. PMP22 exon 3 and 4 specific qPCR resulted in a deletion of one exon3 allele, but presence of 2 exon 4 alleles. Finally MLPA specific for the CMT1A region defined this deletion for the entire 5´ region of PMP22 (exons 1, 2 and 3). Alu-elements have been reported to mediate non-allelic recombination events. Bioinformatic analysis revealed 12 Alu-elements flanking in close neighbourhood the estimated 40 kb deletion region as candidates for recombination events. PCR primers were designed to identify a breakpoint-spanning product including the respective Alu-elements. PCR-driven identification of a junction fragment was succesfull with AluJo-AluSq and AluYb9-AluSq specific primer pairs comprising the same intronic region of PMP22. Sequence analysis of these breakpoint-overlapping PCR fragments revealed a 29 bp motif including a chi-like sequence (GCTGG) present both in the AluYb9 and the AluSq element. These data confirm that low-copy repeats (LCRs) mediate non-allelic homologous recombinations (NAHR).

P306

Giant axonal neuropathy – a novel mutation of a Syrian girl

Stengel C.(1), Marquardt K.(2), Sasse B.(3), Wörle H.(2), Keimer R.(2), Seidel I.(4), Rautenstrauss B.(1), Huehne K.(1)
1) Institut für Humangenetik, Erlangen, Germany
2) Olgahospital, Stuttgart, Germany
3) Universitätsspital, Zürich, Switzerland

4) Heilpädagogische Praxis, Vellberg-Talheim, Germany

Giant axonal neuropathy (GAN) is a rare and severe hereditary autosomal recessive motor and sensory neuropathy with early onset in infancy. A prominent feature of this neurodegenerative disorder is disorganization of the neurofilament network, especially of the cytoskeletal intermediate filaments (IFs). The pathological accumulation of neurofilaments leads to segmental distension of axons affecting both the peripheral nerves and the central nervous system. Keratin IFs also seem to be altered, as patients show characteristical kinky and curly hair. The GAN gene encodes the ubiquitously expressed protein gigaxonin, a member of the cytoskeletal BTB/kelch repeat family. Distantly related proteins sharing similar domain organization have various functions associated with the cytoskeleton. The patient, diagnosed at four years of age and descendant of consanguineous parents, presented with signs of HMSN, as gait disturbances and pes cavus. She developed through the years mental retardation, cerebral seizures, signs of a disturbed myelinisation in NMR and optic atrophy. Giant axons were visible on sural nerve biopsy. The meanwhile twelve year old girl carries a so far unknown homozygous splice-site mutation (IVS 9+1 G>T) changing this canonical sequence to a non-functional nucleotide. In consequence the direct following stop codon in the GAN mRNA leads to premature termination of translation. The failure in splicing between exon 9 and 10 results thus in loss of the functional important kelch domains. This mutation was not present in 50 healthy controls. Therapeutic pedagogy according to Maria Montessori improved mobility as well as mental and social abilities resulting in a higher quality of life for both, the patient and his parents.

P307

Interstitial duplication 15q11-13 in a patient with Asperger Autism and seizures

Oehl-Jaschkowitz B.(1), Freitag C.(2), Göttert E.(1), Martin T.(1), Christmann A.(1)
1) Gemeinschaftspraxis für Humangenetik im Zentrum für genetische Diagnostik Saar, Homburg/Saar, Germany
2) Klinik für Kinder und Jugendpsychiatrie, Universitätklinikum des Saarlandes, Homburg Saar, Germany

We present a boy with the diagnosis of Asperger Autism made at the age of 19 years. Furthermore the boy suffers from seizures since the age of 16 years. Intelligence is in normal range. The patient finished non-classical secondary school with good marks. Afterwards he tried to Graduate from expert school for social sciences which could not be completed because of his special defects in social competence and communication skills and his deficiency of empathy, which, at the end of the initiated diagnostic procedure, led to the diagnosis of Asperger Autism. Since recent studies (1) were able to show a defined interstitial duplication of chromosome 15q11-13 in a few patients with Autism Disorder (1-3%), we investigated this region with respect to this abnormality. We were able to show a duplication of the region of interest with the proof of three alleles for the internal markers D15S122, D15S822 and D15S1234.
Furthermore we could confirm this result with FISH-analysis using the probe GABRB3 (15q11-12) on meta- and interphase chromosomes, which showed three signals. Parental investigation (FISH and molecular analysis) were inconspicuous. Microsatelite-analysis showed that the duplication did arise from the maternally derived chromosome 15, as described in literature (2). The proposed mechanism is misalignment in maternal meiotic recombination. The influence of parental imprinting on the phenotype will be discussed; the variation of the symptoms of the yet published cases will be shown.
1): The Phenotypic manifestations of Interstitial Duplications of Proximal 15q with special Reference to the Autistic Spectrum Disorder; P.F. Bolton et al.: Am.J.Med.Genet.(Neuropsychiatric Genetics)):105:675-685(2001)
2): Interstitial Duplication of Chromosome Region 15q11q13: Clinical and Molecular Characterization; G.M. Repetto et al.: Am.J.Med.Genet.:79:82-89(1998)

P308

Analysis of localization signals in ataxin-3, the affected protein in spinocerebellar ataxia type 3

Antony P.M.A.(1), Schmidt T.(1), Riess O.(1)
1) Universität Tübingen, Medizinische Genetik, Tübingen, Germany

Spinocerebellar Ataxia Type 3 (SCA3), also known as Machado-Joseph disease (MJD), is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the *MJD1 gene* encoding a polyglutamine repeat in the respective ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases. Ataxin-3 is mainly localized in the cytoplasm. However, one hallmark of SCA3 is the formation of ataxin-3 containing aggregates in the nucleus of neurons. In addition, we recently demonstrated *in vivo* using transgenic mouse models that nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3 and that cytoplasmically localized ataxin-3 is not able to induce a phenotype even with a very high number of polyglutamine repeats. Up to now it is not known why and how ataxin-3 leaves the cytoplasm and translocates into the nucleus. The identification of nuclear localization (NLS) or nuclear export signals (NES) within ataxin-3 would make an important contribution to the answer of this question. Recently, using computerized comparison with known sequence profiles, ataxin-3 was proposed to contain both a NLS and a NES. In order to find out whether these proposed signals are functional, we performed nuclear import and export assays in tissue culture. We also tested the consequence of *in vitro* mutagenesis of crucial amino acids within these signals for the localization of ataxin-3 and the formation of intranuclear inclusion bodies. In addition, we performed own searches for novel potential localization signal in ataxin-3 and tested these signals likewise as described above.

P309

Generation and characterization of an inducible mouse model for spinocerebellar ataxia type 3

Boy J.(1), Schmidt T.(1), Leergaard T.B.(2), Odeh F.(2), Nuber S.(1), Holzmann C.(3), Ibrahim S.(4), Grasshoff U.(1), Schmitt I.(5), Zimmermann F.(6), Prusiner S.(7), Bjaalie J.G.(2), Riess O.(1)
1) Universität Tübingen, Medizinische Genetik, Tübingen, Germany
2) University of Oslo, Centre for Moleculare Biology and Neuroscience (and Department of Anatomy), Oslo, Norway
3) Universität Rostock, Medizinische Genetik, Rostock, Germany
4) Universität Rostock, Institut für Immunologie, Rostock, Germany
5) Universität Bonn, Neurologie, Bonn, Germany
6) ZMBH, Heidelberg, Germany
7) University of California, Department of Neurology, San Francisco, United States of America

Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the *MJD1 gene* encoding a polyglutamine repeat in the respective ataxin-3-protein. In order to study the course of the disease we generated an inducible transgenic mouse model using the inducible "Tet-off system". This system is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Tetracycline which allosterically inhibits the tTA protein.
For the ataxin-3-responder mouse lines a full length constructs containing an expanded repeat with the pathological length of 77 glutamine repeats was used. The use of two different promoter mouse lines with known expression in the brain (Prion protein (Prp) promoter, Ca^{2+}/Calmodulin-dependent protein kinase II (CamKII) promoter) allows us to target the transgene expression to different brain regions. In preceding promoter analyses we identified and characterized these brain regions in detail.
Double transgenic SCA3 mice were analysed at different levels: We first analysed the phenotype in behavioural studies. Afterwards we performed immunohistochemical analyses of brain sections to study the expression of the transgene in the brain. In addition the Tet-off system allows us to turn the expression of ataxin-3 on or off at different developmental stages. Using this model we therefore will be enabled to study whether or not the disease phenotype is reversible.

P310

SMN1 and SMN2 gene dosis in autosomal recessive SMA detected by MLPA

Schaafhausen A.(1), Halliger-Keller B.(1), Meng G.(1), Kress W.(1)
1) Institut für Humangenetik, Würzburg, Germany

Autosomal recessive spinal muscular atrophy (SMA) is caused by a homozygous deletion/conversion of the survival of motor neuron gene 1 (SMN1) in over 90% of patients. Disease course is influenced by the copy number of the highly homologous SMN2 gene, which is alternatively spliced loosing then exon 7. The deleted mRNA produces no functional protein. Only a small part of the SMN2 mRNA is still complete and translated in a fully functional protein. So far quantitative analysis of SMN1 or SMN2 gene dosis was based on cumbersome real time PCR protocols on account of the very restricted selection of amplification primers. Using the MLPA method (multiplex ligation-dependent probe amplification, MRC-Holland), which is composed of probes for the whole gene contig on chromosome 5q12.2-q13.3, it is now possible to quantify the SMN genes and their flanking partners.
47 SMA patients with known homozygous deletions (in most cases exon 7 and 8) were investigated, and all the deletions could be confirmed. 6 out of 47 had only a homozygous exon 7 deletion and 1 out of 47 had a single homozygous exon 8 deletion. In some patients the size of deletions could be estimated. 24 parents of deleted SMA patients were found to have only one gene dosis of SMN1 as expected. Therefore MLPA is suitable to detect carriers of SMA. For comparison the distribution of SMN1 gene copies in a normal German control group was determined. In a second step the number of SMN2 gene copies was analysed in a German control group and in SMA patients. Especially patients with SMA type II or III had a high number of copies and the distribution is quite different in comparison to the control group. The highest number of copies was 8 in some patients with type III SMA.
In summary the MLPA test could reliably replace older lab methods for SMA diagnosis with the advantage to get simultaneously an estimate of the size of SMN 1 deletions as well as the copy number of SMN2 genes influencing the disease course.

P311

Autosomal dominant hereditary spastic paraplegia: Mutation analysis of the SPG4 gene in 256 families of Caucasian origin reveals 25 novel mutations

Sauter S.(1), Dörwald N.(1), Neesen J.(1)
1) Institut für Humangenetik, Georg-August-Universität, Göttingen, Germany

The hereditary spastic paraplegias (HSP) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterised by progressive spasticity and hyperreflexia of the lower limbs. Autosomal dominant hereditary spastic paraplegia 4 linked to chromosome 2p (SPG4) is the most common form of autosomal dominant hereditary spastic paraplegia (40 %). It is caused by mutations in the SPG4 gene encoding spastin, a member of the AAA protein family of ATPases. In this study the SPG4 gene of HSP patients from 256 apparently unrelated families of Caucasian origin was analysed. We identified mutations in 64 out of the 256 HSP families. Among the detected 49 different mutations were 29 missense mutations, 14 deletions, 12 nonsense mutations, 5 mutations that affect splicing, as well as 3 insertions and one deletion-insertion mutation. Most of the mutations are located in the conserved AAA cassette-encoding region of the SPG4 gene. Twenty-five of the identified mutations were novel and 7 of the mutations were found in more than one family.

P312

Long-term follow-up of AAV-mediated gene replacement therapy in a mouse model for X-linked juvenile retinoschisis (RS)

Janssen A.(1), Seeliger M.(2), Haverkamp S.(3), Molday R.S.(4), Min S.-H.(5), Hauswirth W.W.(5), Weber B.H.F.(1)
1) University of Regensburg, Institute of Human Genetics, Regensburg, Germany
2) University Eye Hospital, Electrodiagnostics Research, Tuebingen, Germany
3) Max Planck Institute for Brain Research, Frankfurt, Germany
4) University of British Columbia, Department of Biochemistry, Vancouver, Canada
5) University of Florida, Center for Immunology and Transplantation, Gainsville, Florida, United States of America

Objectives: The Rs1h-deficient mouse reveals several features characteristic of human RS, thus representing an ideal model to test the potential of AAV-mediated gene replacement therapy for this condition. A construct containing the human RS1 cDNA under control of the mouse opsin promoter in the adeno-associated virus (AAV) serotype 5 was injected into the subretinal space of one of the eyes of 15 day old knockout mice. The uninjected eyes served as internal controls. With the present follow up study we report on the long term efficiency (over 20 months post-injection) of this therapeutical approach.
Materials and methods: Scotopic and photopic ERGs were performed at different time points. Synaptic plasticity of rod and cone pathways in treated and untreated eyes were analyzed by immunolabeling techniques and electron microscopy (EM).
Results: ERG measurements showed nearly normal b-wave amplitudes in injected eyes suggesting preservation of retinal function for up to 20 month post-injection. Untreated control eyes already completely lacked b-wave responses by the age of 12 month. Consistent with these data immunohistochemistry with several retinal markers revealed intact OFF cone and rod bipolar cells in 20 month old RS1-AAV treated eyes. In the treated eyes, EM shows intact but slightly abnormal morphology of both cone pedicles and rod spherules. Control eyes presented with severe disorganisation and disintegration of bipolar cells with an overall destruction of integrity of photoreceptors and its synapses.
Conclusions: Our study demonstrates that AAV-mediated gene replacement therapy can restore retinal tissue integrity and visual function to nearly normal levels for almost the entire life span of the RS1-deficient mouse. This is a first report on the efficacy of gene therapy during the physiological life span of a treated mouse model.

P313

Expression pattern of OTOF (Otoferlin) in components of the hearing apparatus

Schug N.(1), Braig C.(2), Kalbacher H.(3), Knipper M.(2), Blin N.(1), Pfister M.(2)
1) Human Genetics, Molecular Genetics, Tübingen, Germany
2) Hearing Research Center Tübingen, Tübingen, Germany
3) Biochemistry, Tübingen, Germany

Deafness as a neurosensory disorder is inherited in a majority of prelingual hearing defects in an autosomal recessive mode. Presently, more than 60 genes have been implied in contributing to hereditary hearing impairment. DFNB9 affected individuals were shown to carry mutations in the OTOF gene with 18 mutations (mostly nonsense with a few missense) detected to date. Possible functions of Otoferlin and its specific expression pattern have not been described in any detail until now. On the basis of sequence homology to proteins like Fer-1 (C. elegans), Dysferlin and Myoferlin possible functions like an involvement in membrane trafficking were discussed. We generated for in situ hybridization an Otof-specific riboprobe and an antibody for immunohistochemistry and Western blots. By such means the spatial expression pattern of Otoferlin was investigated in cochlea and brain. The temporal expression pattern was monitored in post-natal mice of varying ages suggesting a down-regulation of Otoferlin within sections of cochlea starting with the onset of hearing.

P314

KIAA1985/SH3TC2 mutations in autosomal recessive Charcot-Marie-Tooth disease type 4C (CMT4C)

Stendel C.(1), Bergmann C.(1), Hahn A.(2), Lochmüller H.(3), Parman Y.(4), Takiyama Y.(5), Urtizberea A.(6), Züchner S.(7), Weis J.(8), Zerres K.(1), Gabrëels-Festen A.(9), Senderek J.(1)
1) Department of Human Genetics, Aachen University of Technology, Aachen, Germany
2) Department of Neuropediatrics, Justus-Liebig-University, Giessen, Germany
3) Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, Munich, Germany
4) Neurology Department, Istanbul Faculty of Medicine, Istanbul, Turkey
5) Department of Neurology, Jichi Medical School, Tochigi, Japan
6) AFM et Généthon, Evry, France
7) Center for Human Genetics, Duke University Medical Center, Durham, United States of America
8) Department of Neuropathology, Aachen University of Technology, Aachen, Germany
9) Institute of Neurology, University Medical Center, Nijmegen, Netherlands

Autosomal recessive Charcot-Marie-Tooth disease type 4C (CMT4C, OMIM 601596) is a childhood-onset demyelinating form of hereditary motor and sensory neuropathy (HMSN). We have recently shown that CMT4C is caused by mutations in the KIAA1985/SH3TC2 gene on chromosome 5q32. Here we report KIAA1985/SH3TC2 mutations in 14 additional families (27 affected patients) and four apparently sporadic cases of various ethnic origins. Eight of these mutations have not been reported earlier while one change, R954X, is a recurrent mutation. Allele sharing

analysis suggests a common ancestor of these families. The sporadic patients all carried R954X in the homozygous or in the compound heterozygous state. Together with our earlier findings on 18 patients from 12 families the present series allows to define the phenotype associated with KIAA1985/SH3TC2 mutations. Age of onset and disease severity are widely variable, even within families and among patients with an identical set of mutations. Spine deformities may be the presenting symptom occurring prior to neurological deficits. Nerve conduction velocities are slowed and sural nerve biopsies display a demyelinating neuropathy and characteristic ultrastructural abnormalities. For clinical testing, the presence of the R954X mutation should be checked in sporadic patients with demyelinating HMSN in whom more frequent causes of HMSN have been excluded.

P315

Hydatid mole and chorionic carcinoma in twin pregnancy: A case report

Hickmann G.(1), Siegmann H.-J.(1), Cramer A.(1), Körtge-Jung S.(1), Haug K.(1), Kozlowski P.(1)
1) Prenatal Medicine and Genetics, Düsseldorf, Germany

A 3 gravida 2 para referred to our institute in 16+3 weeks because of aberrant placental findings. Sonographic examination revealed a former twin pregnancy with one normally developed fetus. The second placenta derived from a vanishing twin showed the characteristics of a hydatid mole. We performed amniocentesis of the normal fetus as well as chorionic villus sampling of the mole. We present the results of cytogenetic and chromosome marker analysis and the course of beta human chorionic gonadotropin level during the observation period. The outcome of the pregnancy is described.

P316

Double trisomies in prenatal diagnostics and spontaneous abortions

Metzke-Heidemann S.(1), Kautza M.(2), Grote W.(1), von Kaisenberg C.(3), Weisner D.(3), Hilpert F.(3), Eckmann-Scholz C.(4), Siebert R.(1), Caliebe A.(1)
1) Institut für Humangenetik, Kiel, Germany
2) Praxis für Humangenetik, Kiel, Germany
3) Klinik für Gynäkologie und Geburtshilfe, Kiel, Germany
4) Gesundheitszentrum Mitte, Kiel, Germany

Double trisomies are rare and mostly seen in spontaneous abortions. In liveborns they generally involve a combination of gonosomal trisomy with autosomal trisomy or mosaicism for autosomal trisomies. This is a describtion of double trisomies in three spontaneous abortions and three vital fetuses. In abortions we found 48,XY,+7,+15; 48,XY,+15,+21 and 47,XX,+22/ 48,XX,+20,+22/ 48,XX+8,+22. Mean gestational age was 10+3 wks, compatible with early fetal demise observed in single trisomies. Mean maternal age was increased, 40 and 39 years respectively. In vital fetuses we found two combinations of gonosomal with autosomal trisomy and one autosomal double trisomy: In a sonographically normal fetus at 25th wks the karyotype was 48,XXY,+21. In a 2nd fetus with intrauterine growth retardation and polyhydramnion at 33 th wks the karyotype was 48,XXY,+18. Both couples decided to terminate the pregnancy. In a 3rd fetus at 13th wks there was hygroma colli, hexadactyly of both hands, microretrognathia and beginning hydrocephalus internus. The karyotype from directly prepared chorionic villi was 48,XY,+13,+18, which was confirmed using FISH in nearly all nuclei investigated. In contrast, chromosomal and FISH analysis in long term cultured chorionic villi only showed trisomy 13. There was subsequent spontaneous intrauterine fetal death at 14 wks. In this autosomal double trisomy the survival period is unexpectedly long. Therefore, a fetal mosaic 47,XY,+13/48,XY,+13,+18 or a single trisomy 13 in the fetus in addition to a second cell line 48,XY,+13,+18 confined to the placenta is more likely. Unfortunately, cytogenetic examination of the fetus was not possible because the parents refused autopsy.

P317

Tetrasomy 12p (Pallister-Killian syndrome): difficulties in prenatal diagnosis

Kunz J.(1), Schoner K.(2), Stein W.(3), Rehder H.(4), Kornmann E.(1), Fritz B.(1)
1) Zentrum für Humangenetik, Marburg, Germany
2) Institut für Pathologie, Marburg, Germany
3) Klinik für Geburtshilfe und Perinatalmedizin, Marburg, Germany
4) Klinisches Institut für chemische Labordiagnostik, Abteilung für Humangenetik, Wien, Austria

Pallister-Killian syndrome (PKS, tetrasomy 12p, OMIM 601803) is a relatively rare aneuploidy syndrome characterized by a tissue-limited mosaicism for an isochromosome 12p [i(12p)]. The first prenatal diagnosis of Pallister-Killian syndrome (PKS) was reported by Gilgenkrantz et al. in 1985. Since this report, about 60 prenatal cases have been described in the literature. In this syndrome, both sonographic and cytogenetic diagnoses remain difficult. The main ultrasound indicators of PKS are nuchal translucency, congenital diaphragmatic hernia, brachymelia, and polydactyly.
Routine prenatal diagnosis of PKS is still complicated: on the one hand in difficulties of discriminating between the supernumerary isochromosome 12p from the inv dup 21q; and on the other hand because of the variable level of mosaicism. No prenatal cytogenetic technique is sensitive enough to ensure prenatal diagnosis and false-negative results have been described on fetal blood, chorionic villi and amniocentesis. We report on a case diagnosed after chorionic villus sampling at 13+4 gw. Indications for prenatal diagnosis were increased nuchal translucency (NT 5,4 mm) and suspicion of cardiac abnormality. Short-term CVS cultures revealed a 46,XX karyotype, whilst long-term CVS cultures showed a 47,XX,+i(12)(p10) karyotype. Retrospective FISH performed on interphase nuclei from the CVS direct preparation confirmed the absence of the i(12p) marker in these cells. Sonografic examination in 17 gw, showed increased nuchal translucency (NT 6,5 mm), brachymelia, diaphragmatic hernia and a marked dextroposition of the heart. After counselling, the parents requested termination of pregnancy. Inspection and autopsy of the fetus revealed many of the characteristic dysmorphic sings and malformations of the Pallister-Killian syndrome. We compare our case with the reported prenatal cases and discuss the prenatal diagnosis of PKS and the feasibility of cytogenetic diagnosis.

P318

Reporting combined risks and isolated risks for nuchal translucency (NT) and biochemistry (PAPP-A, free ßhCG) after first-trimester-screening results in an inadequate increase of invasive procedures

Kitschmann-Prawitt M.(1), Steinberger D.(2), Sancken U.(1)
1) Bioscientia, Zentrum für Humangenetik Ingelheim, Ingelheim, Germany
2) Bioscientia, Zentrum für Humangenetik Ingelheim und, Justus-Liebig Universität, Institut für Humangenetik, Giessen, Germany

First-trimester-screening for chromosomal disorders detects more than 80% of pregnancies affected by fetal trisomy 21 at a screen-positive rate of 5%. In international studies screen results are related to risk calculations that combine information from maternal age, NT, and biochemical parameters (PAPP-A, free ßhCG) in single risk figures. Unlike this procedure, in Germany partial risks are considered in addition. The results of these partial risk calculations are reported to pregnant women as well, leading many of them to request invasive procedures although the combined risk is below the risk cut-off. For the last 4519 first-trimester-screen procedures performed at our laboratory we have analysed the impact on screen-positive rates if in addition to the combined risks partial risks derived from NT and biochemistry are communicated seperately. At a 1:380 risk cut-off 323/ 4519 (7.2%) pregnancies would have been tested “positive” in regard to combined risk assessment. In 530/4519 (11.7%) cases isolated biochemical risks and in 168/4519 (3.7%) cases isolated NT risks alone exceeded the cut-off, altogether making 1021 (22.6%) screen-positive results. At other commonly used cut-offs the high amount of screen-positive results would not be significantly reduced: 20.3% at a 1:300 cut-off, 17.6% at 1:270 or 16.2% at 1:250. Even at a 1:100 cut-off this insufficient method would identify a higher number of screen-positive cases than combined risks alone would do at an applied cut-off of 1:380. In order to yield an expected screen-positive rate of 5% a cut-off between 1:50 and 1:60 would be necessary, too high for detection rates of more than 80%.
The consequences of a method that would accept a 22.6% screen-positive rate instead of 7% are clear: Converting these rates into invasive procedures would result in nearly a threefold higher rate of amniocenteses, leading to a threefold increase in fetal loss with a marginal increase of detected fetuses affected with trisomy 21.

Autorenverzeichnis Index of Authors