Topics

Symposia

S_1 Structural variation

S1_01
Molecular Mechanisms and Clinical Consequences of Genomic Disorders. Implementation of array CGH in Genetic Diagnostics. The Baylor Experience
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Genomic disorders are a group of human genetic diseases caused by DNA rearrangements, ranging in size from an average exon (~100 bp) to megabases and affecting dosage sensitive genes. Three major molecular mechanisms: non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ), and the fork stalling and template switching (FoSTeS)/microhomology-mediated breakage-induced repair (MMBIR) have been described as causative for the vast majority of genomic disorders. Recurrent rearrangements are typically mediated by NAHR between low-copy repeats that are usually >10 kb in size with >97% DNA sequence identity. Nonrecurrent CNVs have been found to be formed by NAHR between highly homologous repetitive elements (e.g. Alu, LINE) and more often by NHEJ and FoSTeS/MMBIR stimulated, but not mediated, by genomic architectural features. Furthermore, simple repeating DNA sequences that have a potential of adopting non-B DNA conformations (e.g. triplexes, cruciforms, left-handed Z-DNA, and tetraplexes) have been shown to lead to gross genomic rearrangements associated with some genomic disorders. Complex rearrangements (e.g. duplication/triplication) can be explained by FoSTeS/MMBIR.
In 2003, a BAC clone-based targeted array CGH called Chromosome Microarray Analysis (CMA) for high-resolution human genome analysis was designed, developed, validated, and implemented at BCM. BCM has strategically positioned coverage on its array to detect the known genomic disorders. Targeted arrays of 356 (V4), 853 (V5) and 1475 (V6) BAC clones have yielded detection rates of 6.5%, 9%, and 12.6%, respectively. In March 2006, utilizing the knowledge of our genome architecture, CMA has been transitioned to genome-wide BAC-emulated oligonucleotide arrays V6 (44 K) and V7 (105 K). The results of CMA in 20,000 patients will be presented.

S1_03
Genome Architecture in Autism Spectrum and Related Neuropsychiatric Disorders
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Autism spectrum disorders (ASDs) are common, heritable neurodevelopmental conditions characterized by impairments in social communication and by a preference for repetitive activities. ASD is observed in ~1 in every 150 newborns with over 30,000 new diagnoses in North America each year. Twin and family data suggest ASD is mainly a genetic condition of complex etiology. A multidisciplinary pan-Canadian group has come together (funded by Genome Canada and partners) with international collaborators in 15 other countries (coordinated by Autism Speaks), to study autism genetics. This 'Autism Genome Project' team has now completed linkage, genome-wide association, and copy number variation (CNV) scans of DNA from thousands of families and the resulting data is beginning to reveal the genetic contributors (or genome architecture) underlying ASD. Our group is also investigating other neuropsychiatric disorders related to- or overlapping- with the clinical presentation of ASD. We find that ~10% of ASD

arises secondary to other disorders, some 10% is due to chromosome abnormalities and CNVs, and another ~2% appears monogenic (arising due to single gene alterations). For the remaining ~80% of cases we are still seeking answers, but there is significant progress. For example, many large rare-CNVs are being observed in ASD families. Some of the ASD genes now identified such as SHANK3 and neuroligin 4, encode proteins functioning at the synaptic complex in neurons, providing new targets for development of therapeutics. Functional experiments including therapeutic- and mutation mechanism- testing will be conducted using induced pluripotent stem (iPS) cell lines from patients with known mutations, as well as animal models. Finally, for some families the CNV and gene discoveries are starting to have clinical utility allowing early identification and refined diagnosis of ASD. These tests are now being transferred to hospital-based diagnostic labs in Canada. Together our genomic discoveries are redefining our understanding of autism and we are working closely with families and their supporters to best realize the benefits.

S_2 Low risk cancer genes

S2_01

Low risk cancer genes – current knowledge and possible implications for genetic counselling in familial breast cancer

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Klinikum rechts der Isar an der Technischen Universität, Frauenklinik, Abt. Gynäkologische Tumorgenetik, Munich, Germany

Except for the high breast cancer risk in BRCA1 and BRCA2 mutation carriers, familial clustering of breast cancer remains largely unexplained. While candidate gene approaches demonstrated moderately increased breast cancer risks for rare mutations in genes involved in DNA repair (ATM, CHEK2, BRIP1, PALB2, and RAD50), genome-wide association-studies (GWAS) identified several single nucleotide polymorphisms as low penetrance breast cancer susceptibility polymorphisms within genes as well as in chromosomal loci with no known genes (FGFR2, TNRC9, LSP1, MAP3K1, TGFB1, 2q35, 6q22). Moreover, some of these low penetrance breast cancer susceptibility polymorphisms additionally act as modifier genes in BRCA1/ BRCA2 mutation carriers (Antoniou et al., Am J Hum Genet 82:937–48, 2008). All of these low risk variants have reached statistical significance only through the analysis of several thousands of sporadic cases and most of them are associated with rather low risks (Easton et al., Nature 447:1087–93, 2007), doubting the clinical importance of such, still ongoing, findings. While functional or biological consequences of most SNPs are as yet unknown, a recent publication (Meyer et al. PLoS Biol May6:e108, 2008) reported the altered binding affinity for transcription factors OCT1, RUNX2 and C/EBP as a result of identified altered SNPs in intron 2 of FGFR2. Currently, further GWA-studies are underway comparing more than thousand of BRCA1/ BRCA2 negative high-risk breast cancer or sporadic breast cancer cases with control cohorts from Germany, identifying certainly new susceptibility polymorphisms. In the meantime, the screening of genetically enriched or familial cases has provided evidence that already known low risk variants (e. g. FGFR2) are playing a more important role in familial cases by interacting with one or more of the moderate penetrant variants. This talk outlines the recent key developments and potential clinical benefit for preventive management and therapy.

S2_02

Low penetrance predisposition genes for colorectal and other cancers

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Recent large-scale screens have shown that the common disease-common variant genetic model is correct for the major cancers. Several SNPs have now been associated with a differential risk of breast, colorectal and prostate carcinomas, and evidence for similar predisposition to other tumour types is accumulating. There appear to be several different mechanisms of raising cancer risk, but some prime candidate genes, such as those involved in DNA repair, are strikingly absent to date. The relative risks associated with cancer susceptibility SNPs are modest (typically up to 1.3-fold) and the variants detected to date can account for a small proportioin of the familial clustering of cancer. The remaining risk may be explained by other types of genetic variant, including copy number polymorphisms and rare (or "private") alleles with modest effects on disease risk.

S2_03

Moleculargenetics of Paraganglial Tumors: Selection of Susceptibility Genes

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Pheochromocytomas and paragangliomas form the group of paraganglial tumors which can occur in any paraganglion from the skull base to the pelvic floor. The terminology is inconstant. The WHO uses pheochromocytoma exclusively for adrenal tumors. In contrast many clinicians and we use pheochromocytoma also for extraadrenal abdominal and thoracic tumors, since by tradition pheochromocytoma is a vasoactive tumor. In contrast, head and neck paraganglioma is mostly only a space occupying mass.

One third of the patients with pheochromocytomas and paragangliomas are carriers of germline mutations in one of 6 genes and have thus hereditary disorders.

The first is neurofibromatosis type 1 (NF 1). About 1% of NF 1 patients have pheochromocytomas. The susceptibility gene NF1 is located on chromosome 17 (#17q11.2) and consists of 59 exons. Germline mutation screening in patients with NF 1 and pheochromocytoma revealed a random distribution over the gene. All pheochromocytoma patients with NF 1 show also cutaneous lesions.

The most wellknown hereditary syndrome associated with pheochromocytoma is the multiple endocrine neoplasia type 2 (MEN 2). About 50% of MEN 2 patients show pheochromocytoma. The dominant lesion is medullary thyroid carcinoma (MTC) or C cell hyperplasia occurring in up to 100% of the patients. The susceptibility gene is the RET gene on chromosome 10 (#10q11.2). Germline mutations occur in exons 8, 10, 11, 13–16, but pheochromocytomas have so far only been described in exons 10, 11, 13, and 16. Patients with mutations in exon 16 are predisposed to an aggressive form of MTC.

Von Hippel-Lindau disease (VHL) is in about 20% of the patients associated with pheochromocytoma. VHL is classified as type 1 (predominantly without) and type 2 (predominantly with pheochromocytoma). Other important components of VHL are hemangioblastomas of the eye and CNS, renal clear cell carcinoma, multiple pancreatic cysts and islet cell carcinoma. The susceptibility gene, VHL, is located on chromosome 3 (#3p25–26), and germline mutations are randomly distributed over all 3 exons.

Since 2000 paraganglioma syndromes (PGL) have been characterized moleculargenetically into types 1–4. PGL 1, 3 and 4 are caused by mutations of the succinatedehydrogenase (SDH) subunit D, C and B genes, SDHD, SDHC and SDHB. These genes are located for PGL 1 on chro-

mosome 11 (#11q13.1) with 4 exons, for PGL 3 on chromosome 1 (#1q21-23) with 6 exons, and for PGL 4 on chromosome 1 (#1p33–25) with 8 exons. The gene for PGL 2 has not yet been identified. Germline mutations occur in nearly all exons of all 3 genes. Paraganglioma syndromes include predisposition to paraganglial tumors in all locations, but PGL 3 patients mostly show only head and neck paragangliomas.
All syndromes associated with paraganglial tumors are autosomal dominantly transmitted, but patients with SDHD mutations develop tumors only, if they inherite the mutation from the father.
In summary, familial paraganglial tumors are characterized by younger age at diagnosis, more frequently multifocal and extraadrenal abdominal pheochromocytoma. Patients with PGL 4 and less frequently VHL are in particular predisposed to malignant pheochromocytoma. Carefully reviewed clinical data are instrumental for selection which gene should in a given patient been tested for germline mutation.

S_3 Transgeneration effects and epigenetic programming

S3_01
Prenatal environment leaves a lasting imprint on DNA methylation in humans

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We are studying the causes and consequences of variation in DNA methylation in various human study designs with the ultimate goal to establish the contribution of epigenetic dysregulation to metabolic disease risk. Epidemiological studies indicate that adult disease is associated with adverse environmental conditions early in development. While the mechanisms behind these relationships are unclear, an involvement of epigenetic dysregulation has been hypothesized.
As a first step in testing this hypothesis, we are studying the methylation of candidate genes among individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944/45. Exposed individuals had significantly altered levels of DNA methylation as compared with their same-sex sibling six decades after the exposure. These changes were observed for both imprinted and non-imprinted genes and both increased and decreased levels of methylation were observed. With one exception, the associations were specific for periconceptional exposure reinforcing that very early mammalian development is a crucial period for establishing and maintaining epigenetic marks. Our preliminary data on the association of maternal periconceptional folic acid use with the child's DNA methylation provide further support for this relationship.
These data contribute empirical support for the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life.

S3_02
Epigenetic Transgenerational Effects of Endocrine Disruptors: Implications on Reproduction and Disease Onset

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Transgenerational effects of environmental toxicants (e.g. endocrine disruptors) significantly amplify the impact and health hazards of these compounds. One of the most sensitive periods to endocrine disruptor exposure is during embryonic gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation. The model endocrine disruptors tested were vinclozolin, which acts as an anti-androgenic compound. Previous studies have shown that maternal vinclozolin treatment can effect embryonic testis development to subsequently cause an increase in spermatogenic cell apoptosis in the adult offspring. Interestingly, this spermatogenic defect is transgenerational (F_1, F_2, F_3 and F_4 generations) and hypothesized to be due to a permanent altered DNA methylation of the germ-line. This appears to involve the induction of new imprinted-like DNA methylation sites that regulate transcription distally. The expression of over 200 genes was found to be altered in the embryonic testis and surprisingly this altered transcriptome was similar for all generations (F_1-F_3). In addition to detection of the male testis disorder, as the animals age transgenerational effects on other disease states were observed including tumor development, prostate disease, kidney disease and immune abnormlities. Recent observations suggest transgenerational effects on behaviors such as sexual selection. Therefore, the transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to alter the epigenetic (i.e DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease development transgenerationally. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational disease is a new paradigm in disease etiology not previously considered.

S3_03
The impact of the early life social environment on our epigenome

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The DNA that we inherit from our parents is programmed during gestation by enzymatic addition of methyl groups. A certain pattern of marking is generated during gestation, which is specific for each cell type and programs gene expression. Proper programming of the epigenome is required for proper physiological functioning; variations in epigenomic markings could lead to differences in phenotype and increased susceptibility to disease and mental pathologies. Aberration in DNA methylation might play a role in many important diseases from cancer to mental pathology. In difference from the inherited genome that is fixed and irreversible, the epigenome is dynamic and is maintained in an equilibrium that could be disrupted by a variety of environmental exposures. The epigenome in early life is especially sensitive to exposure to environmental chemicals, nutrients as well as psychological stress. We propose that not only chemicals but also exposure of the newborn to different social environments, such as maternal care, could affect the epigenome in a manner that would result in a variety of health problems later in life. We previously demonstrated the impact of maternal care on epigenetic programming in the hippocampus of the offspring in rats. We extended this study to wider regions of the genome using high-density oligonucleotide arrays and methylated DNA immunoprecipitation. We will present data from three human cohorts supporting the hypothesis that child adversity early in life might be shaping the way our genomes are marked by the epigenome and affect and program human health. The first study looks at differential

marking of the rRNA locus in the hippocampus in suicide victims with documented childhood adversity. The second study looks at the glucocorticoid recptor gene in the same cohort. The third study looks at subjects from the British cohort of 1958 mapping methylation with respect to social adversity in early life. Since epigenetic programming defines the state of expression of genes, epigenetic differences could have the same consequences as genetic differences. However, since epigenetic changes are reversible by both pharmacological and behavioral interventions, understanding the epigenetic processes that affect human health could lead to a new approach to human health prevention, diagnosis and treatment.
Supported by a grant from the Canadian Institute of Health Research and the National Institute of Cancer of Canada, the Human Science Frontiers Program and the Sackler Program for Epigenetics and Psychobiology at McGill University and the NICHD to MS and M.J Meaney, the CIHR to GT and the MRC to CP.

S_4 Ciliopathies

S4_01
Genetic Defects in Ciliary Structure and Function
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Cilia, hair-like structures extending from the cell membrane, perform diverse biological functions. Primary (genetic) defects in the structure and function of sensory and motile cilia result in multiple ciliopathies. The most prominent genetic abnormality involving motile cilia (and the respiratory tract) is primary ciliary dyskinesia (PCD). PCD is a rare, usually autosomal recessive, genetically heterogeneous disorder characterized by sino-pulmonary disease, laterality defects, and male infertility. Ciliary ultrastructural defects are identified in ~90% of PCD patients and involve the outer dynein arms, inner dynein arms, or both. Diagnosing PCD is challenging and requires a compatible clinical phenotype together with tests such as ciliary ultrastructural analysis, immunofluorescent staining, ciliary beat assessment, and/or nasal nitric oxide measurements. Recent mutational analysis demonstrated extensive genetic heterogeneity. Identification of responsible genes has significantly aided our understanding of the pathogenesis.

S4_02
The ciliopathies: a model to study total mutational load in disease
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Defects of the primary cilium and its anchoring structure, the basal body, cause a number of human genetic disorders, collectively termed ciliopathies, since they are characterized by an overlapping range of phenotypes that include retinal degeneration, polydactyly, renal and hepatic fibrosis, obesity and a complex range of cognitive and neurodevelopmental defects. Recent data have also shown that some ciliopathies overlap not only phenotypically, but also genetically by contributing epistatic alleles that can modulate the phenotypic expressivity and penetrance. As such, the primary cilium and its associated signaling represents a useful model to understand the mechanism of total mutational load in a biological system. Towards that end, we have initiated systematic sequencing and functional evaluation of mutations of ciliary genes in a range of ciliopathy phenotypes and, using a large allelic series, have constructed preliminary models of epistasis in oligogenic disorders. Such studies will ultimately empower the predictive nature of the genotype and inform clinical management and treatment.

S_5 Systems biology

S_6 Molecular processes in meiosis

S6_02
Synaptonemal complex assembly, recombination and fertility
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Complete assembly of the synaptonemal complex is essential for recombination and the completion of meiosis in mammals. In particular the core central element component has been shown to contain at least four proteins which can interact with eachother. Dissection of the formation of this part of the Synaptonemal complex exploiting targeted mouse mutations suggests an essential process of self assembly which may involve components of the recombination machinery. Multicomponent complexes can be disrupted if the stoichiometry of the components are perturbed. Experiments designed to test this hypothesis are underway and may have relevance for human fertility.

Selected Presentations

SEL_1
Parent-of-origin specific DNA methylation of a CpG island within intron 2 of the RB1 gene
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Recently we observed a patient with complex phenotypic features showing hypomethylation at all known imprinted loci in peripheral blood DNA (Caliebe et al., in preparation). Genome-wide CpG methylation analyses (Infinium® HumanMethylation27 BeadChip, Illumina) in the patient and appropriate controls confirmed hypomethylation at known imprinted loci and, moreover, identified additional loci hypomethylated in the propositus. One of these loci was a 1.2 kb CpG island within intron 2 of the RB1 gene. To find out whether this CpG island is differentially methylated in a parent-of-origin specific manner, we performed methylation analysis by bisulfite cloning and sequencing of blood DNA from two retinoblastoma patients with a maternally derived RB1 deletion, three retinoblastoma patients with a paternally derived RB1 deletion, and a normal control. A total of 113 clones were analysed. Clones obtained from the normal control showed methylated and unmethylated sequences (15 and 18, respectively). Almost all clones (26 out of 28) from the patients with a deletion on the maternal chromosome were derived from completely unmethylated sequences, whereas all of the clones (52 out of 52) obtained from the patients with a paternal deletion were derived from completely methylated sequences. We conclude that the CpG island in intron 2 of the RB1 gene is subject to parent-of-origin specific methylation. Methylation analysis of two independent sperm samples revealed that the CpG island is unmethylated in male germ cells, suggesting that the methylation differences represent a germ line imprint. The identification of a differentially methylated CpG island inside the RB1 locus raises the question whether it acts as a promoter of an as yet unknown alternative sense or an antisense RB1 transcript that is subject to genomic imprinting.

SEL_2

Genomic duplications involving a conserved non-coding regulatory element downstream of BMP2 are associated with brachydactyly type A2

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Autosomal dominant brachydactyly type A2 (BDA2, MIM112600) is a limb malformation characterized by hypoplastic middle phalanges of the 2nd and 5th fingers caused by mutations in the Bone morphogenetic protein receptor 1B (BMPR1B) or in its ligand Growth and differentiation factor 5 (GDF5). By linkage analysis in a large Brazilian kindred of German origin negative for BMPR1B and GDF5 mutations we identified a novel locus for BDA2 on chromosome 20p12.3 encompassing the Bone morphogenetic protein 2 (BMP2) gene. Since sequencing detected no point mutations in BMP2 a high density array covering the critical interval of ~1.3 Mb was designed. Array CGH analysis detected a microduplication of ~5.5 kb in a non-coding sequence ~110 kb downstream of BMP2. Screening of other BDA2 patients by qPCR revealed a similar duplication in a second family. The duplicated region contains conserved non-coding sequences which likely function as cis-regulatory element regulating BMP2 expression in the limb. Several studies have identified such elements as essential regulators of developmental gene expression, that have the potential to switch genes off and on in particular types of cells/tissues during certain developmental time points. Given the importance of gene regulation in development it is to be expected that a large number of developmental defects are caused by mutations affecting such regulatory elements. Using a transgenic mouse model we are able to show that this sequence indeed is able to drive expression of a lacZ reporter construct exclusively in the limbs. The lacZ expression pattern resembles that of endogenous Bmp2 supporting the hypothesis of a limb-specific Bmp2 enhancer within the identified duplication. Our results reveal deregulation of the BMP signaling pathway as a novel molecular basis for the pathogenesis of BDA2 and identify duplications of regulatory elements as a novel mutational mechanism for developmental defects.

SEL_3

TNFAIP3 is a novel tumor suppressor gene in 6q23.3 in classical Hodgkin lymphoma

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In cancer genomes homozygous deletions can point to the location of tumor suppressor genes. In order to identify novel, putative TSG loci involved in the pathogenesis of classical Hodgkin lymphoma (cHL), one of the most frequent lymphomas diagnosed in developed countries, we performed array-CGH of four cHL cell lines. Among 17 novel candidate regions of homozygous deletions verified by polymerase chain reaction we identified a biallelic loss of approximately 13 kb in the KMH2 cell line (6q23.3) which includes several exons of the TNFAIP3 gene. This gene encodes the zinc-finger protein A20 - a negative regulator of the nuclear factor-kappaB (NF-κB), which transcriptionally regulates expression of multiple anti-apoptotic factors and proinflammatory cytokines and is known to be constitutively hyperactivated in cHL. FISH and FICTION revealed recurrent deletions of the TNFAIP3 locus also in primary cHL. In order to search for recurrent biallelic inactivation, we sequenced the TNFAIP3 gene in the HL cell lines as well as laser-microdissected HRS cells from primary cHL biopsies. We detected somatic mutations in 12 of 26 cHL (46%), including missense mutations in 2 of 11 Epstein-Barr virus (EBV)-positive cHL and a missense mutation, nonsense mutations and frameshift causing insertions or deletions in 10 of 15 EBV-negative cHL. Functional analyses revealed a considerable decrease in transcripts of selected NF-κB target genes, indicating reduced NF-κB activity following reintroduction of wildtype TNFAIP3 into the cell line lacking functional A20. In conclusion, array-CGH, mutational and functional analyses identify TNFAIP3, a key regulator of NF-κB activity, as a novel tumor suppressor gene in cHL. The significantly higher frequency of TNFAIP3 mutations in EBV-negative than EBV-positive cHL suggests complementing functions of TNFAIP3 inactivation and EBV-infection in cHL pathogenesis.

SEL_4

Genome-wide association scan reveals major susceptibility locus for non-syndromic cleft lip with or without cleft palate on chromosome 8q24

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Non-syndromic orofacial clefts are considered to have a multifactorial etiology with a strong genetic background. The most frequent form is the non-syndromic cleft lip with or without cleft palate (NSCL/P).
We conducted a genome-wide association study involving 224 NSCL/P-patients and 383 controls of Central European origin in order to identify novel susceptibility loci for NSCL/P. A 640-kb region at chro-

mosome 8q24.21 was found to contain multiple markers with strongly significant evidence for association with the cleft phenotype, including three markers which reached genome-wide significance. The 640-kb cleft-associated region was saturated with 146 SNP markers and then analyzed in our entire NSCL/P sample of 462 unrelated patients and 954 controls. In the entire sample, the most significant SNP (rs987525) had a P value of 3.34×10^{-24}. The odds ratio was 2.57 (95% CI: 2.02–3.26) for the heterozygous genotype and 6.05 (95% CI: 3.88–9.43) for the homozygous genotype. The calculated population attributable risk for this marker is 0.41, suggesting that this study has identified a major susceptibility locus for NSCL/P. The NSCL/P susceptibility locus on 8q24.21 is devoid of any known protein-coding genes. We were not able to confirm the existence of transcripts that were found in melanoma cell lines or predicted by computational algorithms in cDNA extracted from normal human tissue. It is possible, however, that the observed association may mediate its effect by as yet unknown transcripts mapping within the region. An alternative explanation is that the disease-associated region contains cis- or transacting elements which control the expression of more distant genes. Interestingly, we found no evidence of any interaction between the 8q24.21 locus and IRF6, the only generally accepted NSCL/P gene to date. This may suggest that the 8q24.21 locus confers its risk through a different pathway to IRF6.
The study was supported by the DFG.

Workshops

W_1 Clinical Genetics

W1_01
Early and severe disease manifestation in autosomal dominant polycystic kidney disease (ADPKD)

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian disorders with a frequency of 1/400–1000. Clinical symptoms usually do not arise until adulthood. The majority of ADPKD patients carry a mutation in PKD1 encoding the 4302 aa Polycystin-1 protein, while about 15% show a mutation in PKD2 encoding the 968 aa protein Polycystin-2. Mutation analysis is cumbersome due to the size and structure of PKD1. PKD2 is regarded to be significantly milder than PKD1. About 2% of ADPKD patients present with early manifestations. Among these cases are fetuses with significant peri-/neonatal morbidity and mortality sometimes clinically and sonographically indistinguishable from those with the severe recessive form of polycystic kidney disease (ARPKD). Early-onset ADPKD was thought to be strictly confined to PKD1. We could recently demonstrate a four-generation family carrying a PKD2 mutation with previously unknown clinically silent disease and perinatal death due to polycystic kidneys in two subsequent pregnancies. Here we present unpublished data of a large cohort of 37 early-manifesting ADPKD families shown to carry a mutation in PKD1 or PKD2. Clinical variability even within the same family is dramatic ranging from prenatal Potter sequence and early death to very mild manifestation in elderly affected family members carrying the same germline mutation. In addition to the above family, we detected PKD2 mutations in five other early-onset patients. The high recurrence risk for early-manifesting ADPKD in affected families argues for a common familial modifying background for early and severe disease expression. Potential modifiers like alterations in genes encoding other cystoproteins will be demonstrated and discussed. Our data provide compelling evidence that early manifestation in ADPKD requires increased attention and is an existing possibility even in PKD2 families, information that should be shared with affected persons and their families.

W1_02
Mutations in FOXG1 cause a broad phenotypic spectrum including microcephaly, frontal pachygyria and agenesis of the corpus callosum

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Heterozygous mutations in FOXG1 have recently been reported in two patients with a congenital variant of Rett syndrome and corpus callosum hypoplasia. FOXG1 encodes a winged helix transcriptional factor of the forkhead protein family which is mainly expressed in brain and regulates early steps in cortical development. We investigated a female patient with a de novo 2;14 translocation [46,XX,t(2;14)(q11.2;q12)]. She had postnatal microcephaly, seizures, and mental retardation. Selected MRI images showed mild pachygyria over the anterior frontal lobe, a mildly thickened cortex in the affected area, and a thin and poorly formed corpus callosum. Breakpoint mapping revealed that the 14q12 breakpoint was located ~265 kb downstream of FOXG1 suggesting that a position effect could cause transcriptional misregulation of FOXG1. By array CGH we identified microdeletions in 14q12 in three patients: a 1-Mb deletion located 36 kb downstream of FOXG1 and two (~8 Mb and ~5.4 Mb) including FOXG1. We sequenced FOXG1 in a cohort of 81 patients with mental retardation, microcephaly and pachygyria as well as in 81 patients tested in a diagnostic lab for MECP2 and found to be negative. We detected the heterozygous de novo missense mutation c.757A>G (p.N253D) in a mentally retarded female from the first cohort. MRI images showed mild pachygyria of the anterior-middle frontal lobe and moderately thickened cortex frontally. Intragenic rearrangements including an ins-del mutation and duplications of one or a few base pairs in FOXG1 were identified in four patients out of the MECP2 negative cohort. The heterozygous c.505_506delGGinsT mutation was found in a male patient who has had infantile spasms and focal seizures since birth, microcephaly and mental retardation. We conclude that de novo mutations in FOXG1 are a rare cause of autosomal dominant mental retardation in patients with microcephaly and various brain malformations that can include frontal pachygyria and abnormal corpus callosum.

W1_03
Chromosome 4p aberrations in Microtia/Oculo-Auriculo-Vertebral Spectrum (OAVS)/Goldenhar syndrome

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Oculo-Auriculo-Vertebral Spectrum (OAVS)/Goldenhar syndrome is a common developmental disorder with an estimated prevalence of 1/5.600. It is still a matter of debate whether isolated microtia is suf-

ficient to establish the diagnosis OAVS, for which the causative gene is still unknown. Over the past seven years we have collected data of 125 patients with OAVS, some of whom have 4p abnormalities. One patient with OAVS and multiple exostoses has a translocation t(4p;8q)(p15.3;q24.1). The chromosome 4 breakpoint maps 76.4 kb distal to the NKX3–2 (BAPX1) gene, which plays an essential role in craniofacial development. Another patient appears to be a somatic mosaic for a very small NKX3–2 dosage change. In fibroblasts from seven of 16 patients, but only from one of 12 controls, we observed strong allelic expression imbalance of this gene (Fisher´s exact test, p=0.048). The analysis of 24 patients and 13 normal controls revealed that NKX3–2 mRNA levels in patients with OAVS are significantly lower compared to normal controls (p=0.0087).In a 9-month-old patient with normal psychomotor development, low birth and postnatal measurements, double outlet right ventricle, left-sided anotia and mild hemifacial microsomia, right-sided microtia and median cleft palate we detected by array CGH an 800 kb deletion in 4p [del(4)(p16.2p16.1)], which comprises nine genes including MSX1. The deletion was also present in the mother, who had oligodontia. We sequenced the remaining copy of the MSX1 gene in the patient, but could not identify a bona fide mutation. In another female OAVS patient, who presented with low birth measurements, right-sided hemifacial microsomia and bilateral microtia, microcornea and corneal opacity, we found a terminal deletion due to an unbalanced translocation [46,XX,der(4)t(4;11)(p15.33;p15.2~15.3),del(11)(p15.2~p15.3)]. The breakpoint in 4p maps 797 kb distal to NKX3–2.Our data suggest that there is at least one locus for microtia/OAVS on 4p.

W1_04

Glomerulocystic disease (GCD) - clinical and molecular heterogeneity

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The term glomerulocystic is morphologically descriptive rather than diagnostically specific. GCD is characterized by cystic dilatation of the Bowman spaces. Glomerular cysts have been described in different types of cystic kidneys (type POTTER II-IV). Cystic kidneys type II (cystic dysplasia, multicystic kidneys) have a heterogeneous etiology and can overlap with type IV kidneys as a consequence of uretheral obstruction. GCD has often been used to describe early onset dominant polycystic kidney disease (PKD). In addition, Rizzoni et al. (Clin. Nephrol. 18: 263–268, 1982) reported two Italian sisters and their mother with what they termed ´familial hypoplastic glomerulocystic kidney disease´, which has been regarded as a separate entity.
On the basis of increasing knowledge about the molecular basis of ciliopathies a molecular based classification of GCD can be given:

- Type Potter II/IV manifestations:
- sporadic (?) as a result of uretheral obstruction
- with HNF1ß mutation
- in syndromes (e.g., tuberous sclerosis, orofaciodigital syndrome 1)
- Type Potter III
- with PKD1 and PKD2 mutation as early onset dominant PKD.
- Familial ‚hypoplastic autosomal dominant glomerulocystic kidney disease' can be caused by mutations in the UMOD gene which can also cause medullary cystic kidney disease type 2 (MCKD2) and juvenile hyperuremic nephropathy as well as in the HNF1ß gene in which a mutation could be identified in the family originally described by Rizzoni et al.

We analyzed cases with GCD and could identify mutations in PKD1, PKD2, UMOD and HNF1ß. We will present typical examples underlining that the evaluation of the family history is of utmost relevance. Before performing a mutational analysis, a careful assessment of available findings including ultrasound of patients and family members is essential. Renal and extrarenal malformations as well as a history of diabetes mellitus and hyperuricemia can be relevant.

W1_05

Schizencephaly as part of the Holoprosencephaly sequence: First report of mutations in SIX3 and SHH

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Schizencephaly (SCH) is a clinically and etiologically heterogeneous brain malformation presenting as unilateral or bilateral hemispheric cleft with direct connection between the inner and outer liquor spaces. In contrast to porencephaly the SCH cleft is lined by gray matter, which appears polymicrogyric suggesting an associated impairment of neuronal migration. The majority of SCH patients are sporadic, but familial SCH has been described. In 1996 one group reported heterozygous mutations in the homeobox gene EMX2 in SCH patients (Brunelli et al.). Here we present our negative results of an EMX2 mutation analysis of a cohort of 52 unselected SCH patients. In concordance with two recent other reports our data confirm that EMX2 mutations do not appear to significantly contribute to the pathogenesis of SCH.
Interestingly, SCH is frequently associated with additional cerebral malformations like hypoplasia or aplasia of the septum pellucidum or the Nervus opticus, suggesting the involvement of genes important for the establishment of forebrain structures. Based on that observation we considered Holoprosencephaly (HPE)-associated genes as potential SCH candidate genes and report here for the first time the identification of heterozygous mutations in SIX3 (E129X and G37C) and SHH (G290D) in two patients and one fetus with SCH; one of them without obvious associated malformations of midline forebrain structures. All 3 mutations have previously been reported in independent patients with HPE. SIX3 acts directly upstream of SHH and the SHH pathway is known as a key regulator of ventral forebrain patterning. Our data indicate that in a subset of patients SCH may develop as one aspect of a more complex malformation of the ventral forebrain and directly result from mutations in HPE genes. We suggest that schizencephaly may be considered as yet another part of the extremely wide phenotypic spectrum of the Holoprosencephaly sequence.

W1_06

Specific mutations of NRAS cause Noonan syndrome

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Background: Gain-of-function mutations in genes encoding molecules of the RAS-MAPK signalling pathway are known to cause Noonan syndrome (NS; OMIM 163950) and related disorders. 70–80% of patients affected by NS carry mutations in PTPN11, SOS1, RAF1, or KRAS, while in the 20–30% of cases the molecular basis has so far remained unclear.
Methods: As part of a systematic search for novel causes of NS, we analyzed NRAS by direct sequencing in a cohort of 280 NS patients

previously tested negative for mutations in the aforementioned genes. Putative mutations were further investigated by the characterization of the mutant proteins' functional properties and consequences on downstream signalling in vitro, including GTP hydrolysis, GST pulldown and ERK/AKT activation assays.
Results: In 4 out of 280 patients from different NS cohorts, we discovered two different NRAS mutations (1 T50I, 3 G60E). Two patients represented sporadic cases with proven de novo occurrence of the mutation, and two had familial NS. Their phenotype was typical of NS with no specific sign or manifestation emerging in this small mutation-positive group. Both mutations predicted a substitution of highly conserved amino acid residues of the protein. Functional assessment provided evidence of a gain of function, eventually leading to increased ERK phosphorylation.
Conclusions: Mutations of NRAS are a novel but uncommon cause of NS. Their functional consequences corroborate the concept that NS and related disorders are the developmental consequence of any kind of constitutional hyperactive RAS-MAPK signalling. The present findings add to a more complete picture of differences and similarities between disorders caused by mutations of RAS genes and provide hints at complementary functions during development.

W_2 Molecular Basis of Disease

W2_01
KIAA1985, a protein mutant in Charcot-Marie-Tooth neuropathy, links peripheral nerve myelination to endosomal recycling pathways
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Charcot-Marie-Tooth neuropathy (CMT) represents a heterogenous group of inherited disorders of the peripheral nervous system. One form of autosomal recessive demyelinating CMT (CMT4C, 5q32, OMIM #601596) is caused by mutations in the gene encoding KIAA1985, a protein of so far unknown function. Here we show that KIAA1985 is exclusively expressed in Schwann cells. KIAA1985 is tethered to cellular membranes through an N-terminal myristic acid anchor and localizes to the perinuclear recycling compartment. A search for proteins that interact with KIAA1985 identified the small GTPase Rab11, a key regulator of recycling endosome functions. CMT4C-related missense mutations disrupt the KIAA1985/Rab11 interaction. Protein binding studies indicate that KIAA1985 functions as a Rab11 effector, as it interacts only with active forms of Rab11 (WT and Q70L) and does not interact with the GDP locked mutant (S25 N). Consistent with a function of Rab11 in Schwann cell myelination, myelin formation was strongly enhanced in mouse dorsal root ganglion (DRG) explant cultures overexpressing Rab11 Q70L. Our data indicate that the KIAA1985/Rab11 interaction is relevant for peripheral nerve pathophysiology and place endosomal recycling on the list of cellular mechanisms involved in Schwann cell myelination.

W2_02
CA8 mutations cause a novel syndrome characterized by ataxia and mild mental retardation with predisposition to quadrupedal gait
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We describe a consanguineous Iraqi family in whom affected siblings had mild mental retardation and congenital ataxia characterized by quadrupedal gait. Genome-wide linkage analysis identified a 5.8 Mb interval on chromosome 8q with shared homozygosity among the affected persons. Sequencing of genes contained in the interval revealed a homozygous mutation, S100P, in carbonic anhydrase related protein 8 (CA8), which is highly expressed in cerebellar Purkinje cells and influences inositol triphosphate (ITP) binding to its receptor ITPR1 on the endoplasmatic reticulum and thereby modulates calcium signaling. We demonstrate that the mutation S100P is associated with proteasome-mediated degradation, and thus presumably represents a null mutation comparable to the Ca8 mutation underlying the previously described waddles mouse, which exhibits ataxia and appendicular dystonia without pathological abnormalities of either the central or the peripheral nervous systems. Subsequently, we identified the mutation R237Q in a highly conserved region of CA8 in an unrelated Iranian family with mild mental retardation and ataxia without quadrupedal gait. Magnetic resonance imaging studies of an affected person revealed no structural cerebral or cerebellar abnormalities. Our findings underline the importance of ITP-mediated signaling in cerebellar function and provide suggestive evidence that congenital ataxia paired with cerebral dysfunction may, together with unknown contextual factors during development, predispose to quadrupedal gait in humans.

W2_03
Cooks syndrome is associated with duplications of SOX9 regulatory sequences
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Cooks syndrome (CS) (%106995) is a rare autosomal dominant developmental disorder of the limbs, characterized by brachydactyly due to missing middle phalanges as well as hypo-/aplasia of the nails of hands and feet. So far the molecular defect in CS was unknown. By Array-CGH we identified a de novo 2 Mb duplication on chromosome 17q24.3 in an Egyptian family with Cooks syndrome in three individuals in two consecutive generations. The duplication was confirmed by quantitative PCR and FISH analysis and characterized molecularly as non-inverted tandem duplication. A duplication of similar size and genomic location was subsequently identified in two patients with clinical symptoms of Cooks syndrome from a Scottish family. Both duplications are in a "gene-desert" upstream of SOX9 encoding a temporal and tissue-specific transcription factor involved in bone formation and male sexual development. Haploinsufficiency of SOX9 is known to cause the clinically distinct campomelic dysplasia. We showed that Sox9 is expressed in the phalanges of mouse embryos at E13.5 as well as in the nail beds at E17.5 which is in line with the clinical symptoms of Cooks syndrome. Recently, a cis-acting regulatory element (SOX9cre1)

located 1.1 Mb upstream of SOX9 has been shown to play an important role in upregulating SOX9 expression via the sonic hedgehog pathway. During skeletogenesis sonic hedgehog regulates the formation of cartilage and bone. We thus suggest that the duplications including SOX9cre1 identified in patients with CS disturb long-range enhancers of SOX9 and interfere with proper limb development.

W2_04

Gerodermia osteodysplastica is caused by mutations in SCYL1BP1, a novel Rab-6 interacting golgin

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Gerodermia osteodysplastica (GO) is a rare autosomal recessive disorder characterized by lax, wrinkled skin, joint laxity, and a typical face with a prematurely aged appearance. Skeletal signs include severe osteoporosis leading to frequent fractures, malar and mandibular hypoplasia, and a variable degree of growth retardation. Using a positional cloning approach in consanguineous Mennonite pedigrees from Germany, Canada, and Mexico, we identified a homozygous interval on chromosome 1q24, which is 5.1 cM in length, with a multipoint lod score of 12.0. Interestingly, all 12 Mennonite patients were homozygous for the same haplotype and the nonsense mutation p.Glu143X in SCYL1BP1, indicating the preservation of an isolated population characterized by common belief and language over several generations and even large distances. In nine additional GO patients from various origins we identified eight other loss-of-function mutations. SCYL1BP1 encodes the soluble protein SCY1-like 1 binding protein 1, which is expressed at high levels in skin and osteoblasts. Co-localization experiments demonstrated that SCYL1BP1 localizes to the Golgi apparatus. Moreover, we have shown that it interacts with Rab6 using a yeast two-hybrid interaction screen, identifying SCYL1BP1 as a novel golgin. These results suggest that protein trafficking and modification play an important role in tissue maintenance of skin and bone. Moreover, our findings associate abnormalities of the secretory pathway with age-related changes in connective tissues.

W2_05

Knockout of the spastic paraplegia gene Reep1 results in a severe motor phenotype in mice

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The Hereditary Spastic Paraplegias (HSP) are caused by degeneration of corticospinal tract axons and thereby result in impaired lower limb movement. They are clinically and genetically heterogeneous. The autosomal dominant subtype SPG31 HSP is caused by mutations in REEP1, a gene of largely unknown function. We previously showed that SPG31 is one of the most frequent HSPs and that the REEP1 mutational spectrum mainly consists of truncating mutations. We have now generated a genetrap-based murine Reep1 knockout. Whereas in heterozygous mice an obvious hind limb movement disorder does not appear earlier than at 6 months of age, homozygous mice develop a strong early onset phenotype starting during the first month of life. When lifted by the tail, they show an abnormal hind limb grasp instead of the normal extension response. At 6 months of age, the hind limbs are rotated outwardly by more than 45° both during rest and movement. The rump cannot be properly supported any more and is dragged behind during movement. The ladder climbing test reveals frequent and strong cloni which are restricted to the hind limbs. These symptoms support a spastic gait disorder due to upper motoneuron involvement, but seem to involve lower motoneurons as well. Attempts to rescue (parts of) this strong and obvious phenotype by adenoviral gene transfer into the M. gastrocnemius have been initiated. In agreement with pathological findings in patients, histological analysis of the Reep1 knockout suggests progressive degeneration of the corticospinal tract. Cortical neuron cultures are currently investigated for axonal outgrowth and transport parameters. The data obtained so far show that our Reep1 knockout is a valid model for SPG31 HSP and a useful tool for a better understanding of the pathologic events causing this disease and, potentially, related neurodegenerative disorders.

W2_06

NPHP3 mutations can cause Ivemark syndrome and may mimic recessive polycystic kidney disease

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Diseases associated with functionally defective cilia have been termed ciliopathies. NPHP proteins belong to the key players in ciliopathies. We and others could recently identify NPHP3 mutations in patients with nephronophthisis and Meckel-Gruber syndrome amongst others. Here we present clinical and genetic data of a cohort of 104 patients originating from 56 unrelated families analysed for NPHP3. The clinical spectrum of patients ranged from isolated cystic kidney disease to early embryonic multi-organ pathology. While the mutation detection rate for the above phenotypes is rather low in NPHP3, clearly pathogenic mutations could be found in all six families analysed with typical features of Ivemark syndrome, an entity whose molecular basis largely remained unknown so far. Ivemark syndrome is characterized by cystic-dysplastic anomalies of the kidney, liver, and pancreas and lateralization defects such as situs inversus and asplenia or polysplenia due to defective left-right axis development. We also present evidence that the clinical and ultrasonographic data of patients with NPHP3 mutations may mimic autosomal recessive polycystic kidney disease (ARPKD) with prenatal Potter sequence and early death. Given the postulated network of cilia proteins, it might be legitimate to claim modifying effects by proteins interacting and/or colocalizing with NPHP3 to be, at least in part, causative for some of the phenotypic variability. Clinical variability even within the same sibship is highlighted by a family carrying heterozygous mutations in NPHP3 and NPHP6. While the first-born son shows isolated Leber congenital amaurosis, the second pregnancy was terminated because of oligo-/anhydramnios and massively enlarged polycystic kidneys later confirmed by autopsy. Experiments on transcript and protein level are under way in this family.

W_3 Cancer genetics

W3_01

Role of common variants for familial prostate cancer susceptibility

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Prostate cancer (PCa) is complex disease with strong heritability. Familial clustering is known for decades now, but hardly explained by any candidate high risk gene. Recent genome wide association studies discovered series of common variants that contribute a small but significant disease effect in several populations. This leaves open a large gap in our knowledge between modification of baseline risks, conferred by discrete low penetrant alleles, and the strong predisposition that runs in hereditary PCa pedigrees. In a cohort of 189 PCa families we addressed the questions if (1) particular common variants tend to cosegregate within affected families, and (2) cumulative effects of multiple low risk alleles could explain a strong susceptibility. Fifteen SNPs from known PCa associated regions were assayed in family based association tests (FBAT) and case control comparisons. Therefore, 559 individuals from PCa families (419 affected), 318 sporadic cases and 213 controls were genotyped. The highest singular risk effect was observed for a rare variant (rs16901979, MAF = 0.03) at 8q24, with an allelic odds ratio of 2,2 (p = 0.02). Further five SNPs at 7p15, 8q24, 11q13 and 17q24 were significantly associated in case control comparisons. The latter three loci showed significant over-transmission of risk alleles towards affected offspring in FBAT analyses. A cumulative risk model was set up by defining proband groups upon the count of risk alleles for the six best candidate SNPs. The extreme groups showed a clear tendency of increased risk (OR = 3.3, p = 0.004; and OR = 5.7, p = 0.0002 for carrying 5–6, and >6 alleles, respectively). For all risk strata the cumulative effect seemed stronger (ORs about 2-fold) when the cases were restricted to familial probands. Their maximum was OR = 8.9 (p = 0.002) for >6 alleles. Multifactorial inheritance could be demonstrated to mimic a high penetrance, even for an incomplete set of candidate variants in a small study cohort as ours.

W3_02

Predisposition for TMPRSS2:ERG fusion in prostate cancer by variants in DNA repair genes

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The oncogenic TMPRSS2:ERG fusion is a common event in prostate cancer. We hypothesized that defects in repair genes lead to an increase of chromosomal rearrangements and thus to the occurrence of the TMPRSS2:ERG fusion. We have previously conducted a genome wide linkage analysis in fusion positive prostate cancer families, revealing potential susceptibility loci on chromosome 5q14, 9p13, 9q21, 10q26, 11q24, 12q15, 13q12, and 18q. In the present study we investigated candidate genes related to DNA damage response and repair from these regions.
A total of eleven candidate genes were screened for mutations in TMPRSS2:ERG positive families. We found 17 non synonymous variants (6 had a MAF <0,05). Fourteen variants were investigated in a case control analysis, including 507 controls versus 327 sporadic and 203 familial cases, as well as in family based association tests (FBAT). Significant association with prostate cancer was found in BRCA2 at 13q12, ESCO1 (Establishment of Cohesion 1) at 18q11.2, RMI1 (RECQ-mediated genome instability) at 9q21.32 and in POLI (Polymerase iota) at 18q21.1. Three variants were associated independently of TMPRSS2:ERG fusion status, so for BRCA2 V2728I (p = 0,028; OR = 6,34; CI95% 1,23–32,82), ESCO1 N191S (p = 0,041; OR = 1,88; 1,03–3,43) and RMI1 N455S (p = 0,022; OR = 1,31; 1,04–1,64), whereas POLI F532S was highly significant associated with TMPRSS2:ERG fusion positive prostate cancer cases (p = 0,0027; OR = 4,10; 1,63 –10,32). The association for POLI remained significant after correction for multiple testing (Bonferroni threshold p = 0,0036).
POLI is required for translesion synthesis during replication of damaged DNA. POLI deficiency might cause DNA double strand breaks resulting from unsolved stalled replication forks, leading afterwards to chromosomal rearrangements like the TMPRSS2:ERG fusion. These results need validation and the variants with high OR may become useful in risk estimates for prostate cancer.

W3_03

Wildtype but not mutant androgen receptor inhibits expression of the hTERT telomerase subunit: A novel role of AR mutation for prostate cancer development

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Androgens play a central role in prostate development and prostate cancer proliferation. Chromosomal translocations and androgen receptor (AR) mutations are associated with prostate cancer development. Further, induction of telomerase is an early event in prostate carcinogenesis and is considered as a marker for both primary tumors and metastases. Interestingly, several reports suggest that telomerase activity is regulated by androgens in vivo. Here, we show that the wild-type human AR inhibits the expression of the catalytic subunit of human telomerase (hTERT) and telomerase activity via inhibition of hTERT promoter activity in the presence of androgen receptor agonists. However, pure androgen antagonists failed to repress hTERT transcription. The androgen-mediated repression of hTERT is abrogated in LNCaP prostate cancer cells that express a mutant AR (T877A) frequently occurring in prostate cancer. We reveal that this single amino acid exchange is sufficient for the lack of transrepression. Interestingly, chromatin immunoprecipitation (ChIP) data suggest that in contrast to the wildtype AR, the mutant AR is recruited less efficiently to the hTERT promoter in vivo indicating that loss of transrepression is due to reduced chromatin recruitment. Thus, our findings suggest that the wildtype AR inhibits expression of hTERT, which is indicative of a protective mechanism, whereas the T877A mutation of AR not only broadens the ligand spectrum of the receptor but abrogate this inhibitory mechanism in prostate cancer cells, revealing a novel role of AR mutations in prostate cancer development and suggests to search for new AR antagonists that inhibit only AR-mediated transactivation but allow its transrepression.

W3_04

Bax inhibitor-1 regulates extrinsic and intrinsic apoptosis pathways and interacts with the catalytic subunit of the protein phosphatase 2A in breast and prostate cancer cells

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The anti-apoptotic protein Bax inhibitor-1 (BI-1) was shown to be overexpressed in mammary and prostate carcinomas. In the present study we concentrated on the question, how BI-1 regulates apoptosis and

which interaction partners are involved in these processes. Therefore, the expression of BI-1 was downregulated in breast (MDA-MB-231) and prostate (PC-3) cancer cells using RNA interference, and an apoptosis-specific cDNA array was performed. Differential expression of candidate genes was confirmed using quantitative RT-PCR. Array results pointed out, that BI-1 has influence on the expression of members of the intrinsic (e.g. BCL-XL, BAG3) as well as the extrinsic apoptotis pathway (TNFRSF12A). This is supported by the results of caspase activation studies showing that caspase 8 and 9 as executors of the extrinsic and intrinsic apoptosis pathway, respectively, are similarly activated. Furthermore, using a yeast-two-hybrid screening on a prostate cDNA library we could identify the catalytic subunit of the protein phosphatase 2A (PP2CA) as binding partner of BI-1. PP2A is known to regulate the activity of BCL-2 proteins via dephosphorylation. The interaction of BI-1 and PP2CA was verified by coimmunoprecipitation in different breast and prostate carcinoma cell lines. The interacting domains were narrowed down to the C-terminal part of PP2CA. In addition, immunohistochemical studies on breast and prostate cancer specimens using a PP2CA-specific antibody revealed that the expression of PP2CA is increased in 68% and 64% of the analysed cases, respectively. Furthermore, a correlation between the tumour stage and PP2CA expression was observed in prostate cancer, suggesting that PP2CA may represent an oncogene rather then a tumour suppressor gene. Taken together, our present results implicate that BI-1 may regulate apoptosis via interaction with PP2CA and may therefore function as a candidate for therapeutical interventions in the future.

W3_05

Integrin α5β1 enhances contractile force generation through increased tumor cell invasion

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The process of metastasis formation that includes cell invasion that causes malignant progression of tumors. The impact of cell mechanical properties on the malignancy of tumor cells has not been investigated systematically. Highly-invasive tumor cells expressed significantly higher amounts of the α5β1 integrin compared to weakly-invasive tumor cells. The hypothesis was that the ability to generate contractile forces is a prerequisite for cell invasion and that α5β1-expression increased cell invasion through enhanced generation of contractile forces. We analyzed whether α5β1-high or α5β1-low expressing breast carcinoma cells differ in their ability to invade into a 3-D collagen fiber matrix. Our results show that higher α5β1 expression increased cell invasiveness. By blocking α5 expression through addition of α5siRNA to α5β1-high cells, we tumor cell invasiveness into a 3-D collagen fiber matrix was decreased. We found that the increased 3-D motility of α5β1-high expressing cells depends on the integrin α5β1 activating traction generation. The increased invasiveness was also inhibited by addition of myosin light chain kinase inhibitor ML-7 or by addition of the ROCK kinase inhibitor Y27632. We analyzed whether α5β1-high and α5β1-low cells formed tumors in mice. The tumor formation and growth is impaired in α5β1-high compared to α5β1-low cells. The integrin α5β1 acts as an enhancer of cell invasiveness, where contractile forces are necessary to overcome the viscous drag, but as a suppressor of primary tumor formation and growth, where increased motility is rather a hindrance for cell clustering to form tumors.

W3_06

Expression of DDX3Y in testicular germ cell tumour cells (TGCT) - a model system for the study of DDX3Y male germ line function

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DDX3Y is a DEAD box RNA helicase located on the Y chromosome with a functional counterpart on the X chromosome, DDX3X. Both proteins have a high homology of >94% and are functionally interchangeable. They are involved in the control of translation and in the control of the G1-S progression during cell division. However they are differentially regulated. The DDX3Y protein is exclusively expressed in the germ line, predominantly in spermatogonia and leptotene spermatocytes. On contrary DDX3X protein is found in somatic cells and in the male germ line only translated in spermatids. Since the deletion of DDX3Y results in pre-meiotic spermatogenic disruption, an essential role of DDX3Y in spermatogenesis is assumed. Our purpose was to examine if the function and mechanisms of translational regulation of DDX3Y might be associated with male germ cell differentiation. Testicular germ cell tumours (TGCTs) are derived from different stages of germ cell maturation and therefore might provide a model system for such germ cell differentiation studies. Consequently, we used a large panel of different TGCTs including the preinvasive carcinoma in situ (CIS) cells to investigate the expression pattern of DDX3Y protein in these specimens.Our results revealed always a strong DDX3Y protein expression in CIS cells but a heterogeneous pattern in the different types of TGCTs, i.e. seminomas, embryonal carcinomas, teratomas. The strong expression in CIS cells is marking their high proliferative activity and clearly designates DDX3Y as a novel marker for these tumour precursor cells. However overt tumours, showed only a small and variable number of DDX3Y expressing cells. An abundant expression in seminomas compared to non-seminomas points to reduction of DDX3Y during tumour progression. Our results indicate a successive vanishing of the male germ cell character of these tumours cells and suggest that translation of DDX3Y is indeed controlled by germ cell specific trans-factors.

W_4 Molecular Basis of Disease

W4_01

Shox2 mediates Tbx5 activity by regulating Bmp4 in the sinus venosus of the developing heart

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Heart formation requires a highly balanced network of transcriptional activation of genes. The homeodomain transcription factor Shox2 is essential for the formation of the sinoatrial valves and for the development of the pacemaking system. Here we identify the Bmp4 gene as the first direct target of Shox2. Shox2 interacts directly with the Bmp4 promoter and activates transcription in luciferase reporter assays. In addition, ectopic expression of Shox2 in Xenopus embryos stimulates transcription of the Bmp4 gene and silencing of Shox2 in cardiomyocytes leads to a reduction in Bmp4 expression. In $Tbx5^{del/+}$ and $Shox2^{-/-}$ mice we show that the T-box transcription factor Tbx5 is required for Shox2 expression in the inflow tract and that Bmp4 is regulated by Shox2 in this compartment of the embryonic heart. This work contributes to the

unravelling of the intricate interplay between the heart-specific transcriptional machinery and developmental signalling pathways.

W4_02

Hereditary hearing impairment (DFNB9): Otoferlin's interaction in the trafficking network

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Mutations within OTOF encoding otoferlin lead to a recessive disorder called DFNB9. Several studies have indicated otoferlin's association with ribbon synapses of cochlear sensory hair cells. Data showing the protein's presence in neurons, nerve fibers and hair cells, suggest a more ubiquitous function. Otoferlin's co-localization not only with ribbon synaptic proteins, but also with additional endosomal (EEA1) or Golgi proteins (GM130) were motivation for a search for further binding partners of otoferlin by a yeast two-hybrid screen in a rodent cochlear cDNA library (P3-P15). This screen identified proteins important to trafficking activities. One novel interacting partner is Rab8b GTPase. Their protein-protein interaction was substantiated by RT-PCR data, transient co-expression and co-localization in HEK 293 cells, application of respective knock-out mouse models and co-immunoprecipitation of the complexes using tagged proteins in vitro and native proteins from cochlea. This finding underlines the observation that otoferlin is a part of components contributing to trans-Golgi trafficking. Dysfunction of this "interactome" leads to one type of the recessive hearing impairments, DFNB9.

W4_03

17β-Hydroxysteroid dehydrogenase type 10 is required for mitochondrial and cellular integrity

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17β-Hydroxysteroid dehydrogenase type 10 (HSD10, also denoted ABAD or MHBD) is mutated in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, a neurodegenerative childhood disorder. HSD10 is localized in mitochondria and is involved in isoleucine metabolism but in addition has yet uncharacterized functions in embryonic development and cellular integrity. Since homozygous mutations of HSD10 in Drosophila (Scully) and mice result in embryonic lethality we analyzed HSD10 function in Xenopus embryos, conditional HSD10 knock-out mice and fibroblasts from MHBD deficient patients. Antisense Morpholino oligonucleotide knock-down in the neuroectoderm of Xenopus embryos caused defects of neural tissue and enhanced apoptosis at tadpole stages. Rescue experiments in which mutated human HSD10 proteins are expressed in HSD10 depleted Xenopus embryos showed no correlation between rescue ability and residual enzymatic activity of the mutated HSD10 proteins. Structural analysis of mitochondria in HSD10 depleted Xenopus animal caps, in conditional HSD10 knock-out mice and in fibroblasts from MHBD deficiency patients demonstrated that HSD10 knock-down or mutation perturbs mitochondrial morphology. Functional analysis of mitochondria in Xenopus animal caps showed reduced pyruvate turnover when HSD10 is knocked-down. As patients with different HSD10 mutations appear to cope differently with exposure to metabolic stress such as infections or vaccinations we analyzed the reaction of fibroblasts from patients to mitochondrial stress by blocking the respiratory chain. Stress response correlated with the clinical data from patients but not with residual enzymatic activity of the respective mutations of HSD10. All these experiments reveal a non-enzymatic role for HSD10 in early development and in mitochondrial and cellular integrity which is likely accountable for the pathogenesis of neurodegeneration in MHBD deficiency patients.

W4_04

Common pathological mutations in PQBP1 induce nonsense-mediated mRNA decay and enhance exclusion of the mutant exon

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Mutations in the polyglutamine binding protein 1 (PQBP1) gene cause X-linked mental retardation. All but one of the PQBP1 mutations known to date cause frameshifts, that give rise to premature termination codons. Most of them affect the AG hexamer in exon 4. Interestingly identical and similar mutations resulted in high clinical variability, ranging from moderate mental retardation (MR) to much more severe forms, including microcephaly, short stature and spasticity. In a first step towards unravelling the pathogenic mechanism of PQBP1 mutations, we evaluated the functional consequences of the mutations on PQBP1 protein and mRNA expression in the patients. We show here that truncated PQBP1 protein is present in most of the patients and remarkably that patients with mutations of the AG hexamer express significantly increased levels of different PQBP1 protein isoforms. RT-PCR experiments confirmed these findings and revealed mutation-specific reduction of normally spliced PQBP1 mRNAs carrying the premature termination codon, which can be partially restored after blocking the nonsense-mediated mRNA decay pathway. In addition, some of the mutations significantly change PQBP1 splicing either by nonsense-associated altered splicing, or by affecting important splicing motifs. In silico searches identified exonic splicing regulatory silencers overlapping the AG hexamer. In the patients, the predicted motifs were partially destroyed or new motifs were added. By transfecting wild-type and mutant PQBP1 minigene constructs into HEK cells, we were able to reproduce the splicing pattern seen in controls and patients, thereby confirming the important role of these motifs in exon recognition. Our results on the molecular mechansims of PQBP1 mutations provide first insights into events contributing to the pathomechanism of the disease.

W4_05

Extended runs of homozygosity encompassing and flanking large NF1 gene deletions

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Large deletions of the NF1 gene region in 17q11.2 are the most common type of recurrent mutation in neurofibromatosis type 1 (NF1). Type-2 NF1 deletions span 1.2 Mb and include the NF1 gene as well as 13 flanking genes. In most instances, type-2 NF1 deletions occur postzygotically and hence are associated with somatic mosaicism. Non-allelic homologous recombination (NAHR) between low-copy repeats (LCRs) has been identified as the major mechanism underlying these deletions. However, it is still unclear why these deletions should occur with such a high frequency. We used Affymetrix SNP 6.0 arrays to reinvestigate 12 previously described NF1 patients with type-2 deletions and precisely

identified breakpoints. We have observed that in 6 of these 12 investigated deletions, the NF1 deletions were flanked by extended regions of homozygosity without copy number loss. These copy number neutral regions of homozygosity surrounding the deletions differed in size between different patients, but in all cases extended beyond the bounds of the deletions themselves, spanning up to several Mb in length. The possibility that these runs of homozygosity were inherited was excluded by analysis of parental DNA. Our findings indicate an as yet unidentified mechanism responsible for the somatic deletions in this chromosomal region: mitotic recombination between allelic sequences centromeric to the NF1 gene region at 17q11.2 would appear to render the region vulnerable to NAHR between LCRs located within the recombining region. We suggest that mitotic allelic recombination may be tightly linked to, or could even trigger, NAHR in genomic regions harbouring LCRs. Since 50% (6/12) of the NF1 deletions analysed were flanked by extended regions of homozygosity without copy number loss, this is likely to be a major cause of somatic deletion in NF1 and may well be an important general mechanism underlying somatic deletions in other types of tumours exhibiting high mitotic recombination rates.

W4_06

TRPS1, a regulator of chondrocyte proliferation and differentiation, interacts with the activator form of GLI3

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The TRPS1 gene on human chromosome 8q24.1 encodes a multi zinc finger transcription factor protein. Mutations in TRPS1 cause the tricho-rhino-phalangeal syndrome (TRPS). Besides typical craniofacial anomalies, skeletal malformations are characteristic hallmarks of patients with TRPS.
Here we show that TRPS1 interacts with Indian hedgehog (Ihh)/GLI3 signalling and regulates chondrocyte differentiation and proliferation. By immunoprecipitation assays using transiently transfected cells as well as native tissue samples from embryonic mouse limbs, we could demonstrate that TRPS1/Trps1 specifically interacts with the activator form of GLI3/Gli3, whereas a direct binding of the repressor form of GLI3/Gli3 could be excluded. GST pull-down experiments were used to verify the interaction of the isolated GLI3 activator domain with TRPS1. Through the use of different truncated TRPS1 constructs, a domain of 185 aa, containing three predicted zinc fingers, was shown to be sufficient for the interaction with GLI3.
Using different mouse models we find that in distal chondrocytes Trps1 and the repressor activity of Gli3 are required to expand distal cells and locate the expression domain of Parathyroid hormone related peptid. In columnar proliferating chondrocytes Trps1 and Ihh/Gli signalling have an activating function. The differentiation of columnar and hypertrophic chondrocytes is supported by Trps1, independent of Gli3. Trps1 seems thus to organize chondrocyte differentiation interacting with different subsets of co-factors in distinct cell types.

W_5 Imprinting

W5_01

High prevalence of germline reprogramming defects in ICSI sperm: Correlation with spermiograms and ART outcome

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Children born after assisted reproduction show an increased risk for congenital malformations, low birth weight, and some rare imprinting disorders. The etiology of these medical problems associated with ART is still unclear. One plausible explanation are epigenetic reprogramming defects during germ cell development that are transmitted to the offspring. We have studied the epigenetic status of sperm DNA from 96 infertile males attending a fertility center for ICSI. The methylation patterns of 5 maternally methylated (Lit1, Snrpn, Peg3, Mest, and Nespas) and two paternally methylated (H19 and Gtl2) imprinted genes, two pluripotency genes (Oct4 and Nanog), one germline-specific gene (BOLL), as well as Alu and Line-1 repetitive elements were analyzed by bisulphite pyrosequencing. Altogether 15 samples exhibited an unusual methylation pattern(s). Most of these samples were endowed with an abnormal methylation of only one of the studied genes. However, three samples displayed abnormal patterns in all imprinted genes and the germline-specific BOLL gene, indicating a genome-wide methylation reprogramming defect. It is noteworthy that oligozoospermic semen samples showed a significantly higher rate (50%) of methylation abnormalities than normozoospermic samples (10%). Similarly, the epimutation rate was much higher (20%) in semen samples with less than 20% sperms with normal morphology than in samples with more than 20% normal sperms (5%). We followed the ART outcome of the analyzed sperm samples. The pregnancy rate was lower (30%) after ICSI with abnormally methylated oligozoospermic samples than after ICSI with oligozoospermic samples with normal methylation patterns (70%). DNA methyltransferases (DNMTs) play an important role in epigenetic reprogramming during spermatogenesis. To find out whether certain DNMT variants/mutations may predispose to methylation reprogramming defects, we have started a systematic genotype analysis in our population of infertile men.

W5_02

A case of familial imprinting disorder (Beckwith-Wiedemann Syndrome) with germline mutation in NLRP2 (NALP2)

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Beckwith-Wiedemann syndrome (BWS) is a fetal overgrowth and human imprinting disorder resulting from the deregulation of a number of genes, including IGF2 and CDKN1C, in the imprinted gene cluster on chromosome 11p15.5. Most cases are sporadic and result from epimutations at either of the two 11p15.5 imprinting centres (IC1 and IC2). However, rare familial cases may be associated with germline 11p15.5 deletions causing abnormal imprinting in cis. We report a family with BWS and an IC2 epimutation in which affected siblings had inherited different parental 11p15.5 alleles excluding an in cis mechanism. Using a positional-candidate gene approach we found that the mother was ho-

mozygous for a frameshift mutation in exon 6 of NLRP2. While germline mutations in NLRP7 have previously been associated with familial hydatidiform mole, this is the first description of NLRP2 mutation in human disease and the first report of a trans mechanism for disordered imprinting in BWS. These observations are consistent with the hypothesis that NLRP2 has a previously unrecognised role in establishing or maintaining genomic imprinting in humans.

W5_03

Considerable natural variation of functionally important DNA methylation patterns

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DNA methylation is an epigenetic DNA modification that plays an important role in gene regulation. Although DNA methylation patterns can be influenced by a variety of external (i.e. nutrition, ageing) and internal factors (i.e. DNA polymorphisms), little is known about the extent and functional implications of natural variation of DNA methylation patterns in imprinting control regions and gene promoters. We have performed a systematic methylation analysis of blood cells from monozygotic (n=24) and dizygotic (n=28) twins, placenta tissue (n=50) from normal newborns, and adult frontal cortex tissue (n=13) from individuals without any evidence for brain pathology. We quantitatively analyzed the DNA methylation patterns of 7 imprinted (GTL2, H19, MEG3, NESP55, NESPAS, PEG3, and SNRPN) and two pluripotency genes (NANOG and OCT4) using bisulphite pyrosequencing. Our twin study revealed that overall methylation patterns are more similar between monozygotic twins than between dizygotic twins (p=0.09) or unrelated individuals (p=0.02). Nine of 52 analyzed blood samples (17%), 4/13 (31%) brain samples and 8/50 (16%) placenta samples repeatedly showed methylation percentages outside the normal range (mean plusminus two standard deviations) in one or two genes, indicating an enormous plasticity of DNA methylation in healthy individuals. This finding may pose considerable problems for the diagnosis of imprinting disorders, in particular of mosaics. Our methylation data allow us to estimate the effects of aging, DNA sequence context, tissue type and developmental state on individual genes. The methylation patterns of some genes (MEG3, NESP55, and NESPAS) appeared to be less susceptible to external and internal influences than those of others (GTL2, H19, and SNRPN). All genes, in particular pluripotency genes showed differences in their DNA methylation patterns between tissues. Compared to other genes, GTL2 and SNRPN methylations patterns exhibited less age-dependent variation.

W5_04

Transcription is required for establishment of germline methylation marks at imprinted genes

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Genomic imprinting depends on the differential marking by DNA methylation of genes in male and female gametes. Most imprint marks (differentially methylated regions or DMRs) are established in the female germline and require the de novo methyltransferase Dnmt3a/3L complex, but the reasons why specific sequences are targeted by Dnmt3a and 3L are not fully known. Amongst the factors thought to be involved are specific histone modifications (lack of methylation of H3K4) and periodicity in CpG spacing, but other evidence indicates that additional factors are also required. Here, we investigated the role of transcription in establishing maternal germline methylation marks. Using the mouse Gnas locus as a model system, we show that truncating transcripts from the upstream Nesp promoter disrupts methylation of the maternal germline DMRs. Moreover, we established that transcription occurs in oocytes across the prospective DMRs at many maternally marked imprinted domains, but not across the paternally marked H19 DMR or unmethylated CpG islands. This suggests a common requirement for transcription events. Interestingly, all transcripts implicated here in gametic methylation are protein-coding, in contrast to the non-coding antisense transcripts involved in the silencing of imprinted genes in somatic tissues, although they often initiate from alternative promoters in oocytes. We propose that transcription is a third essential component for de novo methylation, which might be required to create open chromatin domains to allow the methylation complex access to its preferred targets. Our findings provide a novel molecular explanation for imprinting errors and should encourage a search for similar lesions in other imprinting disorders.

W5_05

Limiting dilution analysis of low amounts of bisulphite modified DNA: Representative Igf2r and H19 methylation patterns from ten oocytes

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Because bisulphite treatment heavily degrades DNA, methylation analysis of a few or even single cells is an unsolved technical problem. To avoid preferential amplification of DNA molecules in the starting sample, we have adopted limiting dilution analysis (LDA) to bisulphite sequencing. The aim of LDA is to dilute an assay down to a binary status: either to obtain a signal or not. We have applied LDA to the methylation analysis of imprinted genes in murine oocytes. To this end, we collected ten oocytes each from superovulated B6/J female mice and converted the DNA using the ZYMO EZ DNA Methylation-Direct Kit with a recovery rate of more than 80%. The eluation volume representing the converted DNA of ten oocytes was diluted 1:10 with water to a final volume of 100 µl. First round multiplex PCR was performed with a template input of 10 µl diluted DNA each. This allowed us to do ten separate second round nested PCRs. The multiplex mixture consisted of one primer pair for Igf2r and two primer pairs for H19. In the ideal case, we should obtain products from ten independent nested PCRs, representing 10 different DNA molecules in the starting sample. In a typical experiment, we could detect 6/10 products for Igf2r, 8/10 for H19 fragment 1 and 4/10 for H19 fragment 2. Bisulphite sequencing of these PCR products revealed that Igf2r was almost completely methylated, whereas both H19 fragments were completely demethylated in oocytes, as expected. Our results show that representative methylation profiles of multiple genes can be generated from a limited number of cells, most likely even from a single cell. This will allow us to study the effects of different assisted reproductive technologies on the epigenetic status and quality of germ cells and early embryos. Our current work is focused on increasing the number of imprinted genes in the multiplex PCR reaction and decreasing the number of cells/DNA molecules in the starting sample.

W5_06

Allelic expression imbalance of the RB1 gene

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The RB1 gene codes for a ubiquitously expressed cell cycle regulatory protein. Humans heterozygous for inactivating RB1 mutations have a predisposition to cancer, most notably retinoblastoma. Genetic analyses of affected families have indicated that genetic factors can explain a considerable proportion of the observed phenotypic variation. Interestingly, RNA analyses in rare families with a specific splice site mutation have shown a higher abundance of transcripts expressed from the

maternal allele. To find out if RB1 gene expression is subject to parent-of-origin effects, we analyzed RNA from individuals heterozygous for expressed single nucleotide polymorphisms (cSNPs).
RNA was extracted from peripheral blood of eight individuals from four families with cSNPs in exons 12, 18, 21, and 23 of the RB1 gene. The relative abundance of the transcripts from the paternal and maternal alleles was determined by single nucleotide primer extension analysis of RT-PCR products. The ratio of peak areas of signals corresponding to the variant nucleotides was normalized using results of primer extension analysis on heterozygous genomic DNA. All eight individuals showed a higher abundance of transcripts expressed from the maternal allele. The mean of the normalized transcript ratios was 2.9:1 (maternal: paternal) with low variation of the degree of skewing between different families and assays (SD ±0.48). These results suggest preferential transcription of the maternal RB1 allele.
Parent-of-origin specific expression is a hallmark of imprinted genes (which hitherto did not include RB1) that are under control of differentially methylated regulatory regions. As the promoter of the RB1 gene is not imprinted we will now search for differentially methylated regions that influence transcript abundance depending on the parent-of-origin.

W_6 Genomics Technology / Bioinformatics

W6_01
The human phenotype ontology
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There are many thousands of hereditary diseases in humans, each of which has a specific combination of clinical features. The great majority of human Mendelian syndromes have been described in detail in the Online Mendelian Inheritance in Man (OMIM) database. However, computational analysis of the data contained in OMIM has so far been hampered by the lack of a controlled vocabulary using consistent annotations with well-defined relationships to one another.
We have therefore developed a Human Phenotype Ontology (HPO) with over 8000 terms representing individual phenotypic anomalies and have annotated all clinical entries in OMIM with the terms of the HPO. Each term in the HPO describes a phenotypic abnormality such as Atrial septum defect. The HPO itself does not describe individual disease entities but rather the phenotypic abnormalities associated with them. Clinical entities are annotated to the most specific terms possible. The true path rule applies to the terms of the HPO. That is, if a disease is annotated to the term Atrial septal defect, then all of the ancestors of this term, such as Abnormality of the cardiac septa, also apply. The structure of the HPO therefore allows flexible searches for disease entities according to phenotypic abnormalities with a broad or narrow focus.
In this presentation, we will introduce the HPO and discuss several important applications of it, including the analysis of phenotypic networks associated with hereditary diseases and their relationship to the underlying genetic and protein networks in the cell, using ontological analysis to aid clinical genetics diagnostics by searching for specific diagnoses based on combinations of features, and combining phenotypic similarity measures with random walk analysis of protein interaction networks to improve predictions of novel disease genes. The HPO is freely available at http://www.human-phenotype-ontology.org.

W6_02
GPGraphics 0.9: A universal graphical backend for SNP microarray analysis
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Whole genome association (WGA) studies using microarray platforms are currently one of the most popular methods to search for disease-associated genes throughout the human genome. Several software packages are available to analyze microarray data; most of them, however, lack both a graphical user interface and graphical output. Considering the processing power required for the large amounts of data generated by microarray platforms, most analysis software is designed to run in high power computing (HPC) environments, where GNU/Linux and a variety of Unix flavors are the predominant operating systems. These systems are rather text- than graphics-oriented, making it difficult for a researcher to get an actual overview of the huge amount of data produced. Last year, we presented GPFrontend and GPGraphics, which, together with a slightly modified version of software also originally written for GNU/Linux systems, greatly facilitated the evaluation of pooling-based WGA studies, while running on Microsoft Windows. We have now expanded GPGraphics (the graphical backend part) to be usable not only with pooling analyses, but also with regular, single sample based WGA software, such as PLINK. GPGraphics provides a series of mathematical filters to visualize even faint signals in noisy data. Due to the modular nature of the software, the more processing intensive analyses may still be performed on a non-graphical HPC system, while the evaluation of the data generated by those systems can be performed in the graphical environment of a Microsoft Windows computer. Since the software is written for the Microsoft .NET framework, it will even run on non-Windows systems, provided they have a .NET runtime environment fully compatible to the .NET 2.0 specification. The presented software has been successfully used at our institute to analyze whole genome microarray data for both qualitative and quantitative traits, such as pseudoexfoliation syndrome and cornea thickness, respectively.

W6_03
The GeneCascade – a comprehensive website for identification of disease genes and evaluation of sequence alterations
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We have developed a software suite for identification of disease genes and prediction of the disease causing potential of sequence alterations. It consists of 3 intuitive tools, is completely web-based and freely accessible at http://neurocore.charite.de/.
HomozygosityMapper allows the rapid identification of disease-linked regions in individuals with an inbreeding background. It is based on a model-free algorithm, robust against genotyping errors and by numbers of magnitudes faster than linkage analysis. HM detects candidate regions and displays the underlying genotypes graphically. For each candidate region, it provides a direct link to our candidate gene database. Researchers can at any point decide to make their results publicly accessible on our website.
GeneDistiller is our candidate gene search engine that integrates data from various biomedical databases. It displays all genes within a genomic region with user-selected gene-specific data such as molecular function or known corresponding human and murine phenotypes. GD can highlight interesting features and score similarities or interactions with known disease genes. Users can sort and filter genes by any of their properties. Besides, GD offers a user-driven ranking of the genes placing the most likely candidates on top of the list. This process remains transparent and researchers can interactively adjust prioritisation settings on behalf of their own background knowledge and expectations.

The last step in identifying disease-causing mutations is the sequence analysis of candidate genes or of whole linkage intervals and the evaluation of every alteration detected, a process that will gain importance with the vast number of variations inevitably detected by deep sequencing. Our tool MutationTaster scores the disease potential of a variation by various tests for different protein and gene properties. It outperforms most similar applications in terms of accuracy (89% correct predictions) and speed (<3 s).

W6_04

Wrongly observed genome variability caused by sequence assembly

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Based on first results on human genomic variation, Venter and coworkers were surprised to find unexpectedly more single nucleotide polymorphisms (SNPs) than predicted. Experimental verification of 26 mutations failed in 6 cases indicating a large false positive error rate. Using a simple leave-one-out experiment where the same set of 500 Roche reads was re-assembled 500 times, using only 499 reads each time, and comparing the contigs generated; we found a large number of reported SNPs to originate from incorrect sequence assembly. Some small sections of alternatively assembled contigs are shown below.
TAGAATAAGCTTCTTGCT-CCCCTTCAA-TTACAACTGCATT-GATTCTGCTAGTAAGCATC
TAGAATAAGCTTCTTGCT-CCCCTTCAAATTACAACTGCATT-GATTCTGCT-GTAAGCATC
TAGAATAAGCTTCTTGCT-CCCCTTCAA-TTACAACTGCATT-GATTCTGCT-GTAAGCATC
TAGAATAAGCTTCTTGCTTCCCCTTCAA-TTACAACTGCATT-GATTCTGCT-GTAAGCATC
Further, incorrectly observed genome variation was studied by comparing 3 sets of contigs derived by the assemblers Phrap, Celera and MIRA2, respectively, from the same set of 91,899 reads of a 10 MB bacterial genome. We could find only 1034 contig triplets, one from each assembler, which admit high quality consistent multiple alignments. The sections common to all members of a triplet are reproducibly assembled regions of the genome but add up to only 40% genome size not considered that many of these may represent overlapping fractions. The 10130 mismatch columns found in all triplets flag possibly wrong SNPs.
Hence, ambiguities of multiple alignments, used in sequence assemblers, represent dangerous sources of wrong SNPs. Advanced alignment scoring is unable to solve this problem. Hence, new software is required that can list local nearly equal quality solutions and help designing targeted experiments for clarification. Not accurate data seriously questions our ability to relate genomic variation and disease. This problem needs to be addressed urgently since molecular diagnostics is moving fast into clinical applications.

W6_05

Identification of novel cholesterol regulating genes by targeted RNAi-screening

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Elevated plasma cholesterol levels are considered responsible for excess cardiovascular morbidity and mortality. Cholesterol in plasma underlies a tight control by cholesterol within cells. Here, we demonstrate an integrative functional genomics strategy that we have established to systematically identify novel regulators of cellular cholesterol levels. Candidate genes were identified by genome-wide gene expression profiling of cholesterol-depleted cultured cells and systematic literature queries. The role of these genes in cholesterol regulation was then tested by targeted siRNA knock-down experiments quantifying cellular cholesterol levels and the efficiency of low-density lipoprotein (LDL) uptake. With this strategy, several genes could be newly identified as functional regulators of cellular cholesterol homeostasis. E.g., one of these factors was characterized as an endoplasmic reticulum localized SREBP target gene that re-distributes to endo-/lysosomal compartments and the plasma membrane upon cellular cholesterol depletion. The molecular mechanisms how this and other genes contribute to regulate cholesterol levels as well as potential relevance for disease are currently explored.

W6_06

Massive parallel bisulfite sequencing of CG-rich DNA fragments reveals that methylation of many X-chromosomal CpG islands in female blood DNA is incomplete

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Methylation of CpG islands (CGIs) plays an important role in gene silencing. Although next generation sequencers allow massive parallel sequencing, the analysis of the complete human genome in a single run is not yet possible. Guided by in silico restriction digests, we used four restriction enzymes and a size selection step to prepare DNA libraries enriched with CGIs from female white blood cells and from sperm. The DNA libraries were ligated to adaptors, bisulfite treated and subjected to a modified Roche Genome Sequencer protocol. We obtained 163,034 and 129,620 reads from blood and sperm, respectively, with an average read length of 133 bp. Bioinformatic analysis by newly developed algorithms revealed that 12,358 (7.6%) blood library reads and 10,216 (7.9%) sperm library reads map to 6,167 and 5,796 different CGIs, respectively. In blood and sperm DNA we identified 824 (13.7%) and 482 (8.5%) fully methylated autosomal CGIs, respectively. Differential methylation, which is characterized by the presence of methylated and unmethylated reads of the same CGI, was observed in 53 and 52 autosomal CGIs in blood and sperm DNA, respectively. In contrast to the methylation of autosomal CGIs, methylation of X-chromosomal CGIs in female blood cells was most often incomplete (25–75%), which appears to be a hitherto unrecognized feature of X-chromosomal CGIs. We verified the results of 12 CGIs with different methylation states by conventional bisulfite sequencing and found complete concordance. Our results show that the extraction of CG-rich fragments and massive parallel sequencing makes it possible to detect tissue and allele-specific methylation differences.

W_7 Complex Diseases

W7_01

Novel susceptibility locus for coronary artery disease on chromosome 3q22.3

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Recent genome-wide association studies (GWAS) of coronary artery disease (CAD) focused on a few chromosomal regions with strong signals.
We hypothesize that applying too stringent statistical thresholds on the association tests´ P-values may prevent one from detecting single nucleotide polymorphisms (SNPs) with modest effects or/and low allele frequency.
We present a three-stage analysis of 1) genome-wide SNP data in 1,222 German cases with myocardial infarction and 1,298 controls, 2) in-silico replication of loci with P<0.001 in three additional genome-wide data sets of coronary artery disease (CAD), and 3) subsequent replication in ~25,000 subjects.
For CAD, we identified one novel locus on 3q22.3 ($P=7.44\times10^{-13}$ [OR 1.15; CI:1.11–1.19]), and a suggestive association on 12q24.31 ($P=4.81\times10^{-7}$ [OR 1.08; CI:1.05–1.11]).
These two new loci were not detected in our recent GWAS (WTCCC & GerMIFS I) because they did not pass the conservative statistical threshold, i.e., a false positive report probability (FPRP) of <0.5, employed in this previous analysis. Further functional work (re-sequencing, fine-mapping) is needed to define the mechanisms by which these loci translate into a higher risk of CAD, and whether this information can be used to improve prevention, prediction or treatment of this common condition.

W7_02

Common variants at ten loci modulate the QT interval duration in individuals of European ancestry: The QTSCD consortium

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The QT interval, a measure of cardiac repolarization, predisposes to ventricular tachycardia and sudden cardiac death (SCD) when prolonged or shortened. Previously, a common variant in NOS1AP (CAPON) influencing QT interval was mapped in a European population. We now analyze genome-wide association data from five European ancestry samples (ARIC, KORA, SardiNIA, GenNOVA and HNR, N = 15,842). We confirm the NOS1AP association (P=1.63×10–35) and identify nine additional loci at P<5×10–8. Four loci map near the monogenic long QT syndrome genes KCNQ1, KCNH2, SCN5A and KCNJ2. Two loci map to ATP1B1 (P=2.18 x10–12), PLN (P =1.97×10–16) that have well known roles in myocardial electrophysiology. The remaining loci are at RNF207 (P =3.57×10–9), LITAF (P=2.92×10–8) and near GINS3-NDRG4-CNOT1 (P=1.26×10–12). Taken together genetic variation at these 10 loci explained 3.3% of corrected QT interval variation across all studies. The ~8% of individuals carrying 14 or more QT prolonging alleles had an OR of 2.52 to have prolonged QT by clinical standards when compared to the ~10% of individuals carrying 8 or less alleles (95% CI 1.74–3.66, P = 4.83×10–7). These results provide new insights into myocardial electrophysiology and provide novel candidate genes for ventricular arrhythmias, long QT syndrome and SCD.

W7_03

Identification of new loci for schizophrenia with genome-wide association study using DNA pooling

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Our aim was to identify new association loci for periodic catatonia (MIM 605419) a subphenotype of schizophrenic psychoses. Previously we identified a schizophrenia locus on chr.15q15 (SCZD10) with genome-wide linkage studies of 12 extended pedigrees. Despite extensive efforts, we failed to identify either point mutations in coding areas or conserved non-genic sequences (CNGs) and CNVs in the identified linkage region. We therefore performed a genome-wide association analysis using SNP Microarray and DNA pooling (SNP-MaP) with 500,568 SNP Affymetrix arrays.
We pooled DNA of 245 cases in three biological replicates (n=84, 84, 77) and 216 controls in two biological replicates (n=108, 108). All pools were processed in three technical replicates. Array data was analysed with a modified version of GenePool (Pearson et al., AJHG 2007; 80(1):126–39). For graphical visualization of GenePool data, we developed the software GPGraphics to facilitate analysis. We determined the mean relative allele signal value (RAS) of the technical replicates of each pool and compared each case pool with the combined controls. We used a 5-SNP sliding window approach and defined clusters as potentially associated loci if they overlapped in all biological replicates.
We then verified pooling data at single genotype level for associated array SNPs at five selected loci using TaqMan assays in an extended cohort (344 cases, 585 controls). Two of these loci showed significant association at single marker (p=0.0002) and haplotype levels (p=0.0042), even after permutation correction (p_c=0.0007; p_c=0.0224). We could further replicate these findings in a second cohort of 1223 psychosis samples (p=0.0184).
Our data show that hybridization intensities of pooled DNA correlate well with individual genotyping results. Thus DNA pooling is a useful strategy for GWAS since we were able to detect significant association loci in a complex disease. Deeper analysis of the associated loci is ongoing.

W7_04

A subgroup of age related macular degeneration is associated with heterozygous mutations in the ABCA4 gene

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Age related macular degeneration (AMD) is a multifactorial disease that leads to severe vision loss due to geographic atrophy (GA) of the retinal pigment epithelium („dry" AMD) or choroidal neovascularisation („wet" AMD). Sequence variants in the genes CFH, C3, and ARMS2 are strongly associated with AMD. On the basis of fundus autofluorescence (FAF) imaging, GA can be classified into different phenotypes. One subgroup is characterized by a fine granular pattern with peripheral punctate spots (GPS) that accounts for 3–5% of GA. Interestingly, the GPS phenotype shows striking similarity to Stargardt disease (STGD) which is caused by biallelic mutations in the ABCA4 gene. The role of ABCA4 variants in AMD etiology is still controversial.
In this study, we sequenced the ABCA4 gene in three groups: group 1 comprised 13 patients with the GPS phenotype; group 2 consisted of 14 patients with AMD but FAF patterns different from GPS; the final group served as control and included 14 patients diagnosed with STGD. In addition, the common AMD risk factors were determined (rs1061170:CFH; rs10490924:ARMS2; rs2230199:C3).
In the GPS group we found a heterozygous pathogenic ABCA4 mutation in 9 patients while 3 patients were heterozygous for two pathogenic mutations, and one patient did not reveal a mutation. In the AMD group only 3 patients were heterozygous for a pathogenic ABCA4 mutation. In the Stargardt disease group a total of 26 pathogenic mutations were identified. The genetic risk variants at CFH, C3 and ARMS2 revealed a significant lower risk in the GPS group compared to the AMD group but frequencies were similar with those in the STGD group.
Our data provide further support for a complex role of ABCA4 mutations in the etiology of retinal degenerations. In particular, this study suggests that the GPS phenotype is associated with a single heterozygous mutation in the ABCA4 gene strongly arguing for a predisposing effect of ABCA4 variants in a minor proportion of AMD cases.

W7_05

A genome-wide association analysis of HDL-cholesterol in the population-based KORA study sheds new light on intergenic regions

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Background: High-density lipoprotein cholesterol (HDLC) is a strong risk factor for atherosclerosis and assumed to be under considerable genetic control. We aimed to identify gene regions influencing HDLC levels by a genome-wide association (GWA) analysis in the population-based KORA Study.
Methods and results: In KORA S3/F3 (n=1,643), we analyzed 377,865 quality-checked SNPs (500 K Affymetrix), complemented by the publicly available GWA results from the Diabetes Genetics Initiative (DGI, n=2,631) and by replication data from KORA S4 (n=4,037) and the Copenhagen City Heart Study (n=9,205). Among the 13 SNPs selected from the KORA S3/F3 500 K p-value list, three SNPs showed consistent associations in subsequent replications: one SNP 10 kb upstream of CETP (pooled p-value=8.5E-27), one SNP about 40 kb downstream of LIPG (p-value=4.67E-10), both independent from previously reported SNPs, and one from an already reported region of LPL (p-value=2.82E-11). Bioinformatic analyses indicate a potential functional relevance of the respective SNPs.
Conclusions: Our GWA study identified two interesting HDLC-relevant regions upstream of CETP and downstream of LIPG which were independent of already known regions. This draws the attention to the importance of long-range effects of intergenic regions underestimated so far which may impact future candidate gene association studies towards extending the analyzed region. Furthermore, our study reinforced CETP and LPL as HDLC genes and thereby underscores the power of our study and of this type of GWA approach to pinpoint associations of common polymorphisms with effects explaining as little as 0.5% of the HDLC variance in the general population.

W7_06

Evidence for positive selection and RNA editing at codon 38 of the human KCNE1 gene

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The KCNE1 protein represents the regulatory beta-subunit of the slowly activating delayed rectifier potassium channel (IKs). The Glycin 38 Serine (G38S) variant of KCNE1 has been linked to congenital Long QT syndrome, a disorder which predisposes to deafness, ventricular tachyarrhythmia, syncope, and sudden cardiac death. Here, we used pyrosequencing to measure allele ratios of the G38S variant in genomic DNAs and cDNAs from 30 lymphoblastoid human cell lines and 14 human frontal cortices. Our results provide evidence for RNA recoding of the 38S (codon AGU) allele to 38G (GGU) by A to I editing in two heterozygous cell lines and one heterozygous frontal cortex. According to population genetic data, including sub-Saharan North Africans, the G38S variant emerged before the radiation of modern humans. Maximum likelihood analysis of the human data with partly newly sequenced orthologs from 18 other eutherian species suggests that the G38S variant evolved under positive Darwinian selection after the human-chimpanzee split. The amino acid exchanges involved are moderately radical, which argues for their functional relevance, probably in the fine tuning of IKs channels. A subtle advantage of heterozygotes might account for the maintenance of the variant in human populations. On the other hand, the 38S allele seems to be detrimental under certain physiological and/or environmental conditions, as suggested by the sporadic 38S/38G recoding and the absence of 38S homozygotes in the sub-Saharan sample. We speculate that individual differences in capability and extent of RNA editing at KCNE1 site 38 might have contributed to conflicting results of recent association studies between the G38S variant and the QT phenotype. The presented findings thus highlight the relevance of mRNA data in future association studies of alleles and clinical disorders. To our knowledge, this is the first report showing polymorphism, positive selection and RNA editing at a single codon site.

W_8 Cytogenetics / Prenatal Genetics

W8_01

Eight years of polar body diagnosis for monogenic disorders in Regensburg – current results and new developments

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Today preimplantation genetic diagnosis (PGD) for both, monogenic as well as chromosomal conditions is an accepted option for a restricted patient cohort. However, in Germany polar body diagnosis (PBD) is currently the only legal way to perform PGD. Here we summarize the results of 35 PBD cycles for monogenic disorders at our center. For each patient an individual multiplex fluorescent PCR system was developed, extensively optimized and tested regarding reproducibility, error rate and potential contaminations. After hormone stimulation oocytes are removed, ICSI is performed and the first and second polar bodies are sequentially biopsied. PCR products are evaluated on an ABI310 or ABI3100Avant sequence analyzer. So far we have performed PBD for Norrie disease, Huntington´s disease, Cystic fibrosis, Tuberous sclerosis, Myotonic dystrophy, Neurofibromatosis, Spinocerebellar ataxia 1, Becker muscular dystrophy and Fragile X syndrome. Retrieval of 268 oocytes resulted in 41 genetically diagnosed transferable embryos. In 23 cycles on average 1.8 embryos per cycle could be transferred and resulted in 7 pregnancies (pregnancy rate 30.4% per transfer cycle), delivery of 8 children (2x twins) and one pregnancy is still ongoing.
An insufficient number of available oocytes is an important issue. This is of particular disadvantage for heterozygous female mutation carriers for autosomal recessive or X chromosomal inherited disorders, where PBD requires to discard all oocytes carrying the mutation, although they may result in a healthy offspring, if the sperm does not carry the mutation or the Y chromosome, respectively. We therefore implemented vitrification to collect oocytes from successive ICSI cycles. We demonstrate that PBD for monogenic disorders can be successfully applied within the strict German legal framework. It requires extensive interdisciplinary cooperation and counseling of the couple regarding all medical, genetic and methodical aspects and limitations of PBD.

W8_02

Disease-associated chromosome rearrangements: Systematic identification of genes for non-syndromic hearing impairment

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The genetic heterogeneity of non-syndromic hearing loss (NSHL) renders identification of new disease genes difficult. In 323 clinically well characterized NSHL patients who attended a specialist clinic, we found five cases with apparently balanced chromosome rearrangements, one case with an unbalanced rearrangement, and two sex chromosome aneuploidies. Because 8/323 (2.5%) are far more than the prevalence in normal populations, it is plausible to assume that in most of these cases the association between NSHL and chromosome rearrangement is not coincidential. A homozygous reciprocal translocation, 46,XY,t(10;11)(q24.3;q23.3),t(10;11)(q24.3;q23.3) was detected in a boy with sensorineural NSHL. The consanguineous parents, who suffer from mild high-frequency hearing impairment, and their four other children, who all hear normally, were heterozygous translocation carriers. The chromosome 10 breakpoint disrupts the PDZD7 gene, which shares sequence homology with the PDZ domain containing genes, USH1C (harmonin) and DFNB31 (whirlin). Protein-protein interaction assays demonstrated the integration of PDZD7 in the protein network related to the Usher syndrome. PDZD7 is a new autosomal-recessive NSHL gene and also a prime candidate gene for Usher syndrome. A reciprocal translocation, 46,XY,t(10;15)(q26.13;q21.1) was detected in a boy with mild sensorineural NSHL. The breakpoint on chromosome 10 was localized in the 3´-region of the ATE1 gene, whereas the breakpoint on chromosome 15 disrupts the coding region of the SLC12A1 gene. We propose that the translocation unmasks a recessive mutation in the 1Na-1K-2Cl cotransporter SLC12A1. A girl with conductive hearing loss and apparently normal development presented with an unbalanced de novo translocation, 45,XX,der(18)t(18;22)(p11.32;q11.21). The deleted 16.4 Mb region on chromosome 22pter-q11.21 contains 10 genes, of which we consider CECR1, CECR2, and CECR6 as candidates for NSHL.

W8_03

Novel mechanisms causing type E brachydactyly in a family with a balanced t(8;12)(q13;p11.2) translocation

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We studied a 3-generation family with type E brachydactyly and identified a t(8;12)(q13;p11.2) translocation. We used FISH with flanking and breakpoint overlapping BACs to characterize the translocation and identified PTHLH on chromosome 12p11.2 and KCNB2 on chromosome 8q13 as candidate genes. KCNB2 was disrupted in intron 2, while the chromosome 12 breakpoint was localized 86 kb upstream of PTHLH; only the latter gene is involved in skeletal development. The 12p11.2 breakpoint is conserved and features an AP1 binding site upstream of PTHLH. Due to the translocation, an EBS core consensus sequence from 8q13 resides near the AP1 site. Since both transcription factors cooperate and interact, we tested if AP1 and ETS1 can activate PTHLH in murine (ATDC5) and human (T/C28-I2) chondrocytes. We used the breakpoint sequences of derivative chromosome 8 and 12 (der(8), der(12)) and the nonaffected chromosome 8 and 12 allele sequences in reporter-gene assays. Four histone modifications (H3K4me1, H3K4me3, H3K9me3, H3K27me3) were present in the breakpoint area. We found the enriched histone H3K4me1 modification at the chromosome 12 breakpoint position in murine and human chondrocytes, while affected fibroblasts showed higher H3K4me1 enrichment at the der(8) breakpoint. Reporter-gene constructs containing the der(8) breakpoint in front of the PTHLH promoter revealed activation in murine and human chondrocytes. Furthermore, the breakpoint sequence bound to recombinant human AP1 and ETS1 in EMSAs. Western blotting after PMA-stimulated AP1 and ETS activation and overexpression of different AP1 and ETS1 combinations showed activated PTHrP expression. We suggest that PTHLH is upregulated by AP1 and EBS in chondrocytes from our patients, which could lead to type E brachydactyly.

W8_04

3-dimensional multicolor banding (3D-MCB) reveals the distribution of chromosomes in human sperm

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Nuclear architecture studies in human sperm are sparse. By now performed ones were practically all done on flattened interphase nuclei. Thus, studies close at the in vivo state of sperm, i.e. on three-dimensionally conserved interphase cells, are lacking by now. Only the position of 14 chromosomes in human sperm was studied, yet. Here for the first time a combination of multicolor banding (MCB) and three-dimensional analysis of interphase cells was used to characterize the position and orientation of all human chromosomes in sperm cells of a healthy donor. We could show, that the interphase nuclei of human sperm are organized in a non-random way, driven by the gene density and chromosome size. Overall, here we present the first comprehensive results on the nuclear architecture of normal human sperm. Future studies in this tissue type, e.g. also in male patients with unexplained fertility problems, may characterize yet unknown mechanisms of infertility.
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W8_05

A novel microdeletion syndrome involving 5q14.3-q15: Clinical and molecular cytogenetic characterisation of three patients

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In 10–15% of patients with mental retardation of unknown aetiology, molecular karyotyping by SNP arrays and array-CGH detects causative copy number variations (CNVs), most of which are scattered across the genome. Clinical interpretation of these novel CNVs is complicated by reduced penetrance of some causative copy number changes and benign copy number variants. Thus, several patients with similar copy number changes and a similar clinical presentation are needed to allow reliable genotype-phenotype correlation.
We report on three unrelated patients, one of them identified through the DECIPHER database, with overlapping de novo interstitial microdeletions involving 5q14.3-q15. All three patients presented with severe psychomotor retardation, epilepsy or febrile seizures, muscular hypotonia with feeding difficulties and variable brain anomalies.
Molecular karyotyping revealed three overlapping microdeletions measuring 5.7, 3.8 and 3.6 Mb respectively. The microdeletions were identified using SNP arrays (Affymetrix 100 K and Illumina 550 K) and array-CGH (1 Mb Sanger array-CGH, deletion size further characterized by BAC-FISH). Confirmation and segregation studies were performed using FISH and quantitative PCR. All three aberrations were confirmed and proven to have occurred de novo. The boundaries and sizes of the deletions in the three patients were different, but an overlapping region of around 1.6 Mb in 5q14.3 was defined. It included five genes: CETN3, AC093510.2, POLR3G, LYSMD3 and the proximal part of GPR98, a known epilepsy gene. Haploinsufficiency of GPR98 is probably responsible for the seizure phenotype in our patients. LYSMD3 shows a high level of central nervous expression during embryogenesis and may be a candidate gene for other central nervous system symptoms such as psychomotor retardation, brain anomalies and muscular hypotonia of the 5q14.3 microdeletion syndrome.

W8_06

ISCN (2009): Important revisions and new additions to molecular methods for copy number detection for cytogenetic analysis

Tönnies H.[1], Campbell L.J.[2], Slovak M.L.[3], Chaabouni M.[4], Fukushima Y.[5], Harrison C.J.[6], Liehr T.[7], Madon P.[8], Mandahl N.[9], Rao K.W.[10], Rosenberg C.[11], Schinzel A.[12], Barber J.C.[13], Shaffer L.G.[14]
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In 2005, the International Standing Committee on Human Cytogenetic Nomenclature published its recommendations for describing results of array-based comparative genomic hybridization (CGH). This nomenclature was mainly developed to describe results from BAC arrays, the predominant array format at the time of the Committee meeting in 2004. Since then, other microarray platforms have proved useful for chromosome analysis including CGH-based oligonucleotide arrays and SNP arrays. Because of these developments, the array nomenclature was updated at the recent meeting of the Standing Committee in October, 2008. The recommendations and revisions will be published in the new International System for Human Cytogenetic Nomenclature (ISCN) early in 2009. The revisions contain nomenclature that encompasses all types of platforms in both a detailed system, which includes the abnormal nucleotides as well as the bordering normal nucleotides and a short system for describing normal and abnormal results. The nomenclature is restricted to a nucleotide-based description of gains and losses. Examples of nomenclature combining G-banding descriptions, FISH and microarray results will be presented. Because of the additional information that can be gained from SNP arrays, new terms and abbreviations have been introduced to describe homozygosity (hmz) resulting from UPD or LOH and situations for the use of heterozygosity (htz). Terms and usage for mosaicism have been provided. Also new to the ISCN, recommendations for describing results after multiple ligation-dependent probe amplification (MLPA) have been introduced.

Educational Sessions

EDU_1
Imprinting Update
Thomas Eggermann (Aachen), Gabriele Gillessen-Kaesbach (Lübeck)

B. Horsthemke, Essen: Grundlagen des Imprinting
G. Gillessen-Kaesbach: Klassische Imprinting-Erkrankungen
T. Eggermann: Seltene und komplexe Imprintingerkrankungen

Während der Großteil der menschlichen Erbinformation unabhängig von der elterlichen Herkunft weitergegeben wird, sind ca. 100 Gene bekannt, bei denen nur die mütterliche oder väterliche Genkopie funktionell genutzt wird. Die balancierte Expression dieser "geprägten" Gene (Genomic Imprinting) gewährleistet eine regelrechte Entwicklung eines Organismus, während eine Störung dieses Prägemusters mit zahlreichen pathologischen Veränderungen einhergehen kann. Zwar sind angeborene syndromale Krankheitsbilder auf der Basis von Prägestörungen seit längerem bekannt, die Dynamik dieses Gebietes zeigt sich in jüngster Zeit abereindrucksvoll u.a durch die Identifikation einer weiteren Imprintingerkrankung, des Temple-Syndroms (Chromosom 14), sowie des Nachweises von Punktmutationen im ZFP57-Gen, die zu generellen Hypomethylierungen geprägter Loci führen. Trotz der unterschiedlichen klinischen Symptomatik weisen alle bisher bekannten Imprintingerkrankungen ähnliche (Epi)mutationen auf, die letztendlich zu vergleichbaren funktionellen Veränderungen führen. Trotz des rapiden Wissenszuwachses auf dem Gebiet des "Genomic Imprinting" sind die funktionellen Zusammenhänge zwischen den wahrscheinlich ursächlichen Veränderungen und der klinischen Auswirkung aber weitestgehend unklar.
In der Sitzung werden die aktuellen genetischen Grundlagen und die klinischen Aspekte der bekannten und neuen Imprintingerkrankungen (Transienter Diabetes mellitus, Silver-Russell-Syndrom, Beckwith-Wiedemann-Syndrom, Temple-Syndrom, Prader-Willi-Syndrom, Angelman-Syndrom, maternales Hypomethylierungssyndrom) referiert und die Bedeutung der molekularen Befunde für die klinisch-genetische Einordnung, Beratung und prä- bzw. postnatale Diagnostik diskutiert.

EDU_2
Risikoscreening für Aneuploidien
Peter Miny (Basel), Bernd Eiben (Essen)

Peter Miny: Der aktuelle Stand aus der Sicht des genetischen Beraters
Bernd Eiben: Organisation und Programme in Deutschland, Österreich und der Schweiz

Diskussion: Seit rund 10 Jahren wird das sog. Ersttrimesterscreening zur Risikoabschätzung für Aneuploidien bereits früh in der Schwangerschaft eingesetzt. In einem verbreiteten Ansatz werden Nackentransparenz, freies beta-hCG, PAPP-A und das mütterliche Alter zur Riskoermittlung benutzt. Zahlreiche Studien haben die Ueberlegenheit dieses Vorgehen im Vergleich zur alleinigen Berücksichtigung des mütterlichen Alters oder der zusätzlichen Verwendung von mütterlichen Serumparametern im zweiten Schwangerschaftstrimenon belegt. Eine Sensitivität von bis zu 90% für Trisomie 21 bei einer Falsch-positiv-Rate von 5% oder weniger sind nach den bisherigen Erfahrungen ein realistisches Ziel in gut organisierten Programmen.
Mit zunehmender Verbreitung des Ersttrimesterrisikoscreenings wurde immer wieder über die abnehmende Zahl von invasiven Eingriffen (Amniozentesen und Chorionzottenbiopsien) spekuliert. Beeindruckende Zahlen liegen seit jüngsten aus Dänemark vor (Ekelund et al. 2008). Die Auswirkungen der Etablierung des Ersttrimesterrisikoscreenings als diagnostisches Angebot sind in einer nationalen Kohortenstudien mit rund 65 000 Schwangerschaften pro Jahr unter Beteiligung von 19 Zentren und dem zentralen Zytogenetikregister systematisch erfasst worden. Die Zahl geborener Kinder mit Down Syndrom nahm von rund 60 in den Jahren 2000–2004 auf 31 in 2005 bzw. 32 in 2006 ab. Die Zahl invasiver Eingriffe (Amniozentesen und Chorionzottenbiopsien) nahm von 7524 in 2000 auf 3510 im Jahre 2006 ab. Die Entdeckungsrate lag 2005 bei 86% und die Falsch-positiv-Rate bei 3,9%. Die entsprechenden Zahlen für 2006 betrugen 93% und 3,3%. Dabei stieg in Dänemark wie in vielen anderen entwickelten Länder das durchschnittliche Alter der Mütter im letzten Jahrzehnt deutlich an. In der Schweiz waren 2006 die Mütter bei 27% aller Schwangerschaften älter als 34 Jahre. Die Befunde belegen die Ueberlegenheit des Ersttrimesterrisikoscreenings bei der Identifizierung von Risikoschwangerschaften eindrücklich.
Nach den Regeln der evidenzbasierten Medizin sollte die Indikationsstellung für invasive pränataldiagnostische Verfahren zumindest bei Schwangeren unter 40 Jahren nicht mehr allein aufgrund des Alters gestellt werden. Allerdings verlangt das Ersttrimesterriskoscreening besondere Anstrengungen im Hinblick auf Organisation und Qualitätssicherung. Insbesondere die Messung der Nackentransparenz erfordert eine gute apparative Ausstattung und eine gezielte Weiterbildung.
Im Workshop soll der aktuelle Stand des Erstrimesterrisikoscreening auch unter Berücksichtigung anderer Aneuploidien sowie zusätzlicher Marker kurz zusammengefasst werden. Darüberhinaus wird die Notwendigkeit einer engen Kooperation mit systematischem Datenaustausch zwischen Frauenarzt, Laborarzt und genetischem Berater begründet. Die in Deutschland, Oesterreich und der Schweiz angebotenen unterschiedlichen methodischen Ansätze und Programme werden vorgestellt. Für Fragen, Kommentare und Diskussion ist etwa ein Drittel der verfügbaren Zeit vorgesehen.
Ekelund CK, Jørgensen FS, Petersen OB, Sundberg K, Tabor A, Danish Fetal Medicine Research Group. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study BMJ. 2008; 337: a2547. Published online 2008 November 27. doi: 10.1136/bmj.a2547

EDU_3
Copy number abnormalities (CNAs) in der Tumorgenetik
Christa Fonatsch (Wien), Reiner Siebert (Kiel), Norbert Arnold (Kiel)

N. Arnold: CNAs bei malignen Tumoren: Biologische Grundlagen
R. Siebert: Methoden zum Nachweis von CNAs in der Tumorgenetik
N. Arnold: CNAs bei soliden Tumoren Biologische und klinische Relevanz
R. Siebert: CNAs bei hämatopoetischen Neoplasien – Biologische und klinische Relevanz

Es wird allgemein angenommen, dass die Progression von Tumoren durch die kontinuierliche Akkumulation genetischer und epigenetischer Veränderungen hervorgerufen wird. Diese Veränderungen können Gene betreffen, die in die Kontrolle von Zellzyklus, DNA-Reparatur, genomischen Stabilität, Zelladhäsion, Angiogenese und transmembrane Signalübertragung involviert sind. Es gibt Hinweise, dass mehr als 30% des Genoms solider Tumore abnormale Kopienzahlen oder andere Aberrationen aufzeigen. Durch neuere Methoden wie array CGH, SNP arrays oder auch next-generation sequencing können kleinere Deletionen und Amplifikationen in einer bislang nicht erreichten Auflösung charakterisiert werden. In Kombination mit klassischen zytogenetischen und molekular-zytogenetischen Verfahren können chromosomale Regionen identifiziert werden, die als mögliche Marker für die Prädiktion des Therapieansprechens und Ziele neuer therapeutischer Ansätze dienen. Ein Musterbeispiel für die Umsetzung von genetischen Informationen zu einer genspezifischen Therapie stellt der Nachweis der Amplifikation und Überexpression von HER-2/neu in Mammakarzinomen und die Behandlung der Patientinnen mit

einem rekombinanten monoklonalen Antikörper (Herzeptin) gegen HER-2/neu dar.
In der Sitzung werden die biologischen Grundlagen der CNA dargestellt. Des Weiteren werden die Nachweismöglichkeiten von CNAs mit verschiedenen Technologien vergleichend gegenübergestellt. Zum Abschuss werden Beispiele für CNAs bei soliden Tumoren und hämatologischen Neoplasien vorgestellt und deren biologische, diagnostische und klinische Relevant diskutiert.

EDU 4
Ionenkanal-Krankheiten
Ortrud Steinlein (München), Bernd Wollnik (Köln)

Dysfunction of ion channels due to causative mutations has been described in a number of human diseases. Although disorders described can affect different organs and tissues, the majority of them are known to affect the electrical stability of neuronal and cardiac excitation. For this reason, our session will focus on pathogenic mechanisms of neuronal and cardiac disorder caused by mutations in ion channel genes.
Dysregulation of the precarious balance between excitatory and inhibitory neurotransmission due to inherited or acquired gene mutations has been implicated in a wide range of neuronal disorders. Mutations in nicotinic acetylcholine receptors have been found in familial nocturnal frontal lobe epilepsy, and several subtypes of this receptor are associated with nicotine dependence, Alzheimer disease or Morbus Parkinson. The GABAergic system has long been implicated in epileptogenesis, and dysregulation of GABAergic neurotransmission, which plays a role in both Angelman syndrome and Rett syndrome, is also suspected to be involved in the pathogenesis of idiopathic autism. Mutations in some voltage gated potassium channel genes can cause a rare seizure disorder of the newborn, while other potassium channels are mutated in inherited deafness. At least three inherited paroxysmal neurologic syndromes have been linked to mutations in the voltage gated calcium channel gene CACNA1A: episodic ataxia type 2, familial hemiplegic migraine and spinocerebellar ataxia type 6. The possible pathophysiological mechanisms underlying channelopathies include loss-of-function mutations in neurotransmitter genes, neurotransmitter receptor subunit genes or genes necessary for the synthesis of neurotransmitters as well as mutations in genes involved in epigenetic regulation of the neurotransmitter system. Several of the neurological disorders that are caused by disruption of either excitatory or inhibitory neurotransmission and their pathomechanisms will be discussed.
Cardiac arrhythmias are a leading cause of morbidity and mortality. Major rhythm disturbances, such as ventricular fibrillation, tachyarrhythmias, bradyarrhythmias, and asystole, represent the most pivotal mechanisms of sudden cardiac death The ability to predict, prevent, and treat these often life-threatening disorders remains a major scientific and medical challenge. Recent molecular genetic led to the identification of genes responsible for the long and short QT syndromes (LQTS; SQTS), inherited cardiac repolarisation abnormalities that are associated with ventricular tachycardias and sudden cardiac death. Functional characterization of identified genes and mutations gave fascinating insides into both, the physiology of hear excitation and pathophysiology of arrhythmias. Subsequently, mutations have been found in other forms of cardiac arrhythmias with a primary electrical cause: e.g. Brugada syndrome, progressive familial heart block, catecholaminergic-induced ventricular tachycardia, and sinus node dysfunction. In this session, the identification and functional analysis of disease causing, predisposing and modifying genetic factors responsible for inherited, sporadic, and complex forms of cardiac arrhythmias will be presented focusing on its application in new molecular genetic tests, genetic counseling services, and development of new therapeutic strategies.

Poster

P001–P068 Clinical genetics

P001
Deletions and mutations of the ZIC1 and ZIC4 genes are a rare cause of Dandy-Walker malformation and related disorders
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The Dandy-Walker malformation (DWM) is the most common malformation of the cerebellum affecting approximately 1:5000 births. DWM is defined by hypoplasia and upward rotation of the cerebellar vermis and a retrocerebellar cyst communicating with the fourth ventricle. DWM has been reported in a wide variety of chromosomal disorders and is also part of many different genetic syndromes. Prenatal exposure to teratogens (rubella and alcohol) are also known to be associated with DWM. But in most cases of DWM the underlying cause is still unknown.
Recently, deletions involving the ZIC1 and ZIC4 genes (= zinc fingers of the cerebellum) on chromosome 3q24 have been described in seven patients with DWM (Grinberg et al. (2004), Nat Genet 36:1053–5). The Zic gene family members are vertebrate homologs of the Drosophila odd-paired gene, which is essential for the parasegmental subdivision during embryonic development. The frequency of ZIC1 and ZIC4 microdeletions in DWM patients is still unclear. Furthermore, patients with DWM have been so far not examined with redard to point mutations or small intragenic deletions in these genes.
We therefore analysed 16 patients with typical DWM and 10 patients with related cerebellar malformations (vermis hypoplasia (n=4), cerebellar hypoplasia (n=2) and cerebellar agenesis (n=4)) for deletions (MLPA) and point mutations (sequencing) within the ZIC1 and ZIC4 genes. All patients had normal karyotypes, no diagnosis of a known genetic syndrome or prenatal exposure to teratogens was reported. We could neither detect a deletion nor a disease causing mutation in any of the 26 patients. In conclusion, deletions and point mutations in ZIC1 and ZIC4 seem to be a rare cause of DWM.

P002
Mycophenolate mofetil – a teratogenic drug? Experience of the Berlin Instiute of Clinical Teratology and drug risk assessment during pregnancy
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Mycophenolate mofetil (MMF) is a newer immunosuppressant mainly used after organ transplantation. Experience with MMF exposure in human pregnancies is limited to case reports and two registries suggesting a high number of both fetal losses and malformations. Summarizing the 15 published cases with birth defects and 3 retrospectively ascertained cases reported to us the following features were observed more frequently: Microtia (13/18), atresia of external auditory canal (10/18), cleft lip and palate (7/18), congenital heart defect (6/18), coloboma or other eye anomalies (5/17), brachydactyly/nail hypoplasia (4/16), tracheo-oesophageal anomalies (4/17), agenesis of corpus callosum (2/17), diaphragmatic hernia (2/17) and kidney anomalies (2/17). The consistent pattern of ear malformations and clefts as well as comparable malformations seen in animal studies raise the question of a possible teratogenicity of MMF. However, so far there are no prospective data allowing an estimation of the teratogenic potential.

We report on 12 prospectively followed pregnancies with maternal exposure to MMF during the first trimester: There were two fetal losses (week 6+7), one early pregnancy termination and 8 liveborn children without major malformations. In three of the 8 newborns intrauterine growth retardation had been described. Three children were born prematurely in gestational week 25, 31 and 33, respectively. One pregnancy with first trimester exposue is still ongoing.
More prospectively followed pregnancies are needed in order to estimate the teratogenic risk. At this stage, we would recommend a detailed fetal ultrasound scan with special focus on cranio-facial structures and heart defects after inadvertent exposure to MMF. The observed higher incidence of fetal losses, intrauterine growth retardation and prematurely born children after MMF-therapy might also be related to maternal disease or the other concomitant immunosuppressive therapy.

P003

The role of genetic polymorphisms and the subgingival occurrence of periodontopathogens

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Background: Periodontitis is characterized as a chronic inflammatory disease. Periodontopathogens induce innate responses amongst others via CD14, TLR 2 and 4 resulting in activation of NF-kB. Functional important SNPs are described for these genes. The aim of this study was to evaluate links between SNP and the subgingival occurrence of periodontopathogens.
Patients and methods: 141 periodontitis patients and 81 healthy controls without periodontitis were included in the study. SNPs in CD14 (c.-159C>T), in TLR4 (Asp299Gly, Thr399Ile), in TLR2 (Arg677Trp, Arg753Gln), and in NF-kB (-94ins/delATTG) were determined by RFLP. Subgingival bacterial colonization was analysed molecularbiologically using the micro-Ident®test.
Results: Associations between genotype and the occurrence of periodontopathogens could be shown: Heterozygous carriers of the Arg-753Gln SNP in TLR2 are at a higher risk for the occurrence of bacteria of the "red complex" (p=0.042). P. intermedia occurred less frequently in individuals positive for the TT genotype of CD14 (p=0.045). Homozygous del/del carriers of the NF-kB SNP had a higher risk for subgingival colonization with A. actinomycetemcomitans (p=0.031). In binary logistic regression analyses, the associations for SNP in CD14 (OR=3.22, CI: 1.23–8.33, p=0.017) and NF-kB (OR=2.7, CI: 1.04–6.98, p=0.042) could be proven considering age, gender, smoking, and clinical attachment loss or approximal plaque index, respectively, as confounding factors. No genotype dependent association with subgingival occurrence of periodontopathogens could be proven for SNPs in TLR4 and TLR2 (Arg677Trp).
Conclusions: SNPs in candidate genes responsible for the bacterial recognition and elimination could be shown to be associated with the subgingival occurrence of periodontopathogens. The analysis of the disease related genetic pattern may help to identify early persons at high periodontal risk and improve the success of the periodontal therapy.

P004

Narrowing the diagnostic criteria of FG (Opitz-Kaveggia) syndrome

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FG syndrome (FGS) is an X-linked mental retardation syndrome, characterized by a particular cranio-facial appearance, hypotonia, broad thumbs and halluces, anal anomalies and/or constipation, and hypoplasia of the corpus callosum. The number of phenotypic anomalies attributed to FGS has increased considerably since its initial description by Opitz and Kaveggia in 1974. As a result, the syndrome definition was blurred and diagnosis complicated.
A recurrent mutation 2881C->4T in the MED12 gene was reported in 9 unrelated males/families. A mutation in the UPF3B gene was found in 2 affected brothers and their mother.
We report our experience with 15 patients who were given a clinical diagnosis of FGS. The main diagnostic criteria were relative macrocephaly, prominent forehead with frontal hair upsweep, hypertelorism, downslanting palpebral fissures, small abnormal ears, broad thumbs, abnormalities of the corpus callosum, anal anomalies/constipation, mental retardation (MR) with a friendly and hyperactive personality and male gender. None of the criteria except of MR and male gender were considered to be obligatory. All but two patients represented sporadic cases and had an unremarkable family history.
MED12 mutation analysis was done by sequencing of all 48 coding exons in nine patients and only of exons 21 and 22 in six patients. Molecular testing of the UPF3B gene by sequencing of the 11 coding regions was performed in all 15 FGS patients.
Only one patient was found to have the recurrent mutation R961 W in the MED12 gene, no mutation in the UPF3B gene was identified.
In summary, our results together with the data from the literature show that

(i) the phenotype appears to be highly consistent in all MED12-mutation positive patients,
(ii) patients with features that overlap with FGS make up a clinically variable and genetically heterogeneous group of different MR syndromes,
(iii) UPF3B mutations are not common in patients with features that overlap with FGS.

P005

Sitosterolaemia and mental retardation – coincidence by chance?

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Introduction: Sitosterolaemia is a rare autosomal recessive sterol storage disease. Clinical manifestations are tendon xanthomas, premature cardiovascular disease, arthralgias and occasionally haemolysis and thrombocytopenia. Sitosterolaemia is caused by mutations in the ABCG5 and ABCG8 genes encoding a heterodimerized form of a transporter which transfers plant sterols into the lumen of the intestine or biliary channels. Sitosterolaemia can be treated by a diet restricted

in plant sterols and application of ezetimibe which blocks the intestinal resorption of sterols.
Case report: We present a 15 year old girl born at 35 weeks of gestation to consanguineous parents, who was brought to our attention because of moderate mental retardation. She showed microcephaly, muscular hypotonia, mild anaemia and mild thrombocytopenia. She had facial dysmorphism with micrognathia, left sided epicanthus, short palpebral fissures, broad eyebrows and a broad nose. The cranial MRI showed periventricular leukomalacia. She had a normal karyotype. Fragile (X)-syndrome – as diagnosed in her younger brother – was excluded. A 600 kb SNP array is pending. Metabolic diagnostics performed for the evaluation of mental retardation revealed significantly elevated levels of the plant sterols sitosterol and campesterol.
Discussion: The laboratory findings and part of the clinical features are consistent with sitosterolaemia. However, to our knowledge, mental retardation has not been reported as a sign of this disease. While in the present case, periventricular leukomalacia is most likely a residue of prematurity, it does not explain the mental retardation completely. Apart from an additional gene defect unrelated to ABCG5 and ABCG8, it is also possible that a deletion encompassing ABCG5 or ABCG8 could be the cause of mental retardation. A detailed mutation analysis of the ABCG5 and ABCG8 genes is currently performed to further elucidate the underlying genetic defect.

P006
Xq28 duplication – an emerging phenotype
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Recently, 14 male patients of five families have been reported with a duplication of Xq28 and a consistent phenotype (Meins et al. 2005, Van Esch et al. 2005). The clinical features include retarded psychomotor development, severe mental retardation, absence of expressive speech, recurrent respiratory infections, seizures, hypotonia in infancy and spasticity at later age. It was suggested that increased gene dosage of MECP2 would be the most likely explanation for the severe mental retardation. Up to date multiple male patients of altogether 50 families have been reported with Xq28 duplications of varying sizes and rather consistent phenotype. Further genes involved in the duplicated Xq region are FLNA, GDI1, L1CAM, IRAK1, SLC6A8 and some others (Meins et al. 2005, Lugtenberg et al. 2005, Van Esch et al. 2005, Del Gaudio et al. 2006, Friez et al. 2006, Smyk et al. 2008, Lugtenberg et al. 2008, Clayton-Smith et al. 2008, Bauters et al. 2008).
Here we present an 11-year-old boy with Xq28 duplication encompassing the MECP2 gene. He presents with severe mental retardation, seizures and frequent respiratory infections. He speaks around five words, his comprehension is rather bad. His EEG pattern was abnormal since the age of three years and he developed treatment resistant seizures at the age of eight years. Body measurements at birth and at present are appropriate for age. Since the age of a toddler he suffered from recurrent bronchitis with a tendency to develop severe pneumonias. His gait is ataxic and he shows progressive spasticity of the legs.
Molecular testing for Angelman and Fragile X syndromes gave normal results. Finally a duplication of the MECP2 region was detected by MLPA. Array CGH analysis using the Agilent 105 k Array revealed a gain of approximately 1 MB. The mother of the patient carries the same duplication in Xq28.
We present a review of the literature with a discussion of the relevance of the clinical features and the role of the involved genes.

P007
Molecular characterisation and phenotypic comparison of a patient with 17q21.31 microdeletion syndrome showing the typical clinical features and a patient with a larger 17q21.31 deletion
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The 17q21.31 microdeletion syndrome (MIM 610443) is a novel genomic disorder that has originally been identified after screening large heterogeneous cohorts of individuals with mental retardation and dysmorphic features using array CGH. The deletion encompasses MAPT (MIM 157140) and CRHR1 (MIM 122561).
We report on a 3 year old girl with facial dysmorphisms including upward slanting palpebral fissures, blepharophimosis and a pear shaped nose with a bulbous nasal tip. Hypotonia with feeding difficulties in infancy and psychomotor developmental delay with pronounced delay of speech development were observed. Further noted features were hip dysplasia and hypoplastic labia majora and minora. Prenatal cytogenetic testing performed because of increased nuchal translucency showed a balanced t(12;13) translocation also seen in the unaffected father. Array-CGH revealed a heterozygous deletion at 17q21.31-q21.32 (~1.04 Mb). FISH analysis of the parents demonstrated that the deletion has occurred as a de novo event.
The second patient with the larger deletion, a 2 year old boy, was more severely affected. Besides some typical signs of the 17q21.31 microdeletion syndrome such as hypotonia, developmental delay and facial dysmorphisms he presented with microcephaly, dystrophy, thumb hypoplasia and unilateral kidney aplasia. Therefore the diagnosis of Fanconi anemia (FA) was initially suspected. Cell cycle analysis by FACS showed some findings compatible with FA. However, these data could not be confirmed by chromosomal breakage analysis. Array-CGH revealed a heterozygous deletion at 17q21.31-q21.32 (~2.7 Mb) encompassing 41 known genes including MAPT and CRHR1 as well as WNT3 (MIM 165330) and WNT9B (MIM 602864). FISH analysis of the parents demonstrated that the deletion has occurred as a de novo event.
Referring to our patients we report in detail clinical and molecular genetic aspects of the 17q21.31 microdeletion syndrome in comparison to the case with the larger deletion.

P008
Detection of gene deletion in the PROS1 gene using Multiplex Ligation-Dependent Probe Amplification (MLPA) assay
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Introduction: Protein S (PS) is a vitamin K-dependent plasma glycoprotein regulating blood coagulation by acting as a non-enzymatic cofactor to activated protein C in the proteolytic degradation of procoagulant factors Va and VIIIa.
PS deficiency is an autosomal dominant disorder linked to the protein S gene (PROS1). Heterozygous PS deficiency is present in approximately 2–5% of patients, suffered from thrombosis (Seligsohn and Lubetsky 2001), whereas the prevalence in the general population has been estimated to be between 0,03–0,13% (Dykes et al., 2001).
Patients and methods: Examination for mutations in the PROS1 gene has been done in 33 patients with suspected PS deficiency. Genomic DNA was extracted from peripheral blood leukocytes followed by PCR amplification of exons including corresponding exon-intron boundaries. Multiplex Ligation-Dependent Probe Amplification (MLPA) SALSA P112 probemix by MRC-Holland was used for detection of deletions in exons 1, 2, 4-7, 9–13 und 15 of the PROS1 gene.

Results: In 11 (33%) out of these 33 patients one mutation within the PROS1 gene was detected. In 5 out of these 11 patients (46%) we identified novel mutations, and 2 patients (18%) carried a heterozygous PROS1 gene deletion.
Conclusion: Large deletions of PROS1 gene are relatively common causes of protein S deficiency. The detection frequency of mutations within the PROS1 gene can be up 33% if medical history and biochemical screening were used in advance to genetic testing. Absence of mutations in sequence analysis of PROS1 gene should be followed by screening for large deletions e.g. by Multiplex Ligation-Dependent Probe Amplification (MLPA) in these patients.

P009

Primary hypoparathyroidism due to a novel germline mutation in the CASR gene – a case study

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Introduction: Mutations in the CASR gene result in gain- or loss of receptor function. Gain of function mutations are associated with autosomal dominant hypocalcemia, while loss of function mutations are associated with familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism.
Patient: We present a 47-year-old male patient from Germany, who was referred to our center to assess and optimise therapy of primary hypoparathyroidism.
In 1993 the patient was in neurologic care because nervousness and tetany had been misdiagnosed as tetany due to hyperventilation. Biochemical screening demonstrated hypocalcaemia, elevated phosphate level and PTH level slightly below normal. Computer tomography excluded a cerebral neoplasia but showed calcification of basal ganglia. A primary hypoparathyroidism was diagnosed and confirmed.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding regions (exons 2–7) and corresponding exon-intron boundaries of the CASR gene. PCR products were sequenced directly.
Results: Sequence analysis of the CASR gene showed a novel heterozygous germline mutation in exon 3, codon 139 (ATT>ATG), leading to the substitution of isoleucine (Ile) to methionine (Met) and therefore to an alteration of the amino acid sequence within the enzyme. Due to genetic counseling and testing for this mutation further family members from three generations with hypoparathyroidism were identified as carriers of this mutation subsequently.
Conclusion: The present case report demonstrates that genetic counselling and molecular diagnostics of the CASR gene is appropriate for the diagnosis of calcium disorders and associated diseases like primary hypoparathyroidism, especially if several relatives are affected.

P010

Severe generalized lipoatrophy and marfan syndrome in a woman with a frameshift mutation at the 3' terminus of FBN1

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We report an unusual combination of Marfan syndrome and an extreme reduction in the amount of subcutaneous fat tissue since birth. The female patient presented with slender fingers and toes, moderate hyperextensibility of the elbows, a narrow thorax, mildly dilated aortic bulb, lens luxation and severe myopia (-20dpt) consistent with Marfan syndrome. Marfan syndrome was confirmed in our patient by mutation analysis in FBN1 which revealed a novel de novo heterozygous two base pair deletion, c.8155_8156del2 in exon 64.
However, another striking problem in our patient was so far neither associated with FBN1 mutations nor described in patients with Marfan syndrome: a congenital and persisting profound reduction of subcutaneous fat. At the age of 24 years, the proband's height was at the 10^{th} percentile, her weight was below the 3^{rd} percentile and the head circumference was at the 10^{th}-25^{th} percentile. Altogether, there was a progeroid appearance with extreme lack of subcutaneous fat tissue.
As the phenotype extends beyond the features of Marfan syndrome, further diagnostic procedures were undertaken. She had an extensive endocrinological workup which did not reveal any abnormalities except for a low free thyroxine (T4) level not requiring therapy. Insulin and insulin receptor studies revealed normal results. Abdominal ultrasound examination, karyotype analysis and Array-CGH were normal. Classical progeroid syndromes were clinically ruled out by lack of premature greying, hypogonadism, and scleroderma-like skin disease. Genes known to be associated with lipoatrophy, BSCL1 (APGAT2), BSCL2 (seipin), FACE1, LMNA, LMNB2, and PPARG did not reveal a causative mutation.
In conclusion, we consider two possibilities:
(1) Marfan syndrome with atypical lipoatrophy due to the FBN1 mutation versus
(2) the presence of two diseases in our patient: Marfan syndrome and a so far unclassified form of lipoatrophy.

P011

A yet unreported region leading to unbalanced chromosomal abnormalities without phenotypic consequences in 10p11.2 to 10q11.2

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UBCA (= directly transmitted unbalanced chromosomal abnormalities) or EV (= euchromatic variants) were recently reported for >50 euchromatic regions of almost all human autosomes. UBCA or EV comprise a few megabases of DNA, and carriers are in many cases clinically healthy. Here we report on partial trisomies of chromosome 10 within the pericentromeric region which were detected by standard G banding. Those were referred for further delineation of the size of these duplicated regions for molecular cytogenetics and/or array-CGH. Partial trisomies of chromosome 10 in the pericentromeric region were identified prenatally in 7 cases. A maximum of three copies of the region between 10p12.1 to 10q11.22 was observed in all cases without apparent clinical abnormalities. The imbalances were either caused by a direct duplication in one familial case or by de novo small supernumerary marker chromosomes (sSMC). Thus, we report a yet unrecognized chromosomal region subject to UBCA detected in seven unrelated cases. To the best of our knowledge, this is the first report of an UBCA in the pericentromeric region of chromosome 10 that is not correlated with any clinical consequences.
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P012

Familial Sotos syndrome caused by a novel missense mutation, C2175S, in NSD1

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Sotos syndrome (SS, OMIM 117550) is characterized by pre- and postnatal overgrowth, macrocephaly, typical facial gestalt, large hands and feet, accelerated skeletal age, and developmental delay, and most patients (50–75%) demonstrate a mutation or microdeletion of the NSD1 gene at chromosome 5q35. Weaver syndrome (WS, OMIM 277590) is much rarer and shows extensive phenotypic overlap with SS especially at young ages. However, slight differences in facial gestalt, camptodactyly, deep set nails and more pronounced accelerated skeletal maturation have been described in WS. In 2003, NSD1 mutations were identified in six patients with Weaver syndrome (Douglas et al. AJHG 2003;72:132–143, Rio et al. J Med Genet 2003;40:436–440), but all patients of Douglas et al. were later reassigned as having SS, not Weaver syndrome (Tatton-Brown et al. AJHG 2005;77:193–204).
We report a familial Sotos syndrome in two children and their 36 year-old mother, who also suffers from type 1 diabetes. The underlying missense mutation, C2175S, occurred in a conserved segment of the NSD1 gene, which was assumed to be a hotspot for mutations causing Weaver syndrome. Our findings confirm that familial cases of SS are characterized by relatively mild or no developmental delay and better reproductive fitness and are more likely to carry missense mutations than nonfamilial cases. Findings promote the idea that NSD1 mutations in the 40-amino acid stretch (residues 2143 to 2183) formerly associated with Weaver syndrome (Douglas et al. 2003) cause SS, not the syndrome of Weaver (Tatton-Brown et al. 2005). Current practice is to perform NSD1 testing in all individuals for whom a diagnosis of Sotos or Weaver syndrome is considered, and if a mutation is identified, the diagnosis of SS is given.

P013

Buschke-Ollendorff syndrome: Genetics in a three generation family and a brief review of the literature

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Buschke-Ollendorff syndrome (BOS, OMIM #166700) refers to the association of connective tissue nevi and osteopoikilosis (OPK), a benign sclerosing skeletal dysplasia, characterized by widespread foci of osteosclerotic trabeculae radiologically showing small rounded or linear opacities. BOS is a rare autosomal dominant disorder (incidence about 1:20'000), with variable penetrance and considerable phenotypic heterogeneity. The first case of BOS was described by Buschke and Ollendorff in 1928. In 2004, heterozygous loss-of-function mutations in the LEMD3 gene, coding for MAN1, were discovered in both BOS and OPK. In 2007, a mutation in the same gene was found in a family with isolated connective tissue nevi (without OPK). Since then 21 germline mutations in LEMD3 were described causing different phenotypes with skin and/or bone involvement, i.e. OPK, BOS with or without melorheostosis, and familial connective tissue nevi.
We present a three generation BOS, caused by a novel mutation in the LEMD3 gene and discuss possible genotype-phenotype correlations reviewing the described mutations in the literature.

P014

Refinement of genotype-phenotype correlation in the non-lissencephalic 17p13.3 contiguous gene deletion syndrome

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Deletions in human chromosome 17p13.3-pter have been recognized to underlie the Miller-Dieker syndrome (MDS), characterized by classical lissencephaly distinct facial dysmorphism and variable further features. Classical lissencephaly occurs also as isolated brain malformation (ILS) and is usually associated with severe mental retardation, intractable epilepsy and spasticity. ILS was shown to be caused by point mutations or deletions of the PAFAH1B1 gene (LIS1) which is located within the MDS region in 17p13.3. Since the ILS deletion-region encompasses the LIS1 and further telomeric genes but never includes CRK and YWHAE, a MDS critical region including these two genes was proposed, while haploinsufficiency of the further telomeric region was thought to be clinically insignificant. Recently, the more severe brain phenotype of MDS was explained by the additional deletion of the YWHAE gene interacting with LIS1 and NUDEL during brain formation.
By molecular karyotyping we identified three novel non-lissencephalic patients with different submicroscopic deletions of the terminal 17p13.3 region allowing further refinement of the 17p- contiguous gene syndrome. Patient 1 showed a terminal 2.2 Mb deletion covering the MDS critical region but not LIS1 and had normal brain MRI, mild to moderate mental retardation, the typical facial characteristics of MDS, short stature and TOF. Patient 2 had an interstitial 1.3 Mb deletion also covering the MDS critical region but not LIS1 and showed also mild to moderate mental retardation, MDS facial features, short stature, PDA and patent foramen ovale. A terminal 1.2 Mb deletion was identified in patient 3 with learning difficulties and mild facial anomalies, only.
Our data confirm association of the MDS facial dysmorphism, mental retardation, short stature and congenital heart defects to the MDS critical region and exclude deletions of YWHAE without deletion of LIS1 as causative of brain malformations.

P015

Neonatal manifestation of multiple sulfatase deficiency

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Multiple sulfatase deficiency is biochemically characterized by the accumulation of sulfated lipids and acid mucoplysaccharides. It presents with combined clinical phenotypes of the different sulfatase deficiencies resulting in features of the following disorders: metachromatic leukodystrophy, Sanfilippo A syndrome, Sanfilippo D syndrome, Morquio A syndrome, Maroteaux-Lamy syndrome, which are autosomal recessive conditions, Hunter syndrome, which belongs to the group of X-linked disorders as well as X-linked types of ichthyosis and chondrodysplasia punctata. Based on the degree of severity and age of onset, neonatal, moderate and mild types of MSD have been differentiated.
So far, the neonatal form of multiple sulfatase deficiency has been described in detail only in two patients. We report clinical, biochemical, and molecular findings in a female newborn affected with a severe form of multiple sulfatase deficiency. She presented with primary microcephaly, facial anomalies including depressed nasal bridge, nasal hypoplasia, anteverted nostrils, smooth philtrum, limited mobility of hip and knee joints, mild ichthyosis as well as muscular hypotonia. The diagnosis is based on detection of excessive mucopolysaccharidurie and enzymatic assays performed in leucocytes which showed complete

deficiency of all of the measured sulfatases. Sequencing of the coding region of the underlying gene, SUMF1, could not identify any mutation. However, failure to detect the corresponding mRNA by RT-PCR proves defective SUMF1 expression.
Conclusion: The diagnosis of neonatal multiple sulfatase deficiency should be considered when dealing with the association of distinct facial anomalies, limited joint mobility, ichthyosis, and muscular hypotonia.

P016

Array CGH in postnatal cytogenetics - 8 additional cases

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In the last years more and more cytogenetically irresolvable chromosome imbalances could be detected by high resolution Array-CGH. We have performed Array-CGH analyses in 68 patients with development delay and/or various malformations and dysmorphisms indicating submicroscopic chromosome imbalance. All patients had been carefully examined by a pediatric clinical syndromologist and had normal conventional chromosome analyses. In all cases the parents of the patients had normal phenotype. 67 patients were investigated by use of the CytoChip arrays from Bluegnome, Cambridge, Great Britain. We add an additional phenotypic female patient (8) with XY sex reversal and dysmorphism, who was analysed by using the Human Genome Microarray Kit 105 K (Agilent).
In 8 of the 67 (~12%) investigated patients euchromatic imbalances have been detected. In 1 patient, the imbalance could already be verified by FISH-analyses performed after knowledge of the imbalance.
Patient 1: Deletion in the region Xp22.33 to Xp22.32 (2.6 Mb) and an amplification of the region 14q32.13 to 14q32.33 (11.5 Mb).
Patient 2: Deletion in the region 1q21.1 (3.7 Mb), FISH analysis confirmed the result.
Patient 3: Duplication in the region 4q34.1 to 4q35.2 (15.3 Mb).
Patient 4: Deletion in the region 3q27.2 to 3q29 (11.2 Mb).
Patient 5: Deletion in the region 16q23.1 to 16q23.3 (5.1 Mb)
Patient 6: Deletion in the region 15q15.3 to 15q21.1 (12.3 Mb)
Patient 7: Amplification in the region 17q25.1 to 17q25.3 (7.2 Mb))
Patient 8: Deletion in the region 17q24.2-q24.3 (3,3 Mb) 0,2 Mb upstream from SOX9, RT analysis confirmed this result.
In summary Array-CGH analyses shows again to be a very helpful method in identifying chromosome imbalances in patients who could formerly not be diagnosed.

P017

Identification of a 7.4 Mb deletion on chromosome 9 via array CGH in a severely retarded boy reveals the need for a thorough dermatologic follow up

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We performed an Array CGH testing on a severely mentally retarded 18 year-old boy with multiple dysmorphologies and seizures. Pregnancy was uneventful and his birth weight, height and head circumference were all above the 97th percentile. At the time of the investigation, the patient had a head circumference of 60 cm (>97th percentile), weight and height were normal. He had a midface hypoplasia, a prominent forehead, a turricephalus due to craniosynostosis, downslanting palpebral fissures and hypertelorism. His incisors were prominent, he had dental crowding and a high palate. Hands and feet were small and he had a bilateral syndactyly of the 2nd/3rd toe. Bilateral cryptorchidism was treated surgically in childhood. Multiple cysts of the maxilla had to be removed and an aortic valve insufficiency and an aneurysm of the aortic arc required surgical treatment. Karyotyping was normal, but Array CGH testing with an Agilent 244 K chip revealed a 7.4 Mb deletion on chromosome 9q22.2-q22.33. This microaberration comprises 100 genes, including the PTCH1 gene. Mutations in this gene were identified as disease causing in the Basal Cell Nevus syndrome (BCNS, Gorlin syndrome, MIM 109400), an autosomal dominant disorder characterized by the presence of multiple basal cell carcinomas (BCC), odontogenic keratocysts, palmoplantar pits, and calcification of the falx cerebri. Another rare, but well known cause for BCNS are larger deletions consisting not only of PTCH1, but also of neighbouring genes. In most cases, individuals with a 9q interstitial deletion show symptoms of BCNS and a mental retardation. Our patient is the first subject with such a microdeletion and a craniosynostosis. So far, our patient has not developed BCCs. Array CGH testing in this patient revealed the need for thorough and regular dermatologic follow ups.

P018

A call for patients: Deletion 4q12–13.1 and/or phenotype including piebaldism and developmental delay or Waardenburg syndrome without mutation

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We report on a boy of 4 years and 10/12 month of age whose phenotypic appearance includes piebaldism (white forelock, depigmented areas of the skin) dystopia canthorum, and impaired hearing suggesting a form of Waardenburg syndrome. Additional features were microcephaly, double sided hernia inguinalis, psychomotoric developmental delay, and constant drooling. Piebaldism is caused by mutations in the cKIT gene but sequencing did not result in any mutation detection in this case. Chromosome analysis revealed a translocation t(3;9)(p13-p14;p13-p21) suggesting the involvement of the Waardenburg syndrome type II locus in 3p14.1-p12.3. However, breakpoint mapping drew us more than 10 Mb away from the critical region and therefore making it unlikely for this locus to be causative. Array-CGH uncovered a submicroscopic deletion on chromosome 4 (4q12–13.1) of 11.5 Mb in size (53.002.654–64.471.364) which was verified by FISH analysis in the patient but not in his parents. This region includes the cKIT gene. To our knowledge we identified the smallest deletion associated with the described phenotype so far. In order to characterize the "4q piebald trait microdeletion syndrome" we are looking for additional individuals with a similar phenotype.
Our call for patients is aiming for individuals with interstitial deletions involving 4q11-q13.3 and/or piebaldism and developmental delay without mutations in cKIT, or suspicion of Waardenburg syndrome without mutations in MITF or PAX3.
Our group is a member of the German Mental Retardation Network (MRNet), a nationwide collaboration that is working on the identification of genes causative of mental retardation and developmental delay.

P019

Alagille syndrome (ALGS) with a partial deletion of JAG1 including several exons and only found by MLPA

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Alagille syndrome (ALGS) is an autosomal dominant multisystemic disorder with varying penetrance and expressivity. Main symptoms are chronic cholestasis, congenital heart anomaly, skeletal defects, eye and kidney abnormalities and a characteristic face. So far, 2 responsible genes were found among the NOTCH-signalling pathway: about 80% of the cases are associated with mutations within JAG1 (ALGS1; MIM #118450) and few cases within NOTCH2 (ALGS2; MIM #610205). We exemplify the potential of a step-wise molecular analysis of this genetically heterogeneous disorder and present the first case of a partial JAG1 deletion including several exons and found solely by MLPA test. The male patient (18y) demonstrated perinatal icterus, embryotoxon posterior, butterfly vertebrae, cholestasis, peripheral pulmonic stenosis, arterial hypertension, renal disorder and a characteristic face. For his older sister as well as his father no abnormality was detected (NAD). His mother was apparently healthy; a compensated aortic valvular defect was only reported. Screening for JAG1 and NOTCH2 exon mutations by cycle sequencing of genomic DNA, analysis of intragenic and flanking microsatellites as well as of intragenic SNPs completed by chromosomal analysis did not reveal any disease associated anomaly in the patient. Subsequent MLPA indicated a deletion at the 3´-site of JAG1-exon 1. A specified PCR test of the JAG1–5´-region completed by sequence analysis revealed a 1693 bp deletion (start promoter region, finish within exon 2) of the patient and his mother.
Conclusion: MLPA is a useful addition to the ALGS mutation screening protocol. Moreover, our study gives further indication of an essentially higher JAG1-mutation frequency as a result of unrecognized mutation carriers with clinical NAD.

P020

A novel autosomal recessive syndrome of severe mental retardation, cataract, coloboma and kyphosis maps to the pericentromeric region of chromosome 4

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We report on three siblings with a novel MR/MCA syndrome who were born to consanguineous Iranian parents. The clinical problems comprised severe mental retardation, cataracts with onset in late adolescence, kyphosis, contractures of large joints, bulbous nose with broad nasal bridge, and thick lips. Two patients also had uni- or bilateral iris coloboma. Linkage analysis revealed a single 10.4 Mb interval of homozygosity with significant LOD score in the pericentromeric region of chromosome 4 flanked by SNPs rs728293 (4p12) and rs1105434 (4q12). This interval contains more than 40 genes, none of which has been implicated in mental retardation so far. The identification of the causative gene defect for this syndrome will provide new insights into the development of the brain and the eye.

P021

Analysis of a patient with mental retardation and dysmorphic signs by array CGH and verification of the imbalances by FISH and quantitative PCR

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Approximately 2–3% of the general population is affected by mental retardation. In 25–35% of the cases the symptoms are caused by a genetic defect. The conventional karyotyping has a resolution of 5–10 megabases and detects chromosomal alterations in only 5% of the patients with unexplained mental retardation. The array-based comperative genomic hybridisation (array CGH) method established during the last years allows the detection of microdeletions and microduplications in the patients, who are cytogenetically inconspicuous.
A 10-year-old female patient with mental retardation, a learning disability, and mild dysmorphisms like epicanthus, broad nose, long philtrum, narrow palate and joint laxity was analysed with a 244 K Agilent oligonucleotide array. The 60 mer oligonucleotides presented on this array, cover the whole genome with a medium distance of 6,5 kb. Chromosomal regions with loss and gain of genomic material were found. Some of them were located at known copy-number variation (CNV) regions and were regarded as non pathogenic. A heterozygous deletion, with a size between 220 kb and 480 kb, was detected on chromosome 16p11.2 and verified by fluorescence in situ hybridisation (FISH) analysis with the BAC clone RP11–22P6. The FISH analysis of the patient´s parents and brother was normal, so the microdeletion had occurred de novo. In the del 16p11.2 there are 9 genes, which have to be studied further for a correlation with the phenotype. Additionally, a 100 kb duplication, which was verified by quantitative polymerase chain reaction (qPCR), was detected on chromosome 17q12. In this case the examination of the family by the qPCR revealed that the father and the brother have a duplication in the same chromosomal region. Therefore the duplication was regarded as a copy-number variant, which was inherited from the father.

P022

Proteus syndrome – a case report

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Proteus syndrome is extremely rare and sporadic congenital disease. In the world it has been described only a several dozen cases. The first manifestations of disease are observed at birth and progress significantly with age. Main feature is partial gigantism of hands and feets, hemihypertrophy and macrocephaly.
The blood sample, a 12-year-old boy treats in A. Jurasz University Hospital in Bydgoszcz, Poland was passed on to our laboratory in the Institute of Human Genetic PAS in Poznań, Poland in order to perform genetic test of PTEN gene. Patient was born to unrelated healthy parents as third child by natural childbirth at 33 week of gestation. Birth weight was 3200 g. The Apgar scale was scored on 9 points. The family history was negative. Proband has three healthy siblings. Since births time it has been observed overgrowth and deformation of lower right limb and left foot. Follow-up examination showed extensive dorsal angioma. Based on these findings at 36 months of age the Klippel-Trenaunay syndrome was diagnosed. Because of progression of skin, vascular and soft tissular changes the diagnosis was changed for Proteus syndrome. Our patient presents main sings of Proteus syndrome like asymmetric overgrowth lower limbs, macrodactyly, protuberant foots, scoliosis, seen skin lesions and vascular neavus. Progression of soft tissue hyperplasia in right limb and foot deformity is watched.

The DNA sample was isolated from peripheral blood cells using standard procedure of phenol-chloroform extraction. Entire coding sequence of PTEN gene was sequenced by direct PCR product sequencing, using MEGABace 500 sequencer (General Electric Health Care USA) according to the manufacturer's specifications. The deletion of one nucleotide was identified at the end of intron 6. The mutation was IVS7–3delT. The deletion occurred at splice junction.
This study was supported by the Ministry of Education and Science Poland (grants number N N401 014435 and 2PO5E02630).

P023

Study of Peutz-Jeghers syndrome in Poland

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Peutz-Jeghers syndrome (PJS) is rare, autosomal dominant disease characterized by occurrence hamartomatous polys. The hamartomatous polyps are manifested during second or third decade of life. The polyps can be located throughout digestive tract. Risk of malignant transformation is lower then others hereditary neoplastic disease, but PJS can be reason of many gastrointestinal discomforts. In PJS a high risk to development malignancies such as the pancreas, the breast, female and male reproductive organs is observed .The second characteristic manifestation of Peutz-Jeghers disease are brown, dark or blue spots. PJS is caused by mutations in the LKB1 (STK11) on chromosome 19. LKB1 gene encodes a serine/threonine protein kinase participating in very important cell signaling pathways. Here we present the study considering 20 patients with PJS diagnosed according to internationally accepted criteria. Patients presented two or more polyps, or one polyp and typical pigmented lesions, or one polyp and a family history of PJS. Mutations screening analysis encompassing SSCP, HA and direct sequencing of the LKB1gene are reveled five mutations and one polymorphism. These mutations are located in different position in gene (1, 2, 7 exons). With the Multiplex Ligation-dependent Probe Amplification (MLPA) - assay we detected additional genomic mutations. For our screening we used the SALSA P101 STK11 kit which contains MLPA probes for most STK11 exons. In four patients we identified exonic deletions or duplications range from one to five exons.
This study was supported by the Ministry of Education and Science Poland (grants number N N401 014435 and 2PO5E02630).

P024

APC gene mutations causing familial adenomatous polyposis in Polish patients

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Familial adenomatous polyposis (FAP) is a well-known hereditary condition characterised by alimentary system tumours. Tens to thousands of polyps occur in the colon and rectum of the patients. There is a high heterogeneity with regard to the number and time of the occurrence of polyps. The occurrence of FAP is associated with mutations in the APC tumour suppressor gene, which was described in 1991. Since then, many studies have been done to analyse the distribution of mutations in individual populations and to determine the function of the gene and a diagnostic approach to FAP. Here the APC gene was studied with respect to the occurrence of small mutations and large rearrangements in 300 unrelated Polish FAP families. Ninety-seven mutations were identified in 164 families. Out of these mutations, 80 were small mutations, including 58 small mutations that were first identified in the Polish population (42 novel and 16 described previously). An increased frequency of mutation c.3927_3931delAAAGA was observed in 10% of the Polish group. Seventeen large rearrangements were found in 29 families. Out of those rearrangements, 8 repeat rearrangements occurred in 20 families. A problem in fast molecular diagnostics of FAP is a high heterogeneity of mutations in the APC gene. It seems that a multiplex ligation-dependent probe amplification test and searching for small mutations by the use of screening methods at the 5' end of exon 15 and exons 14, 9, 11, 13, 5, and 3, help to improve the molecular diagnostics of FAP in Polish patients
The study was supported by the Ministry of Education and Science Poland (grant number 2PO5A10728).

P025

HPGD mutations cause cranioosteoarthropathy but not autosomal dominant digital clubbing

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Cranioosteoarthropathy, clinically classified as a variant of primary hypertrophic osteoarthropathy, is a very rare autosomal recessive condition characterized by delayed closure of cranial sutures and fontanels, digital clubbing, arthropathy, and periostosis. Recently, a locus for primary hypertrophic osteoarthropathy was mapped to chromosome 4q33-q34 and mutations in the gene HPGD encoding the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase were reported in three affected families. Furthermore, an autosomal recessive variant of isolated digital clubbing has been found to be caused by a missense mutation in HPDG in a consanguineous family.
We report the clinical and molecular findings in four patients from two families affected with cranioosteoarthropathy as well as one family with isolated, autosomal dominant digital clubbing. Genome-wide homozygosity mapping identified a locus for cranioosteoarthropathy harboring the HPGD gene in one affected family. We detected two novel homozygous mutations in HPGD in the families with cranioosteoarthropathy: a missense mutation affecting the NAD^+ binding motif, and a frame shift mutation. The clinical presentation in our patients was variable. Digital clubbing and hyperhidrosis were present in all cases. Delayed closure of cranial sutures and fontanels, periostosis, and arthropathy were not consistent clinical features. No HPGD mutation was detected in the familial case of autosomal dominant isolated digital clubbing.
Our data confirm that HPGD is also the causative gene for cranioosteoarthropathy which is, therefore, allelic to primary hypertrophic osteoarthropathy. The failure to identify any mutation in a family with an autosomal dominant type of isolated digital clubbing suggests that HPGD is not the major gene for this condition.

P026

A novel autosomal recessive form of Ehlers-Danlos syndrome with skeletal dysplasia and hand anomalies

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We present the clinical, radiological, biochemical and molecular findings of two patients from a consanguineous family originating from North-Western Iraq. The affected individuals present Ehlers-Danlos syndrome (EDS)-like features and radiological findings of a mild skeletal dysplasia. The EDS-like findings comprise thin and bruisable skin with atrophic scars; hypermobility of the small joints with a tendency to contractures; protuberant eyes with bluish sclerae and keratoconus in the elder patient; hands with finely wrinkled palms, atrophy of the thenar muscles, tapering fingers and varicose veins. Unlike EDS VI the patients show no muscular hypotonia, no spine deformities, and no Marfanoid habitus. The skeletal dysplasia comprises platyspondyly with moderate short stature, osteopenia, and widened metaphyses. The changes in the patients' collagens are intermediate between controls and patients with EDS VI. A genome-wide SNP-scan in our family and in a consanguineous family from South-Eastern Turkey identified the disease locus on chromosome 11 and lead to the identification of the causative gene. The same homozygous 9 bp in-frame deletion in exon 4 of SLC39A13 gene was found in the patients from Iraq and Turkey. The parents and several siblings were heterozygous for the mutation. We suggest naming this entity spondylocheiro dysplastic form of EDS (SCD-EDS).

Reference: Giunta et al., Spondylocheiro Dysplastic Form of the Ehlers-Danlos Syndrome-An Autosomal-Recessive Entity Caused by Mutations in the Zinc Transporter Gene SLC39A13 Am J Hum Genet 82, 1290–1305, 2008

P027

Clinical and molecular characterisation of interstitial deletions 13q in patients with Retinoblastoma

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Retinoblastoma (Rb) is caused by mutational inactivation of the RB1 gene, a tumor suppressor located on chromosome 13q14.2. About 5–15% of the patients are heterozygous for an interstitial deletion that includes the entire or parts of RB1. Previous studies have shown that patients with retinoblastoma and an interstitial deletion 13q show additional clinical features, including mental retardation, short stature, macrocephaly and facial characteristics. These features are highly variable and there is no recognizable phenotype. We have studied 63 patients to analyze genotype-phenotype correlations. We performed chromosome analysis, FISH, MLPA and array CGH analysis in these patients. A customized high-resolution CGH array was used to map deletion breakpoints in 34 patients. Deletion size ranges between 4.2 kb and more than 13 Mb. Locations of breakpoints are variable with no recurrent breakpoints. Sequence analysis of deletion breakpoints in five patients with microdeletions suggested non-homologous end joining as the mutagenic recombination mechanism. In 37 (67%) and 18 (33%) patients the parental origin of the deletion was paternal and maternal, respectively. No correlation between Rb phenotype and parental origin of the deletion was seen. Extensive clinical data were obtained from 40 patients between five months and 47 years of age. We found a variable degree of motor and speech delay, hypotonia, microcephaly and macrocephaly, normal or short stature, recurrent infections and constipation. Patients with intermediate size cytogenetic deletions show high forehead, deep set eyes, short nose, small upper lip and curly hair. In patients with small microdeletions, no recognizable facial phenotype could be seen. Less than 20% of the patients had deafness, seizures, and brain or heart defects. Our data show that interstitial deletions in Rb patients are heterogeneous which corresponds to the heterogeneity of the phenotypic expression of this genomic disorder.

P028

SPONASTRIME Dysplasia- short stature with short dental roots and cataracts – a further case

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SPONASTRIME Dysplasia (SD) (OMIM#271510) is an autosomal recessive skeletal dysplasia of the spondyloepimethaphyseal dysplasia (SEMD). The name was derived from „spondylar and nasal alterations with striated metaphyses".

We report a 14 years old girl with SD, short dental roots and cataracts and add a further case to the expanded spectrum of SD.

The girl was born after a normal pregnancy with discordant twins. She was the first twin and small for gestational age BW 2090 g, L 43 cm, OFC 31,5 cm (<P10), whereas the second twin was appropriate. She developed bilateral cataracts during early childhood, which were treated surgically. Her permanent teeth were small and had extremely short roots, which limited orthodontic treatment.

Midfacial dysplasia with depressed nasal root, short upturned nose and short stature were present. At the age of 14 years she was 142 cm (-2,12 SD), 45 kg (P25–50). Intelligence was normal. The typical radiological abnormalities consisted of platyspondyly, striations in the metaphyseal margin and flattened capital femoral epiphyses. The genetic defect is not known in SD and may be heterogeneous.

P029

Clinical features in MOPD type 2 Majewski caused by compound heterozygosity for mutations in the PCNT (pericentrin) gene: A 10 year follow-up

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Background: Microcephalic osteodysplastic primordial dwarfism (MOPD) type 2 has got its Eponym in honour of Frank Majewski's detailed description. Most remarkable features are severe intrauterine and postnatal growth retardation with short-limb dwarfism, microcephaly, mild to moderate mental retardation, and distinct facial features. A substantial part of patients suffer a stroke as a consequence of various cerebrovascular anomalities. Just recently, the molecular background was clarified by Rauch et al (Science, 2008).

Case report: S.K., unrelated parents, was born after 35 weeks of gestation, with severe IUGR, length 31 cm (-8.8 SD), weigth 1118 g, OFC 25 cm. Growth velocity remained extremely decreased over the first decade, length at 10 y –12 SD, in keeping with an expected final length near 100 cm. Associated obstructive renal complications were confined to the first few years. Aged 3 y the boy experienced right-sided stroke due to ischaemic infarction. Due to this handicap unaided walking started only with 8 years, still presenting a humpling gait. Delayed speech development, aged 10 y he has become able to speak clearly short sentences with a characteristic high-pitched voice. No hypertension was observed so far. Dentition was incomplete with enamel defect. He receives special education with good success. Molecular studies (courtesy of A.Rauch, Erlangen). Molecular analysis confirmed a bial-

lelic loss of function due to two different nonsense mutations in exon 13 and exon 34 of the PCNT gene, respectively. No studies on the protein level were performed so far.
Conclusions: Our observation corresponds well with other reports on this very rare primordial dwarfism including the common complication of cerebral stroke. We stress the spontaneous remission of the neurological burden and the intellectual development in spite of progredient microcephaly. More detailed investigation of the vascular burden will be necessary to possibly prevent further stroke events.

P030

Duplication of the GPC3/GPC4 gene cluster on Xq26.2 detected by Array-CGH in a family with developmental delay/mental retardation and dysmorphic features. A new syndrome?

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Simpson-Golabi-Behmel (SGBS) syndrome is an X-linked overgrowth syndrome characterized by pre- and postnatal overgrowth, a characteristic facial appearance and different congenital malformations. SGBS is caused by mutations or deletions of the glypican 3 (GPC3) gene.
Here, we report on a 2-year-old boy presented with pychomotoric retardation, growth retardation, microcephaly, mild congenital malformations (micropenis, hypospadia) and dysmorphic features (e.g. broad forehead, facial asymmetry, round face, hypertelorism, micrognathia, deep set and posteriorly rotated ears, slight bilateral clinodactyly).
Initially, Silver-Russell-syndrome was suspected. While karyotyping and testing for Silver-Russell syndrome were negative, a 1–4 Mb duplication at Xq26.2 including the GPC3/GPC4 gene cluster was identified by BAC array-CGH. This finding was validated by MLPA and Q-PCR analysis.
For the characterization of the duplication in a much higher resolution we have designed and customized a 60mer oligo array printed in a 105 K format (105.000 oligonucleotides probes) using the eArray technology (Agilent).
Testing of more family members revealed that the mother, the grandmother and a maternal uncle were carrier of the Xq26.2 duplication. All carriers displayed the characteristic features of the syndrome to some extent.
Interestingly, comparison of the Xq26.2 duplication phenotype to the phenotype of SGBS patients revealed partially a „reverse" phenotype to the SGBS.
Recently, it was proposed that the SGBS phenotype is caused by a misregulation of the hedgehog signal transduction pathway. Here we will present a potential explanation of the phenotypical differences between the two syndromes based on the function of glypican 3 in the hedgehog signal transduction pathway.

P031

A 10-year-old girl with clinical features of Ehlers Danlos syndrome and frontal polymicrogyria without FLNA mutation. A new syndrome or coincidence?

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Here we report on a 10-year-old girl with clinical signs of Ehlers-Danlos syndrome (EDS) and frontal polymicrogyria, who was referred to us for diagnostic evaluation. EDS is a group of heterogeneous diseases, in which the collagen production is affected. Apart from the classical types of EDS, rare variants have also been described (i.e. arthrochalastic type, dermatosparactic type). However, involvement of the central nervous system is very rare, even in the non-classical EDS types. Recently, mutations in the filamine A gene (FLNA) on Xq28 have been described in patients with periventricular heterotopia and features of EDS.At birth low normal birth measurements and long fingers were noted in our patient. A mild pulmonary stenosis was diagnosed. Her development was normal until the age of 5, except for hypermetropia and splayfeet. At that age, hyperextensible joints, thin, translucent skin and a tendency to bruises had become apparent. An episode of severe headache and the development of exopthalmus inaugurated a MRT of the brain which revealed frontal polymicrogyria.Clinical examination at the age of 10 years revealed normal body measurements for age (143 cm height, 28,2 kg weight and 54 cm head circumference) and a normal intellectual status. EDS signs were apparent (hyperextensible joints, highly elastic, thin skin, easy bruising, numerous old scars). Moreover, contractures of the left knee, were present.Due to the combination of EDS with a CNS abnormality, sequencing and MLPA analysis of all the coding exons of FLNA were performed, which excluded a disease causing mutation or deletion. We discuss the clinical findings in relation to previously published data in patients with FLNA mutations and consider further tentative diagnoses.

P032

Multiple giant cell lesions in patients with Noonan syndrome and cardio-facio-cutaneous syndrome

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Noonan syndrome (NS) and cardio-facio-cutaneous syndrome (CFCS) are related developmental disorders caused by mutations in genes encoding various components of the RAS-MAPK signaling cascade. NS is associated with mutations in the genes PTPN11, SOS1, RAF1, or KRAS, while CFCS can be caused by mutations in BRAF, MEK1, MEK2, or KRAS. The NS phenotype is rarely accompanied by multiple giant cell lesions (MGCL) of the jaw – denoted as Noonan-like/multiple giant cell lesion syndrome, NL/MGCLS. PTPN11 mutations are the only genetic abnormalities reported so far in some patients with NL/MGCLS. In four NS patients with MGCL, previously tested negative for mutations in PTPN11 and KRAS, we detected SOS1 mutations. In order to further explore the relevance of aberrant RAS-MAPK signaling in syndromic MGCL, we analysed the established genes causing CFCS in three subjects with MGCL associated with a phenotype fitting CFCS. Mutations in BRAF or MEK1 were identified in these patients. All mutations detected in these 7 patients with syndromic MGCL had previously been identified in NS or CFCS patients without apparent MGCL.
This study demonstrates that MGCL may occur in NS and CFCS with various underlying genetic alterations and no obvious genotype-phenotype correlation. This suggests that dysregulation of the RAS-MAPK pathway represents the common and basic molecular event predisposing to giant cell lesion formation in patients with NS and CFCS rather than specific mutation effects. These aspects argue against the existence

of a NL/MGCLS as a separate entity. MGCL are rather a rare manifestation of RAS-MAPK pathway disorders.

P033

Variability of clinical manifestation in 4 affected males with X-linked adrenoleukodystrophy

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Introduction: X-linked adrenoleukodystrophy (X-ALD) (OMIM #300371) is a genetically determined metabolic disorder characterized by progressive neurological disability and primary adrenocortical insufficiency. The tissues and body fluids of patients with X-ALD contain increased level of unbranched saturated very long chain fatty acids (VLCFA).There are six main clinical forms: childhood cerebral, adolescent cerebral form, adrenomyeloneuropathy, adult cerebral form, isolated Addison's disease and asymptomatic form.
Clinical report: We present 2 related affected family (3 male sibs and their cousin) with significant manifestation variability of X-ALD.
Patient 1: 23 years old. Clinical manifestation at 18 year old with progressive skin hyperpigmentation.
Patient 2: At 6 years he had seizures, 2 years later he showed horizontal and vertical nystagmus, right-side central hemiparesis and motor aphasia, have dead at 8 7/12 years old.
Patient 3: 8 years old. Clinical manifestation at the age of 5 years with behavioral manifestations. At 7 years old defective vision and hearing, motor dysfunction, severe stammering and phobic disorder were registered.
Patient 4: Cousin, 15 years old. Clinical debut was at 10 years old: behavioral manifestations and cognitive difficulties. At 14 years old – skin hyperpigmentation, spastic paraparesis and peripheral neuropathy.
The diagnosis of X-linked ALD was confirmed by raised circulating concentrations of VLCFA C22:0, C24:0, C26:0, C24:0/C22:0, C26:0/C22:0 for all patients.
Conclusion: Our patients demonstrated the various ages of disease onset (between the ages of 5 and 18 years), clinical signs (adrenocortical insufficiency or neurological disability) and rate of progression (rapidly progressive childhood cerebral form to the milder isolated Addison's disease and adrenomyeloneuropathy).

P034

Diploid-tetraploid mixoploidy as cause of severe mental retardation

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Background: Tetraploidy is frequently observed in pregnancy loss, but tetraploidy or diploid-tetraploid mixoploidy is rare among live births and patients only exceptionally survive until adolescence. We present a boy with high level diploid-tetraploid mixoploidy who was diagnosed at the age of 16 years.
Clinical report: The boy presented with severe psychomotor retardation from the beginning, reduced peripheral limb muscle bulk, short stature and microcephaly. He was born with bilateral chorioretinitis and multiple contractures. He was now suffering from bilateral spastic cerebral palsy and a severe progredient scoliosis. He had no distinct facial dysmorphisms, no body asymmetry and the skin was normal. The mother remembered however congenital focal skin lesions at the left shoulder and the coccyx, which had disappeared.
Cytogenetic findings: Chromosome analysis from peripheral blood cells revealed a normal male karyotype. However, in cultured fibroblast from the skin in 82% of the metaphases a diploid-tetraploid mosaicism mos 92,XXYY[18]/46,XY[4] was observed. The result was confirmed by interphase FISH analysis.
Discussion: So far, only 12 live born patients with pure diploid-tetraploid mixoploidy have been reported. Most of them did not survive the first years of life. Only one of these patients was older than the boy described here. Despite some common findings as mostly severe retardation, microcephaly, short stature, slender muscle build, and some facial findings there is no specific phenotype indicating diploid-tetraploid mixoploidy. As mixoploidy is frequently undetectable in blood leucocytes, especially in adolescents or adults, the condition may therefore be underdiagnosed. Additional chromosome analysis in cultured skin fibroblasts and/or interphase FISH should be considered in patients with unexplained severe mental retardation and short stature, even in the absence of asymmetry or pigmentation anomaly otherwise indicative for mosaical chromosomal disorders.

P035

Molecular diagnosis of BMD despite normal expression of dystrophin in muscle biopsy

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We report on a 40 year old patient with arrhythmias (Lown IVb) firstly diagnosed by chance at the age of 30 years. At age 40 a dilated cardiomyopathy (NYHA II-III) was diagnosed. There were no other affected family members. Serum creatine phosphokinase (CK) concentration was moderately elevated (371 U/l normal <170 U/l). Neurologically, the nerve conduction velocity was slightly decelerated. No weakness of the skeletal muscles nor muscle hypertrophy or atrophy were initially present. A muscle biopsy was performed. Histologically minor unspecific changes were observed and immunochemistry showed a normal expression of dystrophin (Dys I-III). According to the clinical diagnosis of Emery-Dreifuss muscular dystrophy (EDMD) emerin (X-linked EDMD) was analysed without detecting a mutation. The following analysis of the lamin A/C gene (autosomal dominant EDMD) demonstrated no mutation as well. Since physical examination showed a slight myotonia the differential diagnosis of dystrophia myotonica was taken into account, but there was not the typical expansion in the DMPK1 gene. Despite the results of the biopsy thereupon an analysis of the dystrophin gene by MLPA was performed. The MLPA identified a deletion spanning exons 45–55 (ex45ex55del ->6439-?_817+?del), without frame shift and compatible with Becker muscular dystrophy (BMD). BMD is characterized by skeletal muscle weakness with onset in adolescence. This X-linked disorder can be associated with myocardial involvement potentially resulting in dilated cardiomyopathy (DCM). Usually myocardial involvement remains subclinical in the early stages of BMD. Our case illustrates that cardiac involvement may occur a long time before any symptoms of the skeletal muscles of the disease are perceived. Moreover, a negative staining for dystrophin in muscle biopsy does not exclude BMD. This might be partially explained by the different binding sites of the antibodies used and the structure of the derivative dystrophin molecule.

P036

Second case with maternal uniparental isodisomy 7 and SSMC

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Maternal uniparental disomy of chromosome 7, associated with Silver-Russell syndrome is a rare cytogenetic finding, but among the UPDs, matUPD 7 is the second common feature after matUPD15. The patient was noticeable with postnatal growth retardation and dysmor-

phophic signs. Cytogenetic analysis revealed a marker chromosome (47,XY,+mar[4]/46,XY[46]) in 10 % of the analysed metaphases. Molecular cytogenetic analysis defined that the marker consists of eu- and heterochromatin (7p12–11.1), which results in a partial trisomy for this chromosomal region. Microsatellite investigations for a UPD 7 revealed that eight of thirteen microsatellites were informative for maternal isodisomy 7. Paternity was confirmed using a set of polymorphic markers for eight additional autosomes. Isodisomy in combination with a marker chromosome is a rare finding, because in embryogenesis only two mechanisms could lead to this. First, an error could appear in meiosis II of oogenesis, were two sister chromosomes of chromosome 7 were generated but not shared to two cells. Fertilisation with a normal sperm results in a trisomy 7 and, through trisomy rescue a maternal isodisomy can occur. The second mechanism is due to a postfertilization error, whereas mitotic divisions of the zygote results in two clones. One clone shows a monosomy 7, which is not viable. The other clone with the trisomy 7 undergo trisomy rescue as described for the first mechanism. To get closer to the developing mechanisms we actually try to confirm the paternal origin of the marker chromosome.

P037

CHARGE-syndrome and subtelomeric deletion 7p in two siblings

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Parents with more than one affected child and strikingly different phenotypes are always a special challenge with respect to genetic counselling and diagnosis. We describe a non-consanguinous couple that came to attention in 2004 when their first child was born, suffering of severe asphyxia due to premature placental separation. The boy was hypotrophic and microcephalic with congenital facial nerve paresis and a dysplastic, cup-shaped ear, both on the right side. A CHARGE association was suspected, but due to the lack of a responsible gene defect it could not be proven at that time. In 2008 the second child was born and the little girl showed bilateral cleft lip and palate, a persisting ductus arteriosus, bilateral congenital deafness and a hydronephrosis on the right side. Chromosomal analyses of both brother and sister were normal and DiGeorge-syndrome was excluded. As both children were affected and their phenotypes were quite different, the question arose whether there is a common, familial reason to their sickness. Subtelomeric FISH-analysis was performed on the girl's chromosomes and revealed the absence of one subtelomere 7p without traceable reemplacement by another chromosomal telomere. Subsequent subtelomere-FISH of parents and brother gave no hint on a familial chromosomal rearrangement, the deletion seemed to have arisen de novo in the girl. Array-CGH confirmed a 3,5 Mb microdeletion on chromosome 7. In the case of her brother, molecular analysis of CHD7 resulted in a point mutation (c.6170G>C, pR2057P) in exon 31 of the CHD7 gene, that is considered as probably damaging. The clinical relevance and the recurrence risk depend on the results of the CHD7-analyses of the parents and maybe other family members.

P038

Identification of 11 novel RMRP mutations and update of RMRP mutation analysis in patients with Cartilage Hair Hypoplasia

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Cartilage Hair Hypoplasia (CHH) is an autosomal recessive disorder characterized by short stature, sparse hair, immune deficiency and predisposition to malignancy. CHH is caused by mutations in RMRP which encodes the RNA component of the RNase MRP complex. More than 100 RMRP mutations within the transcript and promoter region are known, only a limited number have been functionally characterized. Clinical findings of CHH patients show pronounced variability and no apparent genotype-phenotype correlation was found.
Here, we present a cohort of 39 CHH patients, diagnosed on the basis of clinical and radiographic findings. RMRP mutation analysis was performed by sequencing the transcript and 150 bps of the promoter. We detected 14 promoter duplications, 58 base pair substitutions and 6 small insertions within the transcript. 11 of these mutations are unpublished: we identified 5 novel promoter insertions and 6 substitutions/insertions in the transcript. The mutations were spread over the whole gene, without clear hotspots. Position 70 was only mutated in 14 alleles (18%), a lower than expected frequency. We evaluated clinical data of 15 CHH patients with regard to the presence of skeletal manifestations, immunodeficiency and sparse hair. Unlike others, we found that patients with at least one mutation located close to position 70 were not mildly affected but suffered from recurring infections and often had sparse hair. Furthermore, in our cohort, promoter insertions do not cause severe immunodeficiency. These results again reflect the variability of clinical findings in CHH patients. However, as the average age of our pediatric patients was 11.8 years, immune deficiency could still evolve later in life.

P039

Severe course of Alagille syndrome associated with a novel NOTCH2 mutation

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Alagille syndrome is a multisystemic disorder with hepatic bile duct paucity and cholestasis in association with other manifestations. In most of the cases haploinsufficiency of JAG1 can be identified as causative for the disease. Only recently also mutations in NOTCH2, the receptor of Jagged 1, have been shown to cause Alagille syndrome in two families. We report on the third mutation in NOTCH2 associated with an unusual severe course of Alagille syndrome with systemic arterial dysplasia.
The male patient was born small for gestational age and the neonatal period was complicated by bilateral pneumothorax, renal insufficiency, persistent ductus arteriosus, arterial hypertension and cholestasis. No ophthalmologic or skeletal anomalies were found. At the age of 6 months the boy was dystrophic and showed jaundice and alopecia. In the further course he developed hyperparathyreoidism and recurrent crises with seizures, hypoxia and lactate acidosis. Extensive metabolic testing showed no abnormal results, liver biopsy showed moderate cholestasis and mild hypoplasia of bile ducts. At the age of 12 months the patient started to sit and spoke first words. Because of increasing

hypertension of the pulmonic arteries intracardiac catheter examination was performed which revealed filiform endings of the peripheral vessels and furthermore hypoplasia and multiple stenosis of the aorta and large arteries. The patient deceased at the age of 15 months.
Karyotyping with FISH-analysis at the JAG1-Locus, JAG1 sequencing and MLPA had revealed normal results. NOTCH2 sequencing showed the novel de novo splice site mutation c.6027G>A. This is the third mutation in NOTCH2, confirming its causative role in Alagille syndrome. If the severe vascular phenotype is an effect of the novel NOTCH2 mutation or an extreme manifestation within the Alagille spectrum regardless of the underlying gene remains unclear.

P040

German patient with Lafora disease

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Lafora disease is an autosomal recessive disorder characterized by seizures and progressive neurologic deterioration, and is usually fatal within 10 years of onset. The disease is a member of the family of progressive myoclonic epilepsies, which are a heterogeneous group of disorders characterized by myoclonic epilepsy, developmental regression, and associated neurologic symptoms. Our 19 year old male patient was born to unrelated german parents and had his first absences at the age of 10 years. Additionally in EEG 3 generalised spike-wave cluster are shown for 1 to 1.5 seconds. Until the age of 17 years only visual auras (probably induced by PC games) and absences were the only symptoms. Two years ago the first myoclonus was observed in both arms. Because of the progressive course of the symptoms a Lafora disease was supposed. Analysing the two known genes EPM2A and EPM2B (NHLRC1), we found no mutation in the EPM2A gene. In the EPM2B gene we found two unkown heterozygous mutations. The first mutation c.943_944insCTCT (p.Tyr315SerfsX15) is an insertion of four bases between the base position 943 and 944. This causes an aminoacid exchange from Tyrosine to Serine and a frameshift mutation. The second mutation c.1142A>G (p.Asp381Gly) leads to an aminoacid exchange on Position 381 from Asparagine to Glycerine. For this variation a computer-based prediction of functional effect of mutations (PolyPhen) revealed that this variants is predicted to be probably damaging.
Although the clinical symptoms were mostly atypical for lafora disease, we found the two mutations causing this severe form of epilepsy. To our knowledge this is the first patient with two compound heterozygous mutations in a children of non-consanguineous parents.

P041

Postaxial polydactyly of feet in two patients with Rubinstein-Taybi syndrome

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We report on two unrelated patients with Rubinstein-Taybi syndrome (RTS) and postaxial polydactyly of both feet with a proven mutation in the CREBBP gene.
Patient one is the second child of an unrelated German couple. The pregnancy was complicated by bleeding in early pregnancy and gestational diabetes in late pregnancy. Birth measurements at term were low, weight 2950 g (10th centile), length 47 cm (<3rd centile), OFC 33 cm (3rd-10th centile). Postaxial polydactyly of both feet and radial deviation of broad thumbs was noted after birth, as well as frontal haemangioma and a nose with a long columella and hypoplastic alae nasi. A heterozygous one bp deletion in exon 31 of CREBBP gene was detected (c.5837delC; p.P1946fsX1975).
Patient 2 is the second child of a non-consanguineous Lebanese couple. The girl was born at 38th week of gestation by caesarean section after premature labour starting in 28th week of gestation. Birth measurements were low normal, weight 2710 g (25th centile, length 47 cm (10th centile), OFC 33 cm (25th centile). The girl had downslanting palpebral fissures, slightly broad thumbs and postaxial polydactyly of both feet. A deletion of exons 15–31 of CREBBP gene was found (CREBBP del e15 - e31). Apart from classical features of Rubinstein-Taybi syndrome like broad thumbs and distinctive facial features (downslanting palpebral fissures and columella extending below the nares) our patients have postaxial polydactyly of both feet. This rarely described feature (Johnson, 1966; Bartsch et al., 1999; Muneuchi et al., 2005) in patients with Rubinstein-Taybi syndrome should be included in the list of Rubinstein-Taybi syndrome clinical signs.

P042

Diastematomyelia in Wildervanck (cervico-oculo-acoustic) syndrome

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Cervico-oculo-acoustic or Wildervanck syndrome (MIM 314600) is characterized by the triad of Klippel-Feil-anomaly, bilateral abducens palsy with retracted globe (Duane anomaly) and deafness. The overwhelming majority of patients were females, raising the question of sex-linked dominance with lethality in the hemizygous male. We here describe a 5 1/2 year old female patient with Duane anomaly, cleft palate, bilateral mixed sensorineural and conductive deafness, short neck with restricted lateral mobility of the neck, mild developmental delay and enuresis. Tendon reflexes of the limbs were normal. Cranial MR showed a diastematomyelia (split cord malformation) of the upper cervical cord and lower medulla and the already known fusions of the cervical vertebrae. We conclude that patients with Wildervanck syndrome should be investigated for craniospinal malformations with MR imaging. All cases with the typical triad of abnormalities are sporadic. However, affected individuals with single components have been described through multiple generations in one family, opening the debate for different modes of inheritance.

P043

An 8 Mb terminal deletion of 6q in a 15-year-old girl with global developmental delay and facial dysmorphisms

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We report on a 15-year-old girl with motor and mental retardation, delayed speech development, and facial dysmorphisms. She is the third child of healthy, non-consanguineous parents, born at term after an uneventful pregnancy (birth weight 2800 g, length and head circumference unknown). Family history was unremarkable. During the first year of life, spasticity and generalized muscular hypertonia were recognized. Motor development milestones and speech were delayed (walking and first words at 28 months of age). At 6 years of age, the patient had a singular generalized seizure, EEG showed focal epileptic activity. The girl remained seizure-free without antiepileptic therapy. Physical examination at 15 years of age revealed microcephaly (OFC 50 cm, <3rd centile), ataxia, areflexia, and dysarthria. She presented with distinct facial dysmorphisms including a long face, large and protruding ears, almond-shaped eyes, high and broad nasal root, broad nasal tip, broad columella, prominent upper lip, oligodontia, and micrognathia. Brain MRI revealed hypoplasia of the cerebellum. Routine analysis of chromo-

somes from lymphocyte cultures showed an apparently normal female karyotype in all metaphases examined. Comparative genomic hybridization using 105 k oligonucleotide array (Agilent) revealed a deletion of 8.626 Mb on the long arm of one chromosome 6 with the putative breakpoint in 6q26-qter. This terminal deletion of 6q was confirmed by FISH analysis. The karyotype reads as follows: 46,XX,del(6)(q26). ish del(6)(q27q27). Conventional cytogenetic and FISH analysis of the parents showed normal results suggesting that the deletion was de novo. Terminal deletions of chromosome 6q are rare. Common clinical features include mental retardation, facial abnormalities, seizures, and brain abnormalities. The phenotype of the patient presented here will be discussed and compared to those of patients with similar terminal deletions of chromosome 6q described in the literature.

P044

Identification of different, subtle chromosomal microdeletions and duplications as a cause of mental retardation and variable additional features in 8 cases

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Eight children with different chromosomal microdeletions or duplications all but one identified by array-CGH will be shown.
Case 1 is a girl with progredient microcephaly, cerebellar hypoplasia and developmental delay who showed a microdeletion Xp11.4 including the 5`- part of the recently identified CASK-gene (see also Uyanik, G. et al., Novel mutations of CASK...).
Case 2 and 3 are two half-brothers with severe developmental delay, severe ataxic gait and severe dysmorphic features with a distinct face who show a 9.5 Mb microdeletion Xq21.1-Xq21.31. The healthy mother carries the same microdeletion.
Case 4 is a 15 month old boy with a 3.2 Mb microdeletion 22q13 proximal to the classical microdeletion 22q13 region which could be detected by array-CGH but not with the classical FISH probe for microdeletion 22q13. The boy shows similar symptoms as the children with the classical microdeletion 22q13, especially macrosomia and develomental delay.
Case 5 is an 11 year old girl phenotypically resembling to Albright-hereditary-osteodystrophy like-syndrome, who shows a 1.25 Mb microduplication on 3q29, which seems to be a common microduplication but none of the hitherto published cases of this microduplication showed the symptoms of our patient.
Case 6 is a boy with a nearly 6 Mb microdeletion 1p36 proximal to the classical microdeletion 1p36. The boy shows different symptoms from the classical 1p36 microdeletion as ptosis of eyelids, microcephaly, mental retardation and behavioural problems like autoaggressive behaviour.
Case 7 is a young man with mental retardation and no severe dysmorphic features with a 1.3 Mb microduplication on 18p11.32.
Case 8 is a girl with an unobvious array-CGH. Further investigations could show a microdeletion 22q13.3 smaller than 300 kb including the SHANK3 gene which was not detectable by our array-CGH. The clinical phenotype was atypical to the classical 22q13 microdeletion syndrome.

P045

A case of a 3.5 Mb deletion 1p31.2p31.3 detected by array-CGH in a girl with macrocephaly and hypoplasia of the corpus callosum

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We report a case of a six months old dysmorphic girl with muscular hypotonia, macrocephaly, ventriculomegaly, and hypoplasia of the corpus callosum. The dysmorphic signs consist of a broad face with broad, prominent forehead, low set ears with dysplastic helices, concave profile of the nose, anteverted nostrils, small chin, small mouth with thin, M-shaped upper lip, high palate and sparse decreased eyebrows. Additionally, cutis marmorata has been observed. Karyotyping, and FISH analyses (22q11.2 and subtelomeric screening) were normal. Array CGH revealed a 3.5 Mb deletion in the short arm of chromosome 1 (1p31.3p32.2) including the NFIA (nuclear factor IA) gene. Haploinsufficiency of NFIA has recently been reported in five individuals with complex central nervous system (CNS) malformations, three of which with urinary tract defects. In all five inividuals, large 12 Mb deletions or involvement of an additional chromosomal translocation has been observed. Our case represents the smallest deletion including the NFIA gene and central nervous system (CNS) malformations.

P046

Deletion 16p12.3p13.11: Severe IUGR, cerebellar hypoplasia and bilateral renal hypoplasia in two sibs

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We report on two fetuses diagnosed with a recurrent malformation pattern by ultrasound in the second trimester of pregnancy. The parents' personal and family history was uneventful except a familial venous angiodysplasia on lower extremities of the father and his mother.
The first fetus was diagnosed with severe IUGR and cerebral malformation at 18+5 weeks of gestation. The pregnancy was terminated at 21+4 weeks and pathological examination confirmed severe growth retardation, microcephaly, bilateral renal and urethral agenesis, agenesis of the occipital lobe, cerebellar hypoplasia and agenesis of the vermis and facial dysmorphisms. The second pregnancy was terminated at 18+3 weeks of gestation after the detection of brain and renal anomalies in ultrasound. Pathological examination showed growth retardation, microcephaly, renal hypoplasia and dysplasia, cerebellar hypoplasia, partial vermis agenesis and dysmorphisms.
Standard karyotyping revealed 46,XX in both cases. Array-CGH analysis (NimbleGen HG18 WG Tiling 385 k CGH v1X1) showed a 3 Mb deletion on the short arm of chromosome 16. The telomeric breakpoint is situated at 15.14–14.40 Mb within an LCR region, the centromeric breakpoint is located at 18.08–18.66 Mb within an LCR region. Another LCR region lies within the deleted region at 16.23–16.76 Mb. The deleted region comprises at least 16 genes and can be defined cytogenetically as 46,XX,del(16)(p12.3p13.11).
Array-CGH analysis of both parents did not show an imbalance. Locus-specific FISH analysis designed for the specific16p region is ongoing for both parents in order to detect a possible balanced translocation or insertion on the submicroscopic level. We discuss the structure, inheritance and significance of the 16p12.3p13.11 deletion in our family in comparison to the previously published deletion 16p13.11 (Hannes FD

et al. 2008; Ullmann R et al. 2007) which has been proposed as a recurrent risk factor for MR/MCA.

P047

Young patients with severe heart problems and abnormal iron parameters should be genetically analysed for juvenile hemochromatosis. Case report of a young male patient with homozygosity for the frequent G320 V mutation in the HJV gene who died after implantation of a cardiac assist device

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Background: Juvenile hemochromatosis (JH) is a severe rare autosomal recessive inherited iron metabolism disorder which causes abnormal iron accumulation in several tissues at young age. A rapid progression and a more severe iron burden due to a stronger iron absorption and storage result in a more malignant prognosis and differentiate JH from adult hereditary hemochromatosis (HH). JH typically becomes noticeable due to heart and/or liver problems. Cardiac complications often lead to death, especially in cases where the disease is detected very late.
Patient: A 21 year old male patient from a consanguineous family initially presented with severe cardiac arrhythmias. After recurrent heart problems over the following ten months the patient received a left ventricular assist device after global heart failure due to cardiomyopathy and after stabilisation with an arteriovenous ECMO (extracorporeal membrane oxygenator). The patient died six days after the first evidence for JH was found by histopathological investigation of explanted heart tissue.
Methods: DNA from EDTA blood was amplified by Polymerase Chain Reaction (PCR). Subsequently, sequencing reactions for the coding exons and the flanking intronic sequences of the hemochromatosis genes HFE, HJV and HAMP were performed and analysed by capillary electrophoresis.
Results: Homozygosity for the common JH-mutation G320 V in Exon 4 of the HJV gene was identified. In addition, heterozygosity for H63D and S65C and homozygosity for IVS2+4T>C were detected in the HFE gene. In the HAMP gene no sequence change was found.
Conclusion: This case emphasises that the diagnosis of JH should be taken into account in every young patient with heart failure and abnormal iron parameters and underlines the importance of HJV testing in these patients. The severe course in this patient could be explainable by modifying effects of the detected variants in the HFE gene (which play a role in HH) in additional to the causative HJV-mutation.

P048

Three patients with overlapping interstitial deletions in 8q22.2–8q22.3

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High-resolution microarray technology has facilitated the detection of submicroscopic chromosome aberrations and characterisation of new microdeletion phenotypes. We present clinical and molecular data of 3 patients with overlapping interstitial deletions in 8q22.2–22.3.
Patient 1 is a 6-year-old girl with severe developmental delay, absent speech, epilepsy, and facial dysmorphism. Birth measurements were normal. During infancy she presented with auto-aggressive behaviour and sleep disturbance. Facial appearance was characterised by a high frontal hairline, thin and brittle hair, telecanthus, epicanthal folds, blepharophimosis, upslanting palpebral fissures, downturned corners of the mouth and poor facial expression. Array CGH revealed a de novo deletion del(8)(q22.2q22.3) of about 5.25 Mb.
Patient 2 was born with normal measurements. A large hiatus hernia, pyloric stenosis, glandular hypospadias and undescended testes were diagnosed. He had short thumbs and toes and was hypotonic. He had severe learning disability and behaviour problems with frequent temper tantrums, hyperactivity and stereotypic hand movements. At age 42 months, his facies were dysmorphic with enophthalmos, narrow palpebral fissures and hypertelorism. Facial movement appeared relatively poor. Array CGH demonstrated a deletion of about 4.9 Mb in 8q22.3.
Patient 3 carries a de novo deletion of appr. 5.26 Mb. She suffers from early-onset myoclonic epilepsy. No further clinical data is available at the moment.
The shortest region of overlap of all 3 deletions spans about 3.2 Mb (localized from 101,355,387 to 104,556,255 Mb) and contains appr. 20 genes. We suggest that patients with a deletion of this region 8q22.2–22.3 have a recognizable facial phenotype. Although there is some similarity in the facial gestalt of patients with Nablus-mask like syndrome and the patients reported here, the deleted regions do not overlap.

P049

Reasons for Fanconi anemia (FA) patients to reach adulthood

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FA is known as a childhood disorder, but there is a growing population of FA patients who reach adulthood. Aim of our ongoing study is to gain insights into factors which contribute to relative longevity of FA patients. Wherever possible, FA patients ages 20 years and older were assigned to a particular FA-complementation group and analyzed for mutations in the corresponding FA gene. To date, our records include 92 adult FA patients from our own records and 42 patients from the literature. Most patients (74) belong to the 20–30 year cohort, 46 patients are ages 30–40, eight reached ages 40–50, and six are 50 or older. In all cohorts there are more females than males, with a general ratio of 2:1. The complementation group (FA-subgroup) is known for 70 of the adult patients. Most of them belong to subgroup FA-A, followed by FA-C, FA-D2 and FA-G. Three of the adult FA patients within the age range of 20–39 belong to subgroup FA-I, and a single FA-J patient was found among the age 20–29 cohort. In the 40–49 cohort there are only FA-A and FA-G patients, and all patients >50 belong to subgroup FA-A. Our study confirms previous observations by Alter et al showing that the majority of adult FA patients display low congenital abnormality scores. Most of our adult FA patients exhibit only 0–3 congenital abnormalites, some to 4–5, and only few have more than 6 or 7. Relative longevity among our adult patient cohort is primarily due to successful HSCT with or without preceding androgen therapy. Other reasons for longterm survival of FA patients include
(a) revertant mosaicism in blood cells due to somatic reversion of one of the two constitutional FA-mutations, and
(b) the existence of "mild" mutations with putative residual protein function.
Squamous cell carcinoma was identified as the major life-limiting factor in our adult FA patients, regardless of whether these patients underwent HSCT or not.

P050

Identification of a novel WTX germline mutation in a family with Osteopathia striata with cranial sclerosis (OS-CS)

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Osteopathia striata has been recognized as part of several bone dysplasias. In association with cranial sclerosis it represents a separate entity, which is not limited to the bones but may affect other structures, leading to abnormal face, cleft palate, deafness, heart defects, and vertebral anomalies. Neurological findings range from normal development to marked retardation with hydrocephalus, cranial nerve deficiencies and deafness. Based on various family observations OSCS had initially been considered an autosomal dominant condition with complete penetrance and high clinical variability. However, the observation of female preponderance and of severely affected sons from OSCS mothers suggested X-linked segregation. Recently, Jenkins et al. (2009) identified loss-of-function mutations in the WTX gene (FAM123B) on Xq11.1 as the underlying cause of this rare disorder. WTX encodes a repressor of canonical WNT signalling and has been shown to be somatically inactivated in 11–29% of cases of Wilms tumor, indicating a role as tumor suppressor gene. We performed mutation analysis of WTX in two families from German origin. A three generation family that included a severely affected male, his mother, aunt and grandmother with OSCS (Rott et al. 2003) and a second three generation family with four affected women and one affected male (König et al. 1996). By direct sequencing of the whole coding region of WTX we identified a so far not reported substitution of a Cytosine by a Thymidine at nucleotide position 1084 (NM_152424) in the second family. This mutation replaces the codon for Glutamine at position 271 by a premature translational stop codon. The nonsense mutation p.Q271X fully segregated with the phenotype in our family. As reported by Jenkins and coworkers our mutation is also located in the 5‘ region of the WTX gene. Up to now no mutation could be identified in the first family, but further analyses of the WTX gene are in progress.

P051

5 new families with the recurrent 15q13.3 microdeletion syndrome

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Recently a recurrent 1.5 Mb 15q13.3 microdeletion syndrome associated with mental retardation, seizures and/or neuropsychiatric disorders was reported in a total of 11 independent probands. We identified the 15q13.3 microdeletion in 3 unrelated patients with mental retardation screening a cohort of 402 patients using a 250 K or a 6.0 Affymetrix SNP array platform. These findings were confirmed by qPCR and/ or FISH. Analyzing further 184 patients by qPCR we identified 2 more patients in whom SNP array analysis confirmed the typical 1.5 Mb deletion as well. Duplications in this highly unstable genomic region were not found in our cohort. All 5 affected probands had mild to moderate mental retardation and mild dysmorphic facial features. In addition, scoliosis was noted in two patients, ADHD in one and aortic and tricuspid valve insufficiency and kidney anomalies in another patient. Familial investigations showed that two patients inherited the deletion from a parent who had learning difficulties. One of these patients had two brothers with mild to moderate mental retardation in whom the aberration was found as well. Her affected mother suffered from depression. Other psychiatric problems were not observed in our patients. Two patients inherited the deletion from their apparently healthy mothers. Abnormal EEG findings reported as being characteristic were verified in two of our patients, only. The frequency of this microdeletion in our cohort of mental retardation cases was 0.85% (5/586) and therefore slightly higher than the reported 0.3% ($p<0.06$). This suggests that the frequency of this 15q13.3 microdeletion disorder is comparable to that of the well known 1p36 microdeletion.

P052

A novel splicing mutation of CYLD in a family with Brooke-Spiegler syndrome

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The Brooke-Spiegler syndrome is a rare, autosomal dominantly inherited disease. It is characterised by multiple neoplasms of the skin appendages, most commonly cylindromas, trichoepitheliomas and spiradenomas.
As a genetic reason for the Brooke-Spiegler syndrome mutations in the CYLD gene were identified. The CYLD gene consists of 20 exons and encodes a cytoplasmic protein with three cytoskeletal-associated protein-glycine-conserved domains that functions as a deubiquitinating enzyme. We report on a new splicing mutation in the CYLD gene in two sisters both showing typical signs of Brooke-Spiegler syndrome.
Molecular investigation of the CYLD gene using DHPLC analysis and automatic sequencing identified a heterozygous point mutation c.1950–2A>G in the highly conserved AG splice acceptor site of intron 13. This mutation has not yet been listed in international data banks. According to formal knowledge about mutations in the splicing region of a gene the formation of a non-functioning gene product can be expected. Therefore a pathogenic effect of this mutation can be postulated.

P053

Next-generation clinical diagnostics with the human phenotype ontology

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It is essential to make the correct diagnosis in persons with dysmorphic features or suspected hereditary disease in order to plan therapeutic approaches, avoid complications, and provide appropriate counseling about the prognosis and family planning. Combinations of features are often used in medical genetics to search for the clinical diagnosis, and a number of computer programs are available to help with this task.
Ontologies appear particularly attractive for clinical diagnostics in medical genetics, where clinicians may be able to describe clinical features in varying levels of detail, and individual patients with a hereditary disease may not show all the features that are potentially associated with a disorder, or may have additional features unrelated to the disorder. Diagnostic algorithms optimally will allow searches at varying levels of detail, weigh specific features more highly than general features, and not be overly sensitive to the fact that individual features may not be present in an individual patient.
We present here a new, freely available, easy-to-use, web-based diagnostics tool called the Phenomizer (http://compbio.charite.de/Phenomizer). The description of the phenotypic abnormalities are taken from our recently published Human Phenotype Ontology (HPO). The >8000 HPO-terms and their synonyms can easily be searched and added to a list of features that the patient shows. Users are able to rank potential diagnoses by a score expressing the similarity to the features entered by the physician to the features of specific diseases listed in OMIM. Each diagnosis is assigned a P-value that expresses the probability of obtaining the same or better clinical similarity score with the same number of random HPO terms. A number of features are provided to improve search results by adding more clinical terms that best distinguish between the possible diagnoses.

P054

Severe mental retardation in a girl with a 0.8 Mb deletion containing the ATRX-gene

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Mutations in the ATRX-gene are a well established cause of syndromic X-linked recessive mental retardation, and characterized by severe mental retardation in males, usually associated with absent speech, microcephaly, hypotonia, growth retardation facial dysmorphism, and genital and skeletal anomalies. Female carriers are usually phenotypically normal and typically show marked skewing of X inactivation. By molecular karyotyping using an Affymetrix GeneChip Mapping 250 K Nsp SNP array we revealed a 0.8 Mb deletion in Xq21.1 containing the ATRX gene in a girl referred for unspecific mental retardation. The ATRX deletion was confirmed by fluorescence in situ hybridisation (FISH) with the BAC probe RP11–135B6. FISH was also performed on the parents' samples demonstrating that this deletion occurred de novo. In accordance with the unusual finding of a severely manifesting female, X-inactivation was found to be random in this girl (53%). To confirm involvement of the ATRX gene in the pathogenesis of her severe mental retardation, immunofluorescence studies with antibodies against the ATRX protein and against the interacting MECP2 protein, respectively, were performed on a lymphoblastoid cell line of the patient. These studies showed nearly absence of the ATRX protein and a marked reduction of the MECP2 protein. At the age of 4 years the affected girl had no speech, was not able to walk alone and showed stereotypic hand movements. She also showed microcephaly, growth retardation, muscular hypotonia and facial dysmorphism compatible with ATRX syndrome. An ASD closed spontaneously and a paraoesophageal hernia resulting in gastrooesophageal reflux was operated twice. To our knowledge this represents the first report of a larger ATRX deletion. Severe mental retardation in the affected girl might be explained by random X-inactivation. Since such larger deletions have never been observed in boys, they might be lethal in males.

P055

Characterization of the clinical and molecular overlap between Stickler syndrome type III and Otospondylomegaepiphyseal Dysplasia (OSMED)

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Stickler syndrome is an autosomal dominant connective tissue disorder resulting in arthropathy, variable orofacial features, ophthalmopathy, and deafness. The most frequent subtype is Stickler syndrome type I caused by mutations in COL2A1. Much less common is Stickler syndrome type II due to heterozygous COL11A1 mutations. Both phenotypes can clinically be distinguished only by specific ocular findings and by the degree of hearing impairment.
Stickler syndrome type III, a subgroup without involvement of the eye is caused by dominantly inherited COL11A2 mutations. Homozygous loss-of-function mutations of COL11A2 lead to autosomal recessive Oto-spondylo-megaepiphyseal Dysplasia (OSMED).
We report on the clinical and molecular findings of two adults, one female affected by Stickler syndrome type III and one male affected by OSMED.
Analysis of the COL11A2 gene in the patient with Stickler syndrome type III revealed a novel heterozygous c.4230+1G>C mutation most probably resulting in an in frame deletion of exon 58 and leading to a dominant negative effect. Besides radiographic findings consistent with mild spondylic dysplasia and a past clinical history with cleft palate, the patient showed a progressive postlingual hearing loss. Symptoms of osteoarthritis manifested in early adulthood of the index patient and of other affected family members.
Analysis of the COL11A2 gene of the patient with typical OSMED showed a homozygous 1 bp deletion c.731_733delC which results in frameshift and termination of translation in Exon 6. This patient has two children, 6 and 1 years old who are suspected to carry a heterozygous COL11A2 mutation and therefore to be affected with Stickler syndrome type III. However, while they are probably to young to show bony changes or hearing impairment they at least do not have cleft palate.
Our discussion focuses on the comparison of the clinical and molecular findings of these two patients.

P056

Parental origin and mechanism of formation of de novo complex chromosome rearrangements

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Complex chromosome rearrangements (CCR) are structural chromosome aberrations characterized by three or more breakpoints located on two or more chromosomes. Coincidence of two simple reciprocal translocations, CCRs with more breakpoints than chromosomes involved, and translocation of three different segments from three chromosomes each to another can be delineated. These aberrations are rare and mostly de novo. So far, parental origin and mechanisms of formation have been investigated in few cases only by indirect methods or theoretical reflection.
Here, we report on the parental origin and assumed mechanism of formation of five cases with de novo CCRs found in four healthy people and in one child with multiple congenital anomalies (46,XX,t(1;1;11), 46,XX ,t(1;14)ins(14;9)t(9;21), 46,XX,ins(8;10),t(10;11;16), 46,XY,t(7;9)ins(8;7), and 46,XY,t(2;3;9)der(5)inv(5)t(5;11)ins(5;3)). Three out of the five have been recorded previously. For each chromosome of interest, microdissected derivative chromosomes and their normal homologs were pooled separately for investigation, which included whole genome amplification (GenomePlex Single Cell Kit®, Sigma-Aldrich, Vienna, Austria), microsatellite mediated haplotype analysis, and visualisation of the products by silver staining subsequent to a 6% polyacrylamide/urea gel electrophoresis. Formation in paternal meiosis was found in three cases investigated so far. The conformity for either maternal or paternal origin for all derivative chromosomes and their normal homologs makes—with respect to the whole number of chromosomes, the number of chromosomal segments, and the number of breakpoints in each case and in total (2–5 and 18, 3–7 and 23, and 3–9 and 27, respectively) - a meiotic formation more likely than a postzygotic formation. In addition, our results confirm the assumed preponderance of CCRs formed in paternal meiosis.

P057

Increased risk for fulminant autoimmune hepatitis and liver failure in patients with 22q13 deletion syndrome

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We report on a 4 year old girl with developmental retardation, absent speech, status post liver transplantation after fulminant liver failure most likely due to autoimmune hepatitis, and microdeletion of chromosome 22qter, resulting in a 46,XX.ish del(22)(q13.32qter)(ARSA-,N85A-,SHANK3-) karyotype. Using the 244 k Agilent array, the deletion breakpoint was mapped between 43,851,501 bp and 43,865,152 bp at chromosome 22q13.32. The deletion extends to the 22q telomere, comprising 5.84 Mb and 68 known genes. The SHANK3 gene, which is haploinsufficient in the girl, has been implicated in causing the severe speech defect in the 22q13 deletion or Phelan-McDermid syndrome, and recently was assumed to play a role in immunological response as well. Earlier this year, Tufano et al. (Eur J Pediatr. 2009;168:225–227) reported a case of liver failure and terminal 22q13 deletion including the SHANK3 gene, and speculated that a deficiency of SHANK3 may result in a deficient T cell receptor signalling predisposing to autoimmune disorders. Our patient is the second case with hyperacute liver failure and 22q13 deletion syndrome. Our findings confirm that the 22q13 deletion syndrome is associated with a deficient immune system integrity and an increased risk to fulminant autoimmune hepatitis. Patients with 22q13 deletion syndrome, or a subset thereof, are at increased risk for liver failure requiring organ transplantation. Very soon after the liver transplantation and despite immunosuppressive therapy, the parents of our patient noted a global catch-up development of their daughter, possibly suggesting generalized beneficial effects of the liver transplantation.

P058

Results of complete AGXT and GRHPR gene sequencing in a cohort of 77 patients with suspected primary hyperoxaluria

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Primary hyperoxaluria type I (PH I) and type II (PH II) are rare but underreported entities of disturbed glyoxylate metabolism with the clinical hallmarks recurrent urolithiasis and/or progressive nephrocalcinosis. PH I regulary results in endstage renal failure (ESRF), PH II carries a better prognosis with a still substantial ESRF risk. Unfortunately PH I shows extreme inter~ and intrafamilial heterogeneity – a significant problem with respect to diagnosis and management. We performed mutational analysis by single exon PCR and subsequent complete sequencing of the AGXT and the GRHPR gene in a cohort of 77 patients (comprising 62 children and 15 adults, of which 12 adults were already in ESRF). Segregation analysis was performed in all available parents. In our cohort the diagnosis was proven in 43 PHI and 3 PHII patients by identifying 2 known mutations. 13 respectively 1 previously unreported mutations were found in the AGXT and GRHPR gene. A (homozygous) major AGXT deletion was detected in a large family with 4 affected children among them dizygotic twins with marked phenotype variation. Segregation analysis identified a heterozygous de novo mutation in the AGXT gene in another patient. In 4 patients the diagnosis of PH I appears likely: one causative mutation was identified, but the second mutation remained unclear. No cases digenic inheritance (AGXT/GRHPR) have been observed in our cohort. Of the remaining 27 patients 10 displayed persistent hyperoxaluria and a phenotype compatible with PH I but AGXT and GRHPR gene sequencing failed to confirm the diagnosis. Mutational analysis is an excellent diagnostic toll for PH I/II but provides little prognostic information on the course of the disease. Out of the 15 adult patients, 10 were diagnosed late with PH I, 8 of them sadly already in ESRF. 10 patients had to be classified as non type I/II hyperoxaluria clearly demonstrating the need to identify additional genetic cause(s) of PH.

P059

Full-blown phenotype of the microphthalmia with linear skin defects (MLS) syndrome and non-penetrance in females with a heterozygous HCCS mutation

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The microphthalmia with linear skin defects (MLS) syndrome is an X-linked disorder with in utero male lethality. Affected females have microphthalmia, scleorcornea and linear skin defects limited to the face and neck. A high intra- and interfamilial phenotypic variability has been described. Segmental aneuploidy of Xp22.2 has been identified in the majority of cases, however, a few individuals have mutations in HCCS encoding the mitochondrial holocytochrome c-type synthase. We performed sequencing of HCCS in two affected girls with normal karyotype. Patient 1 showed the classical MLS phenotype with anophthalmia of the left eye, microphthalmia of the right eye, and linear skin defects on the neck. She died at the age of four months from refractory ventricular tachycardia. Post mortem examination revealed left ventricular non-compaction, oncocytic cardiomyopathy, and cerebral defects. We detected the heterozygous de novo nonsense mutation c.589C>T (p.R197X) in patient 1. Remarkably, the same mutation has been previously found in another patient with MLS. Patient 2 is a 3-year-old girl with bilateral microphthalmia and sclerocornea. Skin or cardiac manifestations were absent. Sequencing did not disclose a mutation in HCCS. However, quantitative real-time PCR indicated that the relative copy number of six HCCS exons in patient 2 was similar to a hemizygous sample. Fluorescence in situ hybridization with fosmids identified an ~850-kb deletion encompassing the HCCS gene. Remarkably, the asymptomatic mother of patient 2 was found to carry the same deletion as her daughter. By X chromosome inactivation analysis we detected a skewed pattern in patients 1 and 2 and patient's 2 mother. Our data suggest that heterozygous HCCS mutations cause a broad spectrum of clinical features ranging from the classic MLS phenotype to isolated ocular malformations and even no clinical feature suggesting that non-penetrance has to be considered in healthy relatives of an affected female.

P060

Intronic BMPR2 mutation and abnormal PASP response to exercise as risk factors for development of the manifest PAH

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Background: In pulmonary arterial hypertension (PAH), germline defects in the BMPR2 gene are detected in about 80% of familial and 10–25% of sporadic cases. Owing to the low penetrance of these mutations further genetic factors have been suggested to play a role in the pathogenesis of PAH.
Methods: In this study we have performed detailed clinical assessment of 45 family members of a large kindred with familial PAH. Pulmonary artery systolic pressure (PASP) at rest and during exercise was measured by echocardiography at two or more time points: at baseline between the years 1996 and 1998 and as follow-up examination 6–13

years later. Genetic analyses of the BMPR2 gene were performed using the direct sequencing, MLPA-analysis and RT-PCR.
Results: At baseline, 6 members of this family were diagnosed with manifest PAH. Additionally in 15 members with normal pulmonary artery pressures at rest, abnormal values have been detected during exercise. In two of them PAH has been newly diagnosed during the follow-up. Molecular genetic analysis revealed an aberrant splicing of exon 6 as a consequence of an ALU element (ALUYb8) insertion in intron 5 of BMPR2 gene. The sequence at the ALU integration site possesses characteristic motifs and signatures of the retrotransposition event. This insertion was detected in all four living affected family members as well as in twelve healthy relatives, which is in line with reduced penetrance of the disease.
Conclusion: We present a large family, for which extensive clinical data have been collected. We show for the first time that an ALU insertion in an intronic sequence of the BMPR2 gene can underlie PAH. Furthermore, the results of our long term follow up study suggest that besides the mutation an enhanced PASP response to exercise may be an additional risk factor for development of the manifest disease.

P061

Neural tube defects (NTD) and iniencephaly in the Meckel Anatomical Collections at the University of Halle, Germany

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The purpose of our paper is to re-evaluate the preparations of the Meckel Anatomical Collections with NTD. MRI, CT scanning and comparative genomic hybridization (CGH) were used to investigate these fetuses. Johann Friedrich Meckel the Younger (1781–1833) studied the closure defects of the neural tube for over 15 years and had assembled a collection of dozens of specimens, which display this disorder either isolated or in combination with iniencephaly or with other conditions not directly related to NTD. Here, we present essential material upon which Meckel and his medical students based their descriptions. Moreover, our re-evaluation of 88 preparations led to the discovery of a wide range of NTD forms: craniorachischisis totalis, craniorachischisis, holocranium, merocranium, encephaloceles, and spina bifida to microforms with sacrococcygeal dysgenesis. In the present paper, we discuss the fetuses with NTD which occurred in combination with iniencephaly. Iniencephaly is defined as a developmental anomaly involving deficiency of the occipital bone and retroflexion of the spine. It is known as to occur frequently in association with the closure defects involving the cervical and sometimes thoracic and lumbar vertebrae. This condition, known as iniencephalus apertus was found in 6 fetuses in the present-day collection. Iniencephaly is a rare congenitale anomaly the etiology of which is still not fully understood. However, it should be noted that the presence of the fetuses with NTD and iniencephaly in the Meckel anatomical collection merely reflects their lethality, rather than their prevalence.

P062

Loss-of-function mutations of the tumor suppressor and major prostaglandine PGE_2 catabolizing enzyme HPGD cause digital clubbing and offer attractive therapeutic options

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Digital clubbing is the most prominent feature in primary (PHO) and secondary (pulmonary) hypertrophic osteoarthropathy (HO). Autosomal recessive mutations in HPGD encoding the major prostaglandine PGE2 catabolizing enzyme have been recently described in familial cases (Uppal et al., Nat Genet 2008). We present comprehensive clinical, genetic and biochemical data of three unrelated PHO families. Rather uncommon for recessive disorders, minor clinical features were also present in some heterozygous family members. The truncating mutation c.173_174del was found in two of our families and in one of the recently described pedigrees, all of European origin. However, we present evidence that c.173_174del is rather a recurrent mutation than an ancient founder allele. We postulate that all HPGD mutations constitute loss-of-function alleles due to protein truncation or missense changes that affect hydrogen bonds lining the HPGD enzyme reaction cavity. In line, all patients demonstrated chronically elevated prostaglandine PGE2 levels. Similarly, loss of HPGD activity can be observed in a number of different tumors with increasing evidence that HPGD is a tumor suppressor. So far, tumor predisposition has not been reported in families with PHO, however secondary HO is a well-known consequence of certain neoplasia and a common pathogenetic mechanism can be postulated for primary and secondary forms of HO. One of our patients showed massive thymus hyperplasia with multiple cysts at the age of 13 years most probably as a result of chronic inflammation. Recent data postulated a direct link between inflammation, the mTOR pathway and tumorigenesis. Answering the question if PHO patients bear an increased risk for tumors is of paramount importance for their management and surveillance. Preliminary data of ongoing therapeutic trials with the nonsteroidal anti-inflammatory drug naproxen in two of our PHO patients are promising; final results will be presented at the conference.

P063

Disruption of PTPRD and GRIN2B in a patient with mental retardation and microcephaly

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Disease-associated, balanced chromosomal aberrations are powerful tools for gene identification. We report a male patient with a balanced de novo 9;12 translocation [46,XY,t(9;12)(p23;p13.1)]. He presents with microcephaly, mild to moderate mental retardation, hyperactivity, and various facial dysmorphism. A genome-wide array CGH with a resolution of 24 kb was done, but did not show any genomic imbalance. We delineated both breakpoints by fluorescence in situ hybridization using fosmid clones and found that the breakpoint in 9p23 directly disrupts the PTPRD gene and that in 12p13.1 the GRIN2B gene. By RT-PCR, we amplified PCR products for both PTPRD and GRIN2B using RNA isolated from leukocytes. In contrast, we were not able to generate reciprocal fusion transcripts between the two genes by using RNA from leukocytes of the translocation patient. PTPRD encodes the receptor-like protein tyrosine phosphatase delta which has an important role in neuronal adhesion and outgrowth of embryonic forebrain neurons

in vitro and is involved in synaptic transmission and induction of long-term potentiation (LTP). LTP is an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. Ptprd-deficient mice show impaired spatial learning and altered hippocampal LTP. The protein encoded by GRIN2B is the NR2B subunit of N-methyl-D-aspartate (NMDA) receptors that are a class of ionotropic glutamate receptors and the predominant excitatory neurotransmitter receptors in brain. Remarkably, Ptprd has been suggested to play a role in the regulation of the activation of NMDA receptors at the synapses providing a possible functional link between PTPRD and GRIN2B. We hypothesize that haploinsufficiency of either PTPRD or GRIN2B or of both genes is of functional importance for the clinical manifestations, in particular mental retardation and microcephaly, in the translocation patient.

P064

Craniofrontonasal syndrome – a genetic disease with an unusual X-chromosomal dominant inheritance pattern: A case report

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Craniofrontonasal syndrome (MIM 304110), a subgroup of craniofrontonasal dysplasia, is a rare syndrome with typical facial dysmorphism and body asymmetries in combination with skeletal, neurological and dermatological abnormalities. It is inherited in an exceptional X-linked manner with female patients more severly affected than male carriers.
We report on a 1 year old girl hypertelorism, broad nasal tip, facial asymmetry, and plagiocephaly. Her paternal great-grandmother, her paternal grandmother, two grandaunts, and two aunts are similarly affected, whereas her father and two brothers of her seem to be unaffected. One of the grandaunts also suffers from seizures and mild mental retardation. Sequence analysis of the ephrin-B1 gene (EFNB1) on the X chromosome revealed a novel heterozygous mutation c.344A>G (p.Q115R) in exon 2 in the indexpatient.
We will describe in detail clinical and molecular genetic aspects of the craniofrontonasal syndrome with a particular focus on the family reported here.

P065

Differential diagnosis of autosomal recessive forms of cutis laxa

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Although all forms of hereditary cutis laxa are characterised by inelastic and wrinkly skin the severity and the involvement of other organ systems are highly diverse. To facilitate differential diagnosis we would like to delineate key features of the different forms of autosomal recessive cutis laxa (ARCL) on the basis of the current clinical and genetic knowledge and present typical cases.
ARCL can be subdivided into two main groups according to the lung involvement. While ARCL type 1 is characterized by emphysema ARCL type 2 does not show significant lung disease. The strongest mental retardation is usually associated with de Barsy syndrome, also referred to as ARCL type 3. ARCL type 2 (Debré type) shows a broad range of very mild to severe mental retardation and even neurodegenerative phenotypes in some cases. In contrast, no mental retardation is found in gerodermia osteodysplastica (GO). GO, on the other hand, displays the strongest skeletal involvement and increased fracture risk. However, bone mass can also be reduced in other ARCL types. The cranial phenotype in ARCL type 2 includes a large fontanelle with delayed closure and a typical facial dysmorphia including downslanting palpebral fissures, while GO patients have a prematurely aged appearance, in part due to jaw hypoplasia. A unique hallmark of de Barsy syndrome is corneal clouding or cataract. Biochemically, ARCL type 2 (Debré type) is the only form of ARCL that is a congenital disorder of glycosylation (CDG). The ultrastructural analysis shows defective elastic fibers, but does not allow to distinguish between different forms of ARCL.
Genetically, ARCL type 1 is caused by mutations in FBLN5 or EFEMP2. ARCL type 2 and GO are caused by loss-of-function mutations in ATP6V0A2 and SCYL1BP1, respectively. Both gene products are localized at the Golgi apparatus suggesting an overlapping cellular dysfunction. The genetic defect underlying de Barsy syndrome/ ARCL type 3 is not yet known.

P066

Inborn errors of metabolism as a cause of mental retardation – what to test?

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Inborn errors of metabolism (IEM) are rare causes of mental retardation (MR) and constitute about 1% of all causes of MR in the Caucasian population. In contrast to recommendations for other diagnostic laboratory tests in MR, e.g. chromosome analysis or fragile X testing, there is no consensus on a national or international level about criteria for metabolic testing. IEM however are potentially treatable and their diagnosis means knowledge on prognosis, recurrence risk and the availability of prenatal diagnosis. We therefore aimed at developing guidelines for metabolic testing in MR.
In a review of available evidence, we identified the following important IEM leading to non-syndromic MR or to conditions with MR as the predominant sign: creatine deficiency syndromes (in particular creatine transporter deficiency), mucopolysaccharidosis III (in particular IIIB), ß-mannosidosis, homocystinuria, CDG Ia, and specific organic acidurias. In addition, urea cycle disorders and sterol synthesis defects (in particular SLO) may be considered. Metabolic testing in unexplained MR thus may include next to basic laboratory tests (liver function, CPK, uric acid, ammonia, lactate, thyroid function), as part of a third level evaluation the investigation of urinary guanidino compounds, organic acids, purines/pyrimidines and glycosaminoglycans incl. electrophoresis; transferrin isoelectric focusing for CDG in serum; and amino acids, homocystein and 7-dehydrocholesterol in plasma. Determination of phenylalanine in the mother should be considered. Additional clinical signs (e.g. seizures, autism, behavioural problems, cerebellar dysfunction, pyramidal signs, dysmorphism) can change the rationale and lead to a stepwise widened investigation.
A prospective study to evaluate the proposed rationale has been started recently and will be presented.

P067

Hennekam syndrome – a case report

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"Hennekam syndrome" is a rare genetic disorder combininglymphedema of the limbs and face, lymphangiectasia and an unusual face.Mental delay and seizures are also part of the syndrome. Familial casesdisplay autosomal recessive transmission. We present the follow-up of a 15-years old patient for further delineation of the phenotype.Proposita (G1;P1, unremarkable) - first child of a non consanguineouscouple was born at term with BW 2800 g; BL 51 cm; OFC 34 cm. He had noclinical signs of protein-losing enteropathy. Growth development andmotor milestones were corresponding to the age. Speech was at a borderlinelevel. Seizures were firstly noted at 3 8/12 years, clinical coursewas characterized by frequent episodes of polymorphic generalizedclonic-myoclonic seizures. Lymphedema of lower limbs developed during the 1-st decade of life, became more pronounced with age spreading to the lower part of the abdomen, scrotum and buttocks.At the age of 13 years weight was 59 kg, length 152 cm, OFC 53,5 cm.The lymphedema remained stable. The patient showed mild mental delay without behavioral problems, learning difficulties, a flat face, prominent ears,epicanthus, concomitant divergent strabism,and myopia.MRI of the brain: cysts, distinct cystic-like enlargement of lateralventricular horns. ECG: sinus tachycardia, conduction abnormality.Sonographic studies: heart -additional chord of left ventricle,regurgitation on tricuspid and pulmonary valves; abdomen-hypotonicgall bladder, cholestasis.Doppler ultrasonography of the lower limb vessels (veins, arteries)revealed normal drainage. Skin biopsy showed a diffuse edema.The results of a selective screening for metabolic defects, lysosomaldisorders, tandem-mass spectrometry analyses, level of serum proteinand cortisol were normal, triglycerides and cholesterol were elevated.The clinical features of our patient and the differential diagnosis will be compared with published cases.

P068

Copy number variation analyses in 4 patients with SHORT syndrome

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SHORT syndrome (OMIM 269880) is characterised by short statue, hyperextensibility of joints and/or hernia, ocular depression, Rieger anomaly, teething delay, and a typical facies. The aetiology of this rare syndrome is still unknown, but multigenerational families described in the literature (including also male-to-male transmission) point to an autosomal dominant inheritance.
Here we present a new patient with SHORT syndrome with short statue, deep set eyes, teething delay, peripheral atrophy of the iris stroma (typical symptom of Rieger anomaly), and distinct facial abnormalities. Human 500 K SNP-array analysis revealed a 0.3 Mb de novo deletion at 3p12.3 from 75.40–75.70 Mb spanning 2 genes (FAM86D and AC131233.3). Molecular investigation of three additional patients with SHORT syndrome from two families did not result in the identification of de novo CNVs or of any pathogenic sequence alterations in the analyzed 2 genes. Since a similar CNV has been found also in healthy controls haploinsuffiency of FAM86D and AC131233.3 seems unlikely to be causative for SHORT syndrome.

P069–P104 Cytogenetics

P069

Identical cryptic partial monosomy 20pter/trisomy 20qter in 3 adult siblings due to a large maternal pericentric inversion: Detection by MLPA and rapid breakpoint mapping by SNP array analysis

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Genotypic and phenotypic data are presented of three adult siblings with mild/moderate mental retardation and mild dysmorphologies. All three siblings showed a gain at the q-telomere and a loss at the p-telomere of chromosome 20 in routine subtelomeric MLPA screening. Analysis of GTG-banded chromosomes did not detect any abnormalities, but subtelomeric FISH confirmed cryptic partial monosomy of chromosome region 20p13 ≥ 20ptel and cryptic partial trisomy of chromosome region 20q13.33 ≥ 20qtel. Furthermore, FISH analysis showed a cryptic inv(20)(p13q13.33) in the mother. This explained the cytogenetic mechanism underlying the chromosomal imbalance in the 3 children, i.e. the meiotic formation of a recombinant chromosome 20 due to crossing-over in the inverted segment. All 3 children thus carried a rec(20)dup(20q)inv(20)(p13q13.33)mat. SNP array analysis enabled rapid and detailed imbalance sizing and showed 1.06 Mb loss in 20p13 and 2.51 Mb gain in 20q13.33, comprising 21 and 78 genes respectively. The maternal inversion is one of the largest pericentric inversions for any chromosome described to date and the largest thus far for chromosome 20, comprising 94.4% of its length. Such large inversions give a particular high risk for live-born unbalanced offspring because the partial monosomy and trisomy segments are small. Moreover, the inversion size is directly related to the percentage of unbalanced gametes due to high crossing-over change within the inverted segment. The fact that 3 children carried the same chromosomal rearrangement has consequences for genetic counselling of large pericentric inversion carriers, as the previously proposed recurrence risk of 5–10% is probably higher.

P070

A 5,6 Mb deletion on chromosome 8q12 including CHD7 causing a contiguous gene syndrome with features of CHARGE syndrome

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CHARGE syndrome (OMIM: #214800) is an acronym for coloboma, heart defects, choanal atresia, growth and developmental retardation, genito-urinary abnormalities, and ear anomalies. The gene CHD7 (Chromodomain Helicase DNA-binding protein 7) on chromosome 8q12 was identified as to be associated with CHARGE syndrome. About 70% of patients show CHD7 coding region mutations. Reports of entire gene deletions are rare.
Here we describe a severely dysmorphic newborn boy with coloboma of the iris, facial asymmetry, micrognathia, malformed ears, laryngomalacia, malformations of upper gastrointestinal tract, and micropenis who died at six month of age of severe immune deficiency due to complete T-cell defect (T-, B+, NK+).
G-banded chromosomal study on cultured skin cells demonstrated an apparently normal male karyotype and microdeletion 22q11.2 was excluded by FISH analysis. However, whole genome array-CGH showed a deletion of approximately 5.6 Mb on chromosome 8q12. Analysis of

both parents demonstrated that the patient's deletion arose de novo. Several RefSeq-genes are located within this 5.6 Mb deletion including CYP7A1, NSMaF, SDCBP, TOX, CA8, RAB2A, CHD7, MGC34646, ASPH, FAM77D, GGH, TTPA and YTHDF3. Even though our patient was much more affected than typical patients with CHARGE syndrome, some morphological findings reminded of CHARGE syndrome that may be due to the deletion of CHD7. Recently, it was shown that also immune deficiency might be a symptom of CHARGE syndrome. However, the spectrum of the T-cell defect is variable. Since it was shown that mice with a knock out of the TOX gene which is located close to CHD7 have no thymus and develop immune deficiency the question raises whether any alteration of TOX gene function might contribute to the immune deficiency. However, whether the deletion of TOX or one of the other genes had any influence on the phenotype in our patient is still unclear.

P071

De novo pericentric inversion leading to monosomy 9pter-p23 in a boy with epilepsy and facial dysmorphism

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We report a 10-months-old boy with an unique chromosomal aberration: an unbalanced de novo pericentric inversion of chromosome 9 with breakpoints in 9p23 and 9q12 and loss of 9pter->9p23 leading to monosomy of this region.
The boy presented with hypotonia of the upper half of the body, developmental delay, hypospadia, widely spaced nipples, abnormal toe position and facial dysmorphism (short palpebral fissures, deep set, posteriorly angulated ears, high palate, thin upper lip, long philtrum and micrognathia). Asymmetric tonic seizures started at the age of 6 months. MRI revealed a marked dilatation of the external cerebrospinal fluid spaces in the frontal regions.
Specific FISH probes and array-CGH were used to characterise this aberration and define the breakpoints.
The deleted region encompasses approximately 9 Mb pairs and contains about 60 genes. 8 of them have been defined as disease associated: DOCK8 (OMIM 611432), which causes mental retardation, SLC1A1 (OMIM 133550), which codes for a glutamate-transporter with high expression in the brain and DMRT1 (OMIM 602424), which causes gonadal dysgenesis. A gene for autism is suspected to be located in this region because this disorder has been found frequently in 9p monosomy.
The phenotype of this patient will be compared with the few patients in the literature with similar deletions in order to define a specific phenotype consisting of distinct facial features (especially thin upper lip, long philtrum, deep set ears), hypotonia, genital anomalies and possibly seizures which have not been described yet in a patient with such a distal chromosome 9p deletion, only in patients with unbalanced translocations involving distal 9p monosomy and partial trisomy of another chromosome.

P072

Generation of murine whole and partial chromosome painting probes based on FISH-microdissection

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Though mouse is one of the most accessible human disease models, cytogenetic studies of this species are still complicated due to the similar acrocentric chromosome morphology. So, FISH is the method of choice to characterize murine chromosomal rearrangements. Murine whole chromosome painting (wcp) probes enable to detect most possible translocations, however, fail to identify intrachromosomal rearrangements and cannot help to identify breakpoints. FISH banding methods such as multicolour banding (mcb) can easily overcome these limitations. Previously, we have generated mcb probes for several mouse chromosomes from somatic cell hybrids (Trifonov et al., 2005). Now we present another approach of generating mouse wcp and mcb probes based on FISH-microdissection. At first, we microdissected all 40 mouse chromosomes from the same metaphase spread, collected them in 40 separate micropipettes and amplified them using whole genome amplification WGA1 kit (Sigma). This led to generation of 40 single chromosome-based mouse wcp libraries. These initial WCPs were used as probes for FISH- microdissection to create fully-representative mouse wcp based on 20 microdissected chromosomes. Further, these wcp probes were labelled with different fluorochromes and co-hybridized together on control mouse metaphases and metaphases with known chromosomal rearrangements to exclude the duplicated probes (as both chromosome homologues were microdissected on the first step) and to assign the dissected probes to correspondent chromosomes (some of the assignments were also confirmed with BACs). Derived wcp were later used to identify mouse chromosomes during FISH-microdissection to generate region-specific libraries for mcb probes. Overall, combined single chromosome-based microdissection and FISH-microdissection have great potential for probe generation in cases when target chromosomes are undistinguishable.
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P073

Microdeletion 13q33.1–13q33.3 associated with Hirschsprung disease, developmental delay, epilepsy, autistic features and minor dysmorphisms

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In the year 1991 Bottani et al. described a 13.5 year old patient with an interstitial microdeletion involving chromosomal bands 13q32.3 to 13q33.2, defined on the basis of GTG banding (resolution of approximately 850 bands). With interstitial deletions 13q, in the literature few patients were described and long term follow up in these patients is rare. We clinically reevaluated the patient at the age of 32 including high resolution molecular genetic analysis. By array comparative genomic hybridisation we had to correct the initially described breakpoints to 13q33.1–13q33.3. Clinically the main features of the 32-year-old patient were Hirschsprung disease, moderate mental retardation, autistic features, epilepsy and minor dysmorphisms. This is the first report about a patient with the chromosomal deletion 13q33.1–13q33.3. This specific interstitial deletion on 13q seems not to be associated with noticeable dysmorphisms nor abnormal growth parameters but rather with moderate mental retardation, epilepsy, autistic features and Hirschsprung disease.

References: Bottani A, Xie Y, Binkert F, Schinzel A (1991) A case of Hirschsprung disease with chromosome 13 microdeletion, del(13)(q32.3q33.2): potential mapping of one disease locus. Hum Genet 87:748–750

P074

Molecular cytogenetic characterization of four new cases with a small supernumerary marker chromosome derived from chromosome 16

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Here we characterized four cases with small supernumerary marker chromosomes (sSMC) derived from chromosome 16. Case 1 had a karyotype mos 47,XX,+r(16)(::p11.1.->q12.1~q12.2::); by array painting the breakpoints of the marker were redefined to 16p11.2 and 16q12.1. Thus, the sSMC led to a partial trisomy of 33.43 Mb to 47.02 Mb on #16. Case 2 was a postnatal case and the sSMC was characterized by centromeric FISH-probes and subcenM-FISH as mos 47,XY,+r(16)(::p11.1->q12::). Case 3 and 4 were diagnosed prenatally and had karyotypes of 48,XY,+min(16)(:p11.1->q11.2:)x2[9]/47,XY,+min(16)(:p11.1->q11.2:)[14]/46,XY[14] or 47,XX,+min(16)(:p10->q12.1:)[12]/46,XX,+min(16)(q12.1->p10::p10->q12.1:)[3]/46,XX[3], respectively. Our results, using molecular cytogenetics, showed that all sSMC were derived from chromosome 16, resulting in the presence of a de novo mosaic partial trisomy of chromosome 16, involving euchromatic material from 16q. Cases 1 and 2 were clinically abnormal, while cases 3 and 4 were postnatally normal. Comparison with other reported cases (http://www.med.uni-jena.de/fish/sSMC/16.htm) confirmed that proximal 16q12.1 is not a dose sensitive region while imbalances involving 16q12.1~12.2 lead to clinical aberrations. Studies like ours are of importance to better delineate does sensitive regions in the centromere-near regions.

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P075

The 'fragile secret' of 32 new molecular mapped aphidicolin induced fragile sites

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The molecular basis of fragile sites (FS) is a still not fully understood phenomenon. Unlike other structural rearrangements within the genome, FS lack clearly defined breakpoints on the DNA-level. In contrary, FS can span up to several mega base pairs and therefore represent a kind of ‚fragility prone regions' within the genome. The three most frequently observed FS are FRA3B in 3p14, followed by FRA16D in 16q23 and FRAXB in Xp22. Therefore it is not surprising that these fragile sites are also part of the already 23 molecular characterized common fragile sites. In the present work 32 yet unmapped FS were aligned to the current genome map by human sequence anchored BAC clones. Moreover for 6 of these regions FRA2H, FRA2 J, FRA4C, FRA7 J and FRA10F we were able to define their distal and proximal boundaries, which enabled a specific sequence analysis and the comparison with other already molecular defined FS. The average size of all mapped fragile sites is 4.948 Mb ranging from 0.353 Mb (FRA9G) up to 10.634 Mb (FRA1H). Overall now 55 FS are molecularly mapped and we start to get an idea of the molecular basis of common FS as a part of the normal chromosome structure.

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P076

Global screening and extended nomenclature for 230 aphidicolin-inducible fragile sites – including 61 yet unreported ones

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Since the first description of human fragile sites (FS) more than 40 years ago (Dekaban et al. 1965) diverse cultivation methods were developed to induce chromosome breaks at these non random breakage prone regions (Sutherland et al. 1977). Up to now, 77 of 88 known common fragile sites that are listed in human genome browsers are inducible by aphidicolin. Here we report a global and comprehensive screening of all aphidicolin inducible FS as detected in lymphocytes of 3 unrelated healthy individuals in not less than 20.765 analyzed metaphase spreads. This is to the best of our knowledge the largest yet undertaken study on aphidicolin induced FS. After analysis we found 230 different FS including 61 yet neither reported nor classified common FS. Additionally we could confirm the existence of 18 FS that were already previously reported (Wyandt and Tonk 2004, Simonic and Gericke 1996) but not classified in genome browsers. Deduced from the alphabetical nomenclature of FS for single chromosomes, we suggest here a new FS designation including also yet unclassified ones and provide a global genome map of FS.

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P077

A 3q21–23 deletion causes BPES with an extended phenotype

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Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (MIM 110100) is a rare genetic disease for which the FOXL2, a forkhead transcription factor gene in 3q23, has been shown to cause both clinical subtypes (BPESI and BPESII). Molecular defects include intragenic mutations (81%), genomic rearrangements comprising both – deletions encompassing FOXL2 (12%) and deletions located outside its transcription unit (5%), as well as chromosomal rearrangements (2%). It is known in this context that chromosomal aberrations are probably the most important underlying cause in patients with BPES and associated mental retardation.

Here we present the clinical, cytogenetic and molecular findings of a sporadic case revealing a 3q21–23 deletion, which includes the FOXL2 gene. To the best of our knowledge, only five further cases with the same genetic loss have been published so far. All of them showed the typical hallmark of severe eyelid malformations. Further characteristics are mental retardation, different congenital malformations including skeletal abnormalities like dislocation of the hip. However, our patient, a 13 years and 7 month old girl, shows additional features extending the phenotype: restrictive eye-motility disturbances and currently developed dislocated lenses as well as radioulnaric synostosis. A congenital unilateral caudal renal ectopia (left kidney), diagnosed in our case, has also never been described before in patients with 3q21–23 deletion. Thus, our case highlights the importance of chromosomal investiga-

tions in patients with an extended BPES phenotype prior to FOXL2 mutation screening for optimal diagnostic management.

P078

A case of pure trisomy 1q42.1-qter – characterisation of the rearrangement and delineation of the specific trisomy 1q phenotype

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Partial trisomy 1q is a rare aberration. In the majority the duplication occurs as a result of an unbalanced rearrangement, and is therefore accompanied by other imbalances.
We report on a 5 1/2 years old girl with pure trisomy 1q42.1-qter due to an intrachromosomal rearrangement. The duplicated segment is translocated to the p-terminal end of one chromosome 1. As proven by FISH with probes of the subtelomeric regions the rearrangement was not associated by a terminal deletion 1p. Our patient is the first child of healthy and non-consanguineous parents. Chromosome analysis was done because of craniofacial dysmorphisms, cardiac defect, and delayed psychomotoric development. Her craniofacial features and the growth retardation were compatible with Silver-Russell syndrome.
To estimate the exact breakpoints, location and size of imbalance, further characterisation was carried out by FISH and customized OligoaCGH analyses.
These investigations revealed a duplication ranging form band 1q42.12 to 1qter, comprising a gain of 20.8 Mb. Beyond that it could be confirmed that the rearrangement does not include a deletion of euchromatic material at the p-terminal end.
The delineation of a distinct phenotype of this condition is difficult because of the coexisting deletions of other chromosomes in most cases. Nevertheless, some clinical findings like macrocephaly, triangular face (prominent and wide forehead, midface hypoplasia, and pointed chin), intrauterine and postnatal growth retardation, developmental delay, and cardiac defects have been reported constantly. To the best of our knowledge, only five cases of pure distal trisomy 1q42 have been reported so far, and only once a comparable intrachromosomal translocation was diagnosed. The more accurate determination of the breakpoints and extend of imbalances by new techniques provides important information for more detailed phenotype/genotype correlations and insights into the emergence of rearrangements.

P079

Haploinsufficiency of 16.4 Mb from chromosome 22pter-q11.21 in a girl with unilateral conductive hearing impairment and development in the normal range

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We present a girl with de novo 45,XX,der(18)t(p11.32;q11.21) and apparently normal development. At birth, mildly low birthweight and ptosis of the right upper lid were noted. Developmental milestones were reached late. Five years and four months old, conductive hearing impairment (60 db) on the left was diagnosed. IQ was 108 by nonverbal intelligence testing. After provision with a hearing aid, she caught up in development and started normal primary school. At 8 years, her speech was age-adequate and her school achievements were good. Her 2 years younger sister who grows faster and is a quicker learner also demonstrated an unilateral ptosis. Subtelomere FISH and MLPA revealed that no essential gene was lost from chromosome 18. FISH with region-specific BAC probes, a customized MLPA assay for chromosome 22q11, and GeneChip Genome Wide Human SNP array analysis were used to delineate the haploinsufficient region 22pter-q11.21. The chromosome 22 breakpoint lies between 16,420,148 bp and 16,425,846 bp. STR analysis with polymorphic markers from 22q11 demonstrated that the de novo rearrangement arose in the paternal germline. The deleted 16.4 Mb region contains 10 genes (POTEH, OR11H1, CCT8L2, XKR3, GAB4, IL17RA, CECR6, CECR5, CECR1, and CECR2) as well as well as many predicted genes, pseudogenes, and retrotransposed sequences with unknown functions. Many of these genes show copy number variation in the human genome (own and published data) and/or have functionally related loci on other chromosomes, which makes them unlikely candidates for a dosage-sensitive gene(s). Duplication of the proximal long arm of chromosome 22 causes cat eye syndrome (CES). The CES phenotype is highly variable, ranging from severe malformations (with lethal outcome) to near normal development. Of the 10 haploinsufficient genes in our patient, we consider CECR1 and CECR2 as candidates for a new hearing impairment-causing gene and also for some features of CES.

P080

Interstitial deletion of proximal 4q characterized by array CGH analysis

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Deletions of the distal part of chromosome 4q result in a defined clinical phenotype comprising craniofacial, digital, skeletal and cardiac anomalies combined with a developmental delay (Strehle et al 2001). There are only few data on deletions of the proximal part of 4q. We report on a 6 months old girl with short limb dwarfism with prenatal onset, brachydactyly, hearing loss and profound developmental delay. No major malformations of heart and brain were detected. Craniofacial features included frontal bossing, midface hypoplasia, low set ears and relative macrocephaly. Moreover, respiratory distress episodes with cyanosis and muscular hypertonia were present. Array CGH analysis using a 105 K chip revealed a deletion of 14.4 Mbp corresponding to the chromosomal region of 4q13.3–4q21.3 hardly visible in G-banding. Proximal interstitial deletions of 4q described in literature resulted in a non-specific phenotype with mental retardation, growth retardation and hypotonia. Interestingly, limb malformations have been described as part of the distal 4q-deletion syndrome and were attributed to the dHAND gene which was not deleted in our patient. We conclude, that the array CGH technique should be used to determine the deletion boundaries in any suspected deletion syndrome and may be helpful in identifying genes of interest in specific phenotypes.

P081

Rare sex chromosome aneuploidies in humans: Report on three patients with 48,XXYY, 49,XXXXY and 49,XXXXX karyotypes

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Birth prevalence of sex chromosome aneuploidies is estimated at about 1:400 livebirths. However, karyotypes with more than one extra X or Y chromosome are rarely described, and relevant clinical information is limited in these cases. Here, we report clinical, cytogenetic and molecular data of three patients with different sex chromosome polysomies

and discuss the data with respect to the mechanism of formation of the additional sex chromosomes.
Patient 1 (48,XXYY) is a 17 year old male who first came to medical attention at the age of 3 years, because of profound speech delay and retarded motor and intellectual development. Due to his aggressive behaviour he is now in permanent psychiatric and forensic care. Using microsatellite analyses we demonstrate, that the origin of the gonosomal aneuploidy was due to non-disjunction in meiosis I/II during spermatogenesis resulting in a XYY gamete.
Patient 2 (49,XXXXY) was first presented at the age of 28 years with hypogenitalism and profound intellectual disability considering Klinefelter syndrome. The most likely origin of the additional X chromosomes is successive non-disjunction at the first and second meiotic division in one maternal germ cell.
Patient 3 (49,XXXXX) is a 10 year old girl with dysmorphic facial features, radioulnar synostosis, heart defect and mental retardation. Molecular analysis on microdissected five X chromosomes indicated that the pentasomy X is most likely the result of three consecutive non-disjunctions involving only one of the maternal X-chromosome.
Conclusion: Aneuploidies with multiple X and Y chromosomes represent a distinct group of disorders that might be recognized in childhood due to their phenotypes and neuropsychiatric profiles. It seems likely that most sex chromosome polysomies are attributable to successive non-disjunctional events of the same parent. However, the specific triggering mechanism remains unclear and appears to be independent of parental ages.

P082

Two clinical cases with mosaicism for 18q deletion syndrome

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The 18q deletion syndrome (MIM 601808) represents a contiguous gene syndrome which is characterized by mental retardation and multiple congenital malformations. The deletion in 18q can be terminal or interstitial, and sizes range from 2 to 36 MB (18q21 or 18q22 to qter). The phenotype is highly variable, including postnatal onset of growth hormone deficiency with disproportionate short stature, muscular hypotonia, hearing impairment, and foot deformities. Further common features are microcephaly, palatal defects, short frenulum, carp-like mouth, short palpebral fissures, and external ear anomalies. Cardiac anomalies include atrial and ventricular septal defects and pulmonary stenosis. Serum IgA levels were also found to be decreased in affected individuals. Both familial occurrence and isolated cases have been described.
Here we present two unrelated cases with mosaicism for 18q deletion with their clinical spectrum as well as a synopsis of the clinical features in comparison to 18q deletion syndrome cases described in the literature. The karyotypes from lymphocytes of the affected individuals presented here are
mos 46,XX,del(18)(q21.2)[7]/46,XX[10] and
mos 46,XY,del(18)(q21.3)[26]/46,XY[15].
In both cases, de novo occurrence was confirmed by cytogenetic examination of the parents. In the affected girl´s blood sample arrayCGH using an oligoarray (Agilent) defined a deletion size of ~24 Mb, but in spite of the mosaic status, the presence of a normal cell line would have been missed using arrayCGH alone. Concerning the clinical phenotypes, the affected boy who had mosaicism for a smaller deletion (~9.8 Mb) showed a more severe mental retardation and more clinical abnormalities than the girl with mosaicism for the larger deletion. Possible explanations could be the higher proportion of aberrant cells in the affected boy with the smaller deletion or divergent mosaicism ratios in different tissues of both affected children.

P083

Parental origin and mechanism of formation of a 46,X,der(X)(pter->q21.1::p11.4->pter)/45,X karyotype in a female with mild Turner syndrome

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Turner syndrome is characterized by pre- and postnatal growth retardation, oedema of hands and feet, webbed neck, short 4th and 5th metacarpal, cubitus valgus, gonadal dysgenesis leading to infertility, cardiac and renal abnormalities, and various dysmorphic features. Half of the cases show a 45,X karyotype, the rest reveals mosaicism with a normal XX or XY cell line, or a normal cell line and a derivative X chromosome in a second cell line. Few cases have more complex gonosomal chromosome complements.
Here, we report on the rare case of an Xp/Xq translocation in a female with normal height, gonadal dysgenesis, and mild facial dysmorphisms. Conventional karyotyping, molecular-cytogenetic investigations with an X chromosome library, subtelomeric probes for Xp, Xq, and the X inactivation centre (ToTelVysion®, Vysis, Downers Grove, IL), locus specific FISH-probes localized on Xp11.4, SNP microarray analysis with the Affymetrix Human Mapping 50 K SNP-Array, and molecular investigations of genomic DNA with microsatellite markers mapped to the X chromosome revealed a 46,X,der(X)(pter->q21.1::p11.4->pter)[23]/45,X[8] karyotype and a lack of the paternal alleles distal to Xq21.1. By investigation of separately microdissected and whole genome amplified der(X) and its normal homolog with appropriate microsatellite markers we were able to demonstrate for the first time that such karyotypes are formed by recombination between the sister chromatids of the paternal X chromosome, most likely during meiosis. In line with the demonstrated occurrence of the X inactivation centre on the der(X) and the presence of the pseudoautosomal region of Xp in a considerable proportion of cells, our patient clinically shows only mild Turner syndrome features like slightly reduced height. The ovarian failure is rather the consequence of the lack of Xq21.1->qter as Xp duplication is not known to have an influence on female fertility.

P084

Deletion 7q11.21-q11.23 and infantile spasms without deletion of MAGI2

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We report on the clinical and cytogenetic findings as well as on the array-based characterization of an interstitial 7q11.21-q11.23 deletion initially recognized by standard karyotyping in a 15 months old female patient. Beginning at the age of 3 months and 2 weeks the patient suffered from severe infantile spasms.
Recently, it was reported that infantile spasms are associated with deletion of the MAGI2 gene on chromosome 7q11.23. Nevertheless, not all patients reported with deletions of MAGI2 developed infantile spasms and at least one reported patient with a deletion 7q11.23 without missing the MAGI2 gene was diagnosed with infantile spasms. Molecular karyotyping of our patient confirmed a large 13 Mb deletion encompassing the 7q11.21-q11.23 region without involvement of MAGI2. Critical review of published data and the results of our patient underline the importance to map precisely the deletion boundaries of further patients to reevaluate the significance of MAGI2 hemizygosity in the pathogenesis of infantile spasms.

P085

FISH-analysis for the role of the inversion of the WBS-critical region in the origin of the deletion

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Williams-Beuren syndrome (WBS), characterized by craniofacial dysmorphisms, developmental delay, typical personality profile and cardiovascular abnormalities like supravalvular aortic stenosis (SVAS) and peripheral pulmonal stenosis (PS), is caused by a chromosomal microdeletion of an approximately 1,5 Mb-sized region in 7q11. The WBS deletion is thought to be derived from an unequal meiotic recombination. In 2001, Osborne et al. (Nat Genet, 2001) suggested that parents of children affected by the WBS have an increased likelihood to carry an inversion at the WBS locus. Other groups later confirmed these results though the numbers of patients and controls analyzed so far are comparably low.
To further investigate whether the WBS inversion polymorphism increases the risk to develop a microdeletion of the WBS locus, we examined 24 pairs of parents of children with WBS, who were recruited by cooperation with the Bundesverband Williams-Beuren-Syndrom e.V. The control group included 51 set of parents, the majority (35/51) with at least 2 healthy children. 3-color interphase FISH was applied to detect the inversion. In the group of parents of WBS patients, in 21% of the pairs a carrier of the inversion polymorphism of the WBS locus was detected. This frequency closely resembles that described in the literature (Osborne et al., Nature Genetics, 2001: 33%; Bayés et al., Am. J. Hum. Genetics, 2003: 28%). Interestingly, also in the control in 14% of the couples the inversion of the WBS critical region was detected in one parent. There was no significant difference between the frequencies of the inversion in parents of children with WBS as compared to the controls. Furthermore, in one patient with WBS, we could show that the WBS deletion did not originate from the parent with the inversion of the WBS region. Our results suggest that the inversion polymorphism of the WBS locus does not play that an important predisposing role as assumed.

P086

Pallister-Killian syndrome (PKS): 4 cases of pre- and postnatal cytogenetic diagnostics

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PKS is a rare sporadic disorder first described by Pallister (1977) and Killian, Teschler-Nicola (1981), first prenatal case – by Gilgenkrantz et al. (1985) The syndrome is clinically recognizable, phenotype features include profound mental retardation, seizures, abnormal pigmentation, facial anomalies are characterized as "coarse" face. The main ultrasound prenatal indicators are hydramnios, congenital diaphragmatic hernia, and micromelia of a predominantly rhizomelic type.
Cytogenetically PKS is specified as mosaic tetrasomy due to isochromosome 12p, the mosaic status is characterized as tissue-limited mosaicism: fibroblasts have cell clone with 47 chromosomes including the extra supernumerary marker chromosome (SMC) and normal clone.
4 PKS cases using conventional cytogenetic techniques and FISH (WCP, locus specific probes, M-FISH, MCB, cen M-FISH) are presented. We report girl 2 2/12 years old with mitotically stable neocentromeric SMC (peripheral blood lymphocytes) and 3 prenatal cases with age of gestation 12 weeks (skin fibroblasts postmortem), 18 weeks (amniocytes), 20 weeks (amniocytes).
Cytogenetic diagnostics of PKS is still problematic. Following circumstances should be in the focus of attention during prenatal and postnatal conventional/FISH karyotyping:
- tissue-limited mosaicism
- karyotype of lymphocytes is usually normal, but presence in lymphocytes of SMC derived from 12p was discovered in number of cases
- involving of various segments of 12p in SMC formation: common i(12)(p10); cases with analphoid neocentromeric SMC
- cell clones with variable degree of 12p imbalance: tetrasomy, trisomy
- difficulties of GTG-discriminating between the supernumerary isochromosome 12p and the duplication 21q
- variable size of mosaic cell clones
- loss of the i(12p) in the course of amniocyte subculturing in some of PKS cases.

Summarized points are substantial for standard karyotyping and FISH interpretation.
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P087

Molecular cytogenetic investigation of a balanced complex chromosomal rearrangement involving chromosomes 2, 4, 14, 15 in a pregnant woman without phenotypic abnormalities

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Complex chromosomal rearrangements (CCRs) are constitutional structural rearrangements involving three or more chromosomes or having more than two breakpoints. CCRs are rare in individuals with normal phenotype. Carriers are considered to have a considerable reproductive impairment through disturbance of meiosis and a high risk for producing unbalanced gametes. Here, we report on a complex chromosomal rearrangement involving chromosomes 2, 4, 14, and 15 ascertained in a 21 years old pregnant woman without phenotypic abnormalities. Amniocentesis was carried out in the 16th week of gestation because of increased fetal nuchal translucency and conspicuous maternal serum markers. The karyotype of the fetal cells was determined by conventional and molecular cytogenetics: 46,XY,der(14) (4qter→4q31.3::14p10→14qter). Chromosome analyses of the parents revealed a normal paternal chromosome complement 46,XY and a maternal CCR: 46,XX,t (2;4;14;15) 2qter→2p11.2::15q10→15q22.1::15p10→15pter; 4pter→4q31.3::2p11.2→2pter; 14qter→14p10::4q31.3→4qter; 15pter→15q10::15q22.1→15qter). To elucidate the complexity of this CCR we used Comparative Genomic Hybridization (CGH) and Fluorescence – in situ – hybridization (FISH). We also discuss the possible segregation products and their predictive value concerning the viability of the outcomes.

P088

Habitual abortions and familial translocation t(13;18)(q12;q11.2): Synergism of different methods in prenatal analysis

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In about 50% of abortions, studied by conventional karyotyping, chromosomal aberrations are detectable. In 4–6% of the couples with abor-

tions, balanced familial translocations are found as a cause of their miscarriages. These chromosomal abnormalities are without clinical relevances for the carrier himself. However, they can cause an unbalanced translocation in the offsprings.
Translocations with acrocentric chromosomes present specific challenges for the cytogeneticists, because of frequent heterogenities in the short arm.
Here we report the results of the prenatal analyses (first trimester screening, rapid aneuploidy screening by fluorescence in-situ hybridization (interphase FISH), conventional chromosome analysis and metaphase FISH) in a 36 year old Caucasian woman. Amniocentesis was done at week 15 of gestation because of an enhanced nuchal translucency at the first trimester ultrasound. The result of the biochemical first trimester screening was unsuspicious. Interphase FISH showed three clearly separated signals indicating a free trisomy 18. Interestingly, the conventional chromosome analysis revealed a male chromosome complement unremarkable for numerical chromosome anomalies. One chromosome 13 was suspicious due to an elongated short arm.
Reflecting the interphase FISH results, we assumed, that this chromosome is a derivative chromosome 18. To verify the nature of this conspicuous chromosome, different metaphase FISH studies were performed. FISH results confirmed our assumption and showed that the observed derivative chromosome 18 was caused by a t(13;18). Therefore, the fetus showed a partial monosomy of chromosome 13 (p13->q12) and a partial trisomy of chromosome 18 (p11.3->q11.2). Chromosome analyses of the parents revealed a balanced translocation t(13;18)(q12;q11.2) in the mother which which was unidentified up to now.
As there have already been two unclear abortions so far in this couple, the results of the studies mentioned above offer the conclusion.

P089

Mosaicism for an unbalanced structural rearrangement and a normal cell line associated with infertility – a case report

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Mosaicism for an unbalanced autosomal structural rearrangement and a normal cell line is rarely reported. If the abnormal cell line contributes to gonadal tissue it can lead to reproductive failure.
We describe the case of a 30-year-old infertile woman referred to our laboratory for routine karyotyping before assisted reproductive technology. Except for treated hypothyroidism and occlusion of one fallopian tube, she was in good health. Family history was unremarkable. Analysis of GTG banded metaphases from peripheral blood lymphocytes revealed an aberrant chromosome 13 in approx. 37% of the cells. Repeated karyotyping of two different lymphocyte cultures showed the same aberrant chromosome 13 in about 7% and in about 27% of the cells respectively. After investigations by fluorescence in situ hybridization (FISH) and Multi-Colour-Banding-FISH (MCB-FISH), the karyotype 46,XX,der(13)(qter->q14.1::p11.1->q14.1::q22->qter)[16]/46,XX[44] was estimated, resulting in a mosaic partial trisomy 13q22->qter in approx. 27% of cells. FISH studies on urine epithelial cells and buccal mucosa failed.
We present results of chromosome and FISH analyses and discuss correlation with infertility.

P090

3,53 Mb de novo microdeletion Xq27.3-q28 including FMR1 and FMR2 in a girl with mental retardation, muscular hypotonia and obesity

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Patients with mental retardation (MR) of unknown etiology are increasingly analyzed by molecular karyotyping using SNP arrays and array-CGH in order to identify novel disease- causing microaberrations and related syndromes. Here, we report on a 16 10/12 year old girl with MR, muscular hypotonia, as well as obesity due to hyperphagia and a lack of feeling of satiety. She showed mild dysmorphisms, such as a long face, mild hypertelorism, flared eyebrows, a bulbous nasal tip and a prominent chin. Tapering fingers, mild joint hyperextensibility of fingers and hands as well as hypoplastic nails of the 4th and 5th toes and foreshortened appearance of the 4th and 5th toes, possibly due to shortened metacarpalia 4 and 5 were noted. Molecular karyotyping using an Illumina 550 k SNP chip detected a 3,53 Mb deletion in Xq27.3-q28. qPCR confirmed the deletion and demonstrated a de novo occurrence. The deletion contained 9 genes including FMR1 (the fragile X syndrome gene), AFF2/FMR2 and IDS. Mutations in FMR2 have been described in mentally retarded males. Loss of function-mutations of IDS (iduronate 2-sulfatase) cause Hunter disease / mucopolysaccharidosis type II.
A tentative genotype-phenotype-correlation based on the comparison of our patient to published female patients with comparable deletions will be presented. In these, both the extent of the deletion and the X-inactivation status of the deleted X chromosome influence the clinical appearance. X-inactivation analyses and FMR1-repeat analysis of our patient are in progress.
Our report confirms the importance of molecular karyotyping in the etiological research and diagnostics of mental retardation and highlights the significance of X-chromosomal copy number variants in female mental retardation patients. (AZ and EW contributed equally to this poster.)

P091

Implication of the teamwork hypothesis of the VCX/Y genes for X-linked mental retardation – a male patient with a microdeletion of the VCX-A and VCX-B1 genes in Xp22.31 and a pseudodicentric Yp chromosome with loss of the VCY genes

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It is well known, that male patients with a terminal Xp22.3 deletion suffer from mental retardation additionally to short stature and X-linked ichthyosis. This MRX locus has been mapped to the STS gene region in Xp22.31, and the VCX-A gene was considered to be responsible for this X-linked mental retardation (Fukami et al. 2000). Recently, several papers reported on the microdeletion of the STS gene region in Xp22.3 including the VCX-A gene in patients without mental retardation (Tobias et al. 2001, Lesca et al. 2005, Esch et al. 2005, Cuevas-Covarrubias & González-Huerta 2007, Mochel et al. 2008). The teamwork hypothesis of the VCX/Y genes (Lahn & Page 2000) has been put forward to explain the contrary phenotypic manifestations of VCX-A gene deletions (Esch et al. 2005). According to the teamwork hypothesis, the loss of the VCX-A gene could possibly be compensated by the othe VCX/Y genes, and may influence the phenotypic manifestation.

We report on a 10-year-old phenotypically normal male patient with mosaicism 46,X, psu dic(Y)(q11.2) (89%)/45,X (11%). In spite of the 45,X cell line (also presented in amniotic fluid cells), the patient has normal male internal and external genitalia. The main clinical findings were behavioural abnormalities, i.e. an attention-deficit-disorder and aggressive behaviour.
The breakpoint of the pseudodicentric Yp chromosome in Yq11.21 has been specified by FISH-analysis and Molecular Karyotyping using Array-CGH. Thus, the loss of the VCY and the VCY-1B genes in Yq11.221 was confirmed. In addition to the psu dic(Y)(q11.21) chromosome, we observed the common 1.9-Mb STS microdeletion in Xp22.31, including the genes VCX-A, HDHD1A, STS, and VCX-B1, but not of VCX-B and VCX-C.
We demonstrate the loss of four of the six VCX/Y genes in our patient without mental retardation. Therefore, the absence of mental retardation in our patient could obviously not be explained by the teamwork hypothesis of the VCX/Y genes.

P092

Study of 820 abortions and cytogenetic findings

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A total of 820 abortions between the 5th and 24th week of gestation were analysed by conventional chromosome analysis. For chromosome analysis, material of chorionic villi, parts of umbilical cord, facia lata and/or Achilles tendon were used.
In summary, the frequency of abnormal karyotypes was 53,2% (436). In the majority of cases, autosomal trisomies were found (67%), followed by monosomy X (11,2%), triploidies (9,2%, together with near-triploidies: 11,7%) and structural chromosome aberrations (6,4%).
Among the trisomies, trisomy 16 (24,7%), trisomy 22 (13,2%), trisomy 21 (12,1%) and trisomy 13 (10,4%) were predominant.
With advancing age the percentage of trisomies increased from 31% in women younger than 25 years to up to 80% in those 36 years and older.
Average maternal age was increased for trisomy 8, trisomy 9, trisomy 21 and trisomy 22, and was highest for trisomy 18, trisomy 15, trisomy 13 and trisomy 7, whereas no maternal age effect was observed in some chromosomal aberrations such as tetraploidy, triploidy, monosomy X and structural chromosome rearrangements.
Tetraploidies, structural aberrations, triploidies and trisomies 2, 4 and 7 have been found in early stages of aborted pregnancies.
For trisomy 21 and trisomy 18, pregnancies were aborted significantly later compared to those described before.
Interestingly, among the unbalanced structural chromosome aberrations, in 7 out of 26 cases (27%), a chromosome 18 was involved, leading to a partial deletion of 18p (11,5%) or to a deletion of 18p and a trisomy of 18q (i(18)(q10); 15,5%), being in line with the observation that 18p- is the second most common autosomal deletion syndrome found after the Cri-du-chat syndrome.
In summary, our findings are in agreement with those reported in literature. The cytogenetic analysis of abort material can be considered a rapid, comprehensive and reliable method to detect the broadest spectrum of chromosome aberrations in abortions.

P093

Deletions on chromosome 18p in three non-related patients

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The de Grouchy syndrome 1 refers to a chromosomal disorder, which results in a monosomy 18p or just a partial deletion of the short arm of chromosome 18 and has been first described in 1963. In two thirds of the cases, the 18p- syndrome occurs de novo and is mostly restricted to a terminal deletion. The residual one third is caused by a de novo translocation with loss of 18p, malsegregation of a parental translocation or inversion, or by a ringchromosome 18.The phenotypes vary widely, but the major clinical manifestation includes mental retardation, growth deficiency and facial dysmorphic features.Here we report three non-related cases with a deletion of the short arm of chromosome 18 identified by chromosome analysis, FISH (fluorescence in situ hybridization) and MLPA (Multiplex Ligation-dependent Probe Amplification). We found two deletions and one derivative chromosome 18, which resulted in an inbalance of 18p.The comparison of the clinical features of our patients with the cases previously described demonstrates the variable phenotypical expression of this syndrome.

P094

Three familial cases of 22q11.2 duplication syndrome

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We report on three families with an inherited microduplication of chromosome region 22q11.2, well-known from the microdeletion syndrome 22q11.2.
Two of the index patients presented mental retardation and a heart defect. The third patient- a fetus at 23+2 weeks of gestation- showed signs of intrauterine retardation and a VSD. Chromosome analyses yielded normal karyotypes. We suspected a microduplication in all of our patients by means of Fluorescence – in situ – Hybridization (FISH) with the TUPLE 1 microdeletion probe (Vysis) either in interphase nucleus or in both nucleus and metaphase. Using Multiplex Ligation-dependend Probe Amplification (MLPA) we confirmed the duplications in all patients (kit from MRC-Holland).
Each of our index patients owns a microduplication (~3–4 Mb). Interestingly, the father of one has a smaller microduplication than the son. It is situated at the distal end of the son's duplication. Therefore, in this family the microduplication has enlarged from one generation to the next. In the other two families the mothers and in one of them an additional brother own the same duplication as the index patient.
We present clinical data and discuss the relation with the regions of Low-Copy-Repeats (LCRs) on chromosome 22q11.2.

P095

Molecular cytogenetic analysis of three murine trophoblast stem cell lines reveals chromosomal abnormalities possibly promoting proliferation

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Trophoblast stem cells are derived from the polar trophectoderm of the preimplantation blastocyst and give rise to all trophoblast subtypes of the placenta. The derivation of mouse trophoblast stem (TS) cell lines

offers excellent possibilities to study the genetic regulation of placental development and the molecular pathogenesis of placental failure. Although research on trophoblast stem cells has significantly advanced in recent years, a detailed cytogenetic characterization of available mouse trophoblast stem cell lines has not been performed to date. Here, we set out to determine the chromosome complement of three different mouse TS cell lines (TS-Rs26, TS-G28, and TS-GFP) by whole chromosome painting (WCP) and fluorescence in situ hybridization (FISH) with gene-specific BAC probes. We detected trisomies of chromosomes 1 and 11 in both the cell lines TS-G28 and TS-GFP. In cell line TS-Rs26, we found a hypotetraploid karyotype with mosaic pentasomy of chromosome 8, trisomy of chromosome 16, and two translocation chromosomes most probably containing parts of chromosomes 3 and 9. Interestingly, metaphases with pentasomy of chromosome 8 always displayed two of the five chromosomes 8 situated close to each other, a finding suggestive of chromosome-specific somatic pairing. In cell line TS-G28, we further observed a partial trisomy of the central and distal part of chromosome 7 associated with a translocation most probably also involving chromosome 8. Since some of the chromosomes involved contain known important positive proliferative regulators of placental development (e.g. Igf2 on distal chromosome 7 and Egfr on chromosome 11), we hypothesize that the observed chromosomal changes provide selective growth advantages for TS cells and may also be a frequent feature of mouse in vivo placentation.

P096

Deletion 22q13.3 syndrome due to ringchromosome 22 - a case report

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Deletion 22q13.3 syndrome is a microdeletion syndrome characterized by neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech, and minor dysmorphic features. The prevalence of this disorder is unknown, but it is likely under-diagnosed. Males and females are equally affected. The 22q13.3 deletion syndrome can accompany a simple chromosome deletion, an unbalanced translocation or a ring chromosome. Ring chromosomes are rare cytogenetic findings. In patients with ring chromosome 22, variable clinical manifestations may be seen dependent on the amount of deleted material during ring formation.
Here, we report a case of deletion 22q13.3 syndrome due to a de novo ring chromosome 22 in a female infant with muscular hypotonia, retarded motor-/ mental development, mild zygodactyly of both feet and minor facial dysmorphism. Magnetic Resonance Imaging (MRI) showed hypoplastic corpus callosum and delayed myelinisation. All ultrasound investigations during pregnancy were normal and outcome was in terms.
Chromosome analysis was performed on peripheral lymphocytes. GTG-banded metaphase chromosomes showed a female karyotype with a ring chromosome 22: 46,XX,r(22)(p11.2~11.1q13.3). Fluorescence in situ hybridization (FISH) analyses were carried out using the LSI dual-color DiGeorge Region Probe (TUPLE1, ARSA) and the TelVysion22q subtelomeric probe (Abbott). FISH revealed intact signals for TUPLE1 (22q11.2) on both chromosomes 22, but ARSA (22q13.3) as well as TelVysion22q were absent in the ring chromosome. Multiplex Ligation-dependent Probe Amplification (MLPA) with SALSA P188 MLPA KIT 22q13 (MRC Holland) was done subsequently to determine size and extent of genetic material deleted from the ring chromosome. MLPA uncovered a deletion spanning area of approximately 6–8 Mb, including ARHGAP8 proximal and RABL2B distal to ARSA. Chromosome analyses in the parents excluded cryptic rearrangements and parental mosaicism as well.

P097

Analysis of congenital anomalies of the kidneys and urinary tract (CAKUT) using array-based comparative genomic hybridization (Array-CGH)

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are frequently associated with malformations of other organs. The etiology of maldevelopment often remains unknown. Therefore, we wanted to identify novel chromosomal regions associated with the CAKUT phenotype. We analyzed 30 unexplained CAKUT-patients with at least one additional extrarenal symptom using genome-wide array-based CGH. In 3 patients, causal imbalances were detected. Patient HD1 was affected by hypospadias, microhematuria and scrotum bipartitum, cryptorchidism, eye anomalies, cleft palate, laryngomalacia, pectus excavatum, mental retardation (MR), and agenesis of corpus callosum. Array-CGH detected a terminal loss of 0.6 Mb in chromosomal band 1q44 and a terminal gain of 6.5 Mb in 16q23.3-q24.3 shown to result from an unbalanced 1;16-translocation by karyotyping and FISH analysis (46,XY,der(1)t(1;16)(q44;q23.3)). The patient's brother, who has a similar phenotype, was shown to carry imbalances of 1q and 16q with the same breakpoints by array-CGH. The 1;16-translocation was detected in both patients' father in a balanced form using GTG-banding and FISH analysis. In patient HD16 presenting with renal hypoplasia and proximal ureteral stenosis in addition to MR, macrocephaly, atresia of the auditory canal, microtia, and multiple hypopigmented skin lesions, array-CGH detected a gain of 3.1 Mb in 1q21.1. In the patient's phenotypically normal father, a gain of 1.3 Mb in 1q21.1-q21.2 was detected involving the distal part of the patient's gain, for which benign copy number variation has been described. In patient HD24 affected by a multicystic-dysplastic kidney associated with MR, microcephaly, growth retardation, multiple joint contractions, blepharophimosis, and ptosis, array-CGH identified a loss of 11.9 Mb in 3q23-q25.1, confirmed and shown to be de novo by FISH analysis. In summary, four chromosomal regions were identified that potentially harbor genes associated with CAKUT malformations.

P098

Comparative DAZ and CDY mapping discloses recurrent rearrangements on Y chromosomes of the common chimpanzee

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Large-scale chromosomal rearrangements are thought to be a major force in primate evolution. In human, such chromosomal rearrangements were shown to occur frequently in genomic regions enriched in segmental duplications. Here, we present the results of a comparative FISH analysis involving DAZ and CDY genes on the Y chromosomes of a variety of common chimpanzee individuals. Surprisingly, our analysed chimpanzee sample revealed highly diverse results, regarding Y chromosomal location and quantity of signal copies of these two genes. Additionally, a structurally altered Y chromosome of a fertile chimpanzee was detected in our study. Our results suggest that the Y chromosome of the common chimpanzee is prone to frequent rearrangements and is more diverse than previously thought. It may be concluded that the ampliconic regions of the chimpanzee Y chromosome including DAZ and CDY present a highly variable structural organization. Thus,

care should be taken when drawing general conclusions from studies based on the Y chromosome of a single chimpanzee individual.

P099

Microdeletion 12q12q13.12: Clinical and molecular findings

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We report on a 16 year old boy with psychomotor retardation and congenital malformations. Dysmorphic features are microcephaly, hypertelorism, short nose with prominent nasal root, malalignment of teeth, myopia, coloboma lentis, pterygium colli, kyphosis, pectus excavatum, joint contracture of the elbow and clinodactyly. The child underwent surgical treatments for severe retrognatism, cleft palate, aortic valve stenosis and pes cavus. Cytogenetic analysis and molecular cytogenetic analysis for DiGeorge critical region gave normal results. No subtelomeric rearrangements were identified. To analyse interstitial copy number alterations an array-CGH analysis using a BAC-Array with a genome wide resolution of at least 1 MB was performed. An approx. 7.7 Mb deletion with breakpoints in 12q12 and 12q13.12 was identified. FISH analysis was performed for verifying the deletion region. Molecular cytogenetic analysis of lymphocytes from the parents with FISH probes assigned to the deletion region revealed normal karyotypes.
Three younger patients (age 20 months, 24 months and 10 years) with a similar microdeletion have been reported in the recent literature. The shared minimal deleted region spans approx. 4.5 Mb in 12q12q13.11. All patients showed psychomotor retardation. Growth retardation was already recognized prenatally in these cases. Common clinical features include microcephaly, cleft or high arched palate, ophthalmologic alterations, malalignment of teeth and inguinal hernia.

P100

Paternal balanced insertion (5;7) resulting in Greig cephalopolysyndactyly-contiguous gene syndrome in the son

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Greig cephalopolysyndactyly syndrome (GCPS) is characterised by craniofacial dysmorphism and polysyndactyly of hands and feet. The cause of GCPS are mutations, duplications or deletions of GLI3 in 7p13. When the deletion is large and affects additional genes, it is termed Greig cephalopolysyndactyly-contiguous gene syndrome (GCPS-CGS). Such patients show craniofacial malformations, hypertelorism, post- or preaxial polydactyly of the hands and feet as well as developmental impairment. The size of the deletion seems to correlate with the level of disability.
We report on a six years old boy with a GCPS-CGS and severe developmental delay. Facial dysmorphism comprises microcephaly with frontal bossing, hypertelorism, broad nasal tip, long philtrum and posteriorly rotated ears. The feet display a bilateral duplication of the big toes as well as a syndactyly between the 2nd and 4th toe. In addition, a heart defect and cryptorchism were noted.
Conventional chromosome analysis and fluorescence in-situ hybridization revealed a deletion comprising bands 7p13 and 7p14. Haplotype analysis determined the breakpoints between the distal markers D7S2252 and D7S2250 and the proximal markers D7S667 and D7S519 on the paternal genetic background. The size of the deletion is therefore estimated to have an extent of approximately 9 to 14 Mb.
Nearly all reported cases of GCPS-CGS result from de novo deletions. In our case, however, chromosome analyses of the parents revealed a balanced insertion of the 7p13–14 segment into the long arm of chromosome 5 in the father.

P101

Mother and daughter with karyotype: 46,X,inv(X)(p22.1q22) arr cgh delXp22.2p22.11

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A case of prenatally diagnosed interstitial deletion of the short arm from chromosom X and a pericentric inversion is presented. We received amniotic fluid from a 33 years old patient (indication: advanced maternal age). The patient and her husband were reported to be healthy, there was no consanguinity and the family history was negative. The amniotic fluid cells exhibited a X-chromosomal inversion (46,X,inv(X)(p22.1q22).The same inversion was also detected in the blood cells of the mother, but not in one of her parents. Array-CGH analysis revealed an interstitial deletion of the short arm of chromosom X of about 12 MB in the fetal cells as well as in maternal blood cells. The karyotype from mother and fetus was 46,X,inv(X)(p22.1q22) arr cgh delXp22.2p22.11. X-inactivation analysis showed that in all maternal cells the X-chromosom, carrying the inversion and deletion, was inactivated. The child has now borne healthy and the X-inactivation analysis is pending.

P102

Unusual pattern of inheritance in a family with SHOX deletion

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Background: The SHOX gene is located in the pseudoautosomal region 1 (PAR1) of both sex chromosomes. Haploinsufficiency of SHOX leads to different phenotypes ranging from isolated short stature to Léri-Weill dyschondrosteosis with short stature, mesomelia and Madelung deformity. Usually females with SHOX deletion show a more severe phenotype than males. A SHOX mutation can be located in Xp22.3 or Yp11.3 but is more frequently found on the X chromosome. We describe a family with an originally Y linked deletion of SHOX that has been transmitted from father to daughter by crossing over during meiosis.
Clinical report: The index patient is a 15-year old boy with severe short stature (5 cm<3rd centile), disproportionate shortening of the limbs and mild Madelung deformity. His father had a normal height but slight disproportionate short legs. A sister presented with marked Madelung deformity. However, the girl showed normal height which is in contrast to most published case reports.
Genetic findings: MLPA identified a complete SHOX deletion (37.3 kb) in the father, his affected son and daughter. FISH analysis was performed to characterize the deleted region on the X or Y chromosome. Metaphase spreads of the index patient and his father showed a deletion of the SHOX gene on Yp11.3. In the affected daughter FISH analysis showed the SHOX deletion in the PAR1 of the X chromosome.
Discussion: This unusual pattern of inheritance can be explained by a meiotic crossing over of the SHOX gene region between X and Y chromosomes. Due to the obligatory crossover between the sex chromosomes during male meiosis, the PAR1 is a known recombination "hot spot". In our family the recombination involving SHOX led to an uncommon transmission of a Y linked SHOX deletion in the father to an X linked SHOX deletion in the daughter. In summary, our results indicate the importance to verify MLPA detected SHOX deletions by FISH analysis to identify the involved sex chromosomes.

P103

Duplication 11p15 in a Silver-Russell syndrome patient due to a maternal translocation 11;15

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The role of 11p15 disturbances in the aetiology of Silver-Russell syndrome (SRS) is meanwhile well established: in addition to hypomethylation of the H19/IGF2 differentially methylated regions, meanwhile five patients with a duplication of maternal 11p15 material have been reported. However, all these cases were sporadic. We report on the first case of SRS carrying a maternally inherited duplication of 11p15. The patient showed the typical clinical picture of SRS including severe intrauterine and postnatal growth retardation, relative macrocephaly, a prominent forehead, a triangular face, downturned corners of the mouth and clinodactyly V. Asymmetry was not observed. Family history was negative. By molecular genetic analyses including MLPA and microsatellite typing a duplication of 11p15 was identified, cytogenetic analysis revealed an unbalanced translocation 11;15. The healthy mother carried a balanced reciprocal transolation 11;15. Thus, an increased risk for further children with SRS due to a 11p15 duplication can be delineated. Additionally, the family has a risk for offspring with a 11p15 deletion and a severe BWS phenotype. The phenotype will furthermore be influenced by haploinsufficiency of further genes in 11p15 affected by the deletion.

P104

False-negative findings on chorionic villus sampling: Report of a case of a deletion 18q

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Complete fetal-placental discordance for a structural non-mosaic chromosome rearrangement is an extremely rare finding. We report a case of a 26-year-old mother who was referred to CVS sampling because of an increased fetal nuchal translucency. Standard cytogenetic analyses showed normal female karyotype on direct chromosome preparation and cultured CVS. Quantitative PCR performed at the same time excluded a foetal trisomy 13, 18 or 21. At 36 weeks of gestation amniocentesis was performed because of a malformation of the corpus callosum in order to detect a possible subtelomeric rearrangement. Cytogenetic analysis on amniocytes revealed a female karyotype with large non-mosaic deletion in 18q: 46,XX,del(18)(q21). Pregnancy was terminated. Post-interruptionem phenotypic signs of the 18q deletion syndrome were observed. Several fetal tissues were investigated cytogenetically to confirm the prenatal findings. A normal female caryotype was found again on placental tissue, whereas caryotypes performed on skin, lung and umbilical cord showed the non-mosaic 18q deletion. Findings in all tissues were confirmed using quantitative PCR. Clinical data and a review of the literature will be presented.

P105–P153 Cancer genetics

P105

100 K single nucleotide polymorphism arrays identify genes encoding microtubule-associated proteins as recurrent targets of chromosomal aberrations in mantle cell lymphomas

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The translocation t(11;14)(q13;q32) is the genetic hallmark of mantle cell lymphoma (MCL) but is not sufficient for inducing lymphomagenesis. Here, we performed genome-wide 100K GeneChip Mapping in 26 t(11;14)-positive MCL and six MCL cell lines. We could demonstrate for the first time that partial uniparental disomy (pUPD) leads to TP53 inactivation in a primary MCL and thus might probably important for prognosis and therapy Furthermore, we identified twelve novel regions of recurrent gain and loss as well as twelve high-level amplifications and eight homozygously deleted regions hitherto undescribed in MCL. Interestingly, these findings point to different genes, encoding proteins involved in microtubule dynamics, such as MAP2, MAP6 and TP53, as targets for chromosomal aberration in MCL. Further investigation including mutation analyses, fluorescence in situ hybridisation as well as epigenetic and expression studies, revealed additional alterations frequently affecting these genes. In total, 19 of 20 MCL cases, which were subjected to genetic and epigenetic analyses and five of six MCL cell lines harboured at least one aberration in MAP2, MAP6 or TP53. Our study provides evidence that alterations of microtubule dynamics might be one of the critical events in MCL lymphomagenesis contributing to chromosomal instability.

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P106

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

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Mutations (mts) that impair RB1 gene function can cause hereditary retinoblastoma (rb). In a few families, rb is caused by mts in the promoter-region of the gene. These families can show incomplete penetrance and milder expressivity compared to families with RB1 null mts. In addition, RB1 promoter mts have been identified in isolated cases. In vitro assays can help to decide if an alternation of the promoter sequence is pathogenic or a rare variant allele (va). Moreover, it is interesting to test if any of the few promoter variants identified in cis to bona fide pathogenic mts (bystanders) have distinct regulatory activity and thus may modify tumor predisposition.
The promoter activities of vas and mts were tested with Dual Luciferase Assays in four cell lines (CHO, HeLa, HEK 293 and Y-79 Rb). Protein-DNA interactions were assayed with Electrophoretic Mobility Shift As-

says (EMSA). We tested 11 variant sequences comprising 6 oncogenic mts, 4 bystanders plus one rare va.
Five out of 6 oncogenic mts and SNP (g.1718G>A) consistently showed reduced reporter gene activities with the Dual Luciferase Assay. g.1910G>C, an oncogenic mt, showed a higher promoter activity. The bystander mts showed specific responses. With exception of c.2101C>T [p.Ala14Ala] all mutant sequences tested altered protein-DNA interaction.
For the first time our EMSA results confirmed transcription factor binding sites (TFBS) within the region harbouring vas g.1848G>T and g.1821G>A that had previously been predicted in silico only. In addition our results indicate that there is protein-DNA interaction in areas assumed not to contain TFBS (Exon1 and outside the essential promoter region). Luciferase Assay results point out clearly that the rare va g.1718G>C is functional. In all assays VA c.2118C>T [p.Pro20Leu] showed a drastically reduced promoter activity and, therefore, this appears to be an oncogenic mt.

P107

Functional analysis of genes in chromosomal regions amplified in head and neck squamous cell carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is a malignant neoplasm, which belongs to the six most common human types of cancer. Characteristic for these carcinomas are amplifications of specific chromosomal regions, e.g. 11q13, within which several known oncogenes and potential candidate genes are contained that may be involved in the development and progression of HNSCC. Comprehensive profiling of chromosomal aberrations was performed on 22 cell lines derived from patients with HNSCC by CGH on metaphase chromosomes. In 7/22 cell lines an amplification of 11q13 was detected. In further analyses the expression of the candidate genes was assessed by qRT-PCR. Within 11q13 two amplified genes were also very high expressed: CCND1 and CTTN. Gene copy number and expressions were confirmed by FISH and immunocytochemistry, respectively. Subsequent transient transfection using siRNA for the candidate genes showed a specific knockdown in the cell lines with amplification in 11q13. To analyse the functional impact of the candidate genes on progression and metastasis in HNSCC, the generation of stably transfected cell lines that currently being established will be the basis for long term studies. In these studies, the candidate genes will be permanently over expressed or knocked down and the function of these genes concerning invasion, migration and proliferation of HNSCC characterized.

P108

A novel MSH6 frame shift mutation in a family with Lynch and mismatch repair cancer syndrome

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Lynch syndrome (LS) is the most common origin of hereditary colorectal cancer caused by heterozygous germline mutations in mismatch repair (MMR) genes. Carriers of a homozygous mutation develop more severe phenotypes designated as mismatch repair cancer syndrome (MMRCS). This syndrome is characterized by childhood onset of leukaemias/ lymphomas or brain tumours, early onset of LS-associated malignancies, and phenotypic features of neurofibromatosis type 1. We report here on a novel pedigree with a homozygous germline mutation in MSH6 in a Turkish family.
The index patient, having several café-au-lait spots, developed T-cell lymphoblastic lymphoma at age 6. Following chemotherapy, she relapsed and underwent haematopoietic stem cell transplantation from a matched sibling donor. By the age of 13, she was referred for genetic counselling due to diagnosis of colorectal cancer. Her consanguineous parents reported on colorectal cancer at age 42 in a paternal uncle married to the sister of the index patient's mother, but LS or MMRCS was not yet considered.
The index patient's colon cancer showed high grade microsatellite instability (MSI). Immunohistochemistry demonstrated a loss of MSH6 both in normal and cancer cells. Homozygous germline mutation c.691delG (p.Val231TyrfsX15) causing a premature stop codon was detected in MSH6. Subsequently, heterozygous germline mutation was also found in the parents and the affected paternal uncle whose colorectal cancer displayed MSI and loss of MSH6.
We report here on a novel MSH6 mutation and provide clinical information of a further family with Lynch and mismatch repair cancer syndrome. Although LS is well known, up to now there are only few reports on MMRCS. This report further emphasizes how important it is to be aware of MMRCS in pedigrees with Lynch syndrome-associated cancer, e.g. early-onset colon cancer, and childhood leukaemia/ lymphoma and/ or brain tumour, especially in consanguineous families.

P109

Novel "hot-spots" for genetic testing for RET protooncogene in "sporadic" multiple endocrine neoplasia type 2 and medullary thyroid carcinoma

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Introduction: Medullary thyroid carcinoma (MTC) accounts for 5–10% of thyroid carcinoma. It occurs alone or as multiple endocrine neoplasia type 2 (MEN2). According to the literature the majority of MTC cases seem to be "sporadic". In about 20% of patients suffering from MTC or MEN2 germline mutations within the RET protooncogene were detected, which are inherited in an autosomal-dominant manner. Early genetic testing is very important for relatives of the affected gene carrier to detect asymptomatic gene carriers to plan thyroid management (e.g. total thyroidectomy), because of a strong genotype-phenotype correlation in these diseases. Until now genetic testing of exons 10, 11, 13, 14, 15 and 16 was recommended to identify disease-causing mutations. In addition mutations in exons 5 and 8 are known as cause of disease in some cases but are not tested routinely so far. Therefore we wanted to clarify the relevance of mutations in these exons, suspected to be novel "hot-spots" for mutations within the RET protooncogene.
Patients: Genetic testing for RET mutations was performed in 290 unrelated apparently sporadic index cases, suffering from MTC or MEN2.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification and direct sequencing of exons 5, 8, 10, 11, 13, 14, 15, and 16 including corresponding exon-intron boundaries.
Results: In 290 patients 29 heterozygous RET mutations were detected in exons 5, 8, 10, 11, 13, and 14. These mutations include four novel mutations, three of which were found in exon 5 and one in exon 8.
Conclusion: Recognition of germline mutations within the RET protooncogene is important to differentiate sporadic from hereditary MTC/ MEN2 to confirm the diagnosis and define asymptomatic gene carriers for early therapy. For this purpose genetic testing and genetic counselling for RET mutations should include exons 5 and 8 in addition to the "established hot-spots" for RET mutations.

P110

Mosaicism in hereditary non-polyposis colorectal cancer: De novo MLH1 mutation in cis to unclassified variant MLH1:c.1321G>A (p.A441T)

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Hereditary non-polyposis colorectal cancer (HNPCC) is linked to germline mutations of mismatch repair (MMR) genes. Monoallelic mutations of a MMR gene generally shows no effect on MMR function, while. MMR deficiency occurs through inactivation of the wild-type allele in tumour tissue. De novo germline mutations in MMR genes have only occasionally been reported.
We report on a 36-year-old female with rectal carcinoma, a family history of endometrial and breast cancer, and high microsatellite instability. She was found to harbour the MLH1 variant c.1321G>A (p.A441T) as well as a pathogenic mutation, c.475_476delAT, in lymphocyte DNA. Subsequently, a single cDNA fragment spanning both mutations was PCR-amplified and subcloned. Sequencing of clones led to the detection of three different alleles indicating somatic mosaicism in the individual's peripheral blood cells. The de novo mutation c.475_476delAT occurred in cis relative to c.1321G>A. Sequence analysis of DNA derived from tumour tissue displayed loss of the wildtype allele. The remaining MLH1 allele contained both c.1321G>A and c.475_476delAT.
DNA sequence variations leading to amino acid substitutions often are of uncertain relevance unless the pathologic effect is proven. The MLH1 missense alteration c.1321G>A (p.A441T) had originally been described to be pathogenic since compound heterozygosity for this mutation and an additional missense mutation in MLH1 appeared to be associated with breast cancer in the index patient and her father (Hackman et al., Nature Genetics 17, 1997). Later, an additional BRCA1 germline mutation was found in both individuals (Borg et al., Int. J. Cancer 85, 2000). Mangold and colleagues listed the substitution of the non-conserved amino acid as unspecified variant since they had found additional heterozygous pathogenic MLH1 mutations in two further individuals (Int. J. Cancer 116, 2005). Our finding of a pathogenic monoallelic postzygotical mutation event supports this evaluation.

P111

Microarray-based DNA methylation profiling of 368 cases of common subtypes of hematological neoplasms

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Aberrations in the DNA-methylation pattern are a typical hallmark of cancer, including hematological malignancies. Using the GoldenGate Cancer Panel I BeadArray (Illumina Inc.) we have here analyzed the DNA methylation status of 1,505 CpG loci in 368 cases of common subtypes of hematological neoplasms (204 B-cell, 30 T-cell and 134 myeloid tumors) and 30 normal hematopoietic controls (13 normal B-cell, 5 normal T-cell and 12 PB/BM/CD34 samples). A subset of samples has been additionally analyzed using the HumanMethylation27 DNA Analysis BeadChip allowing the analysis of 27,578 CpG loci. Lymphoid malignancies were associated with higher levels of de novo DNA methylation than myeloid malignancies. Interestingly, high levels of de novo DNA methylation seemed to be prevalent especially in germinal center derived B-cell lymphomas like diffuse large B-cell lymphoma, Burkitt lymphoma and follicular lymphoma as well as precursor lymphoid neoplasias like B- and T- lineage ALL. Furthermore, hierarchical cluster analysis indicates that although some entities tend to cluster together (e.g. myeloid tumors or T-PLL), there is a marked heterogeneity of methylation within subtypes, especially in lymphoid tumors. Besides hypermethylation was more frequent than hypomethylation in all entities analyzed but T-PLL, hypomethylation is a recurrent event in hematopoietic neoplasias. In contrast to the other hematopoietic malignancies, the number of hypomethylated genes in T-PLL clearly exceeds the number of hypermethylated genes. Between 32% and 59% of the genes suffering de novo methylation in hematological tumors were PRC2 targets in embryonic stem cells. This enrichment of PRC2 target genes was statistically significant in all hematological neoplasias studied. This might support the model that hematopoietic malignancies derive from a precursor cell with stem cell-like characteristics.

P112

3D-interphase studies of chromosomes 8 and 21 in AML patients with trisomy 8

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Trisomy 8 is the most frequent numerical chromosome aberration in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). It is often associated with other karyotypic abnormalities but also can occur as the sole abnormality. Little is known about the prognostic impact of trisomy 8 as the sole change in AML and MDS. Another frequent cytogenetic abnormality involving chromosome 8, the reciprocal translocation (8;21) usually correlates with AML M2. To analyze the position of chromosome 8 and to investigate the co-localization of chromosomes 8 and 21 in nuclei here we performed for the first time a three-dimensional interphase studies in bone marrow cells of AML cases with trisomy 8 and ALL cases with normal karyotype as control group. A combined application of multicolor banding (MCB) and suspension-fluorescence in situ hybridization (S-FISH) was done. In our study the chromosome 8 has peripheral localization in bone marrow cells. Moreover, in trisomy 8 cells two chromosomes 8 were co-located. Unlike chromosome 8, chromosome 21 took in positions more in periphery of interphase nuclei with a high probability co-localize to each other. Although t(8;21) is very specific for AML, surprisingly, we could show that one chromosome 8 and 21, each, have the tendency co-localize in bone-marrow, especially in cases with AML and ALL. In a previous study in interphase nuclei of haploid human sperm we showed that chromosomes 8 and 21 are not colocalized there, but their positioning is driven by gene density and chromosome size (Manvelyan et al., Mol Cytogenet 2008, 1:25). The obtained results are a hint for tissue specific differences of chromosome localization and co-localization.
Supported in parts by Stefan-Morsch-Stiftung, DFG (LI 820/21–1), IZKF, Dr. Robert Pfleger-Stiftung, DLR/BMBF (ARM 08/001, BLR 08/004) and DAAD (D/07/09624).

P113

Validation of two novel unbalanced whole arm-translocations in cervical smears

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Background: Persistent infection with a high risk human papillomavirus (HR-HPV) type is a prerequisite for the development of cervical

cancer (CxCa). This process is accompanied by numerous genetic alterations including chromosomal aberrations. Recently, we identified two novel unbalanced translocations, der(10;14) and der(7;21), in HPV-immortalized cells. Der(10;14) could also be detected in approximately 40% of low grade cervical intraepithelial lesions (CIN1), 80% of CIN2/3 and 60% of CxCa and der(7;21) in about 20% of CIN1, 53% of CIN2/3 and 47% of CxCa.
Objective: The aim of this project was to establish a protocol for the detection of these chromosomal translocations in cervical smears as a basis for further diagnostic evaluation.
Methods: Cervical smears from 31 patients (healthy controls (n=15), CIN1 (n=6), CIN2/3 (n=7) and CxCa (n=3)) were collected and stained according to standard procedures for "Papanicolaou" (Pap)- staining. Cytological images of the areas of interest were made and the respective XY-coordinates were recorded. Coverslips were then removed using xylene and the slides were prepared for fluorescence in situ hybridization (FISH). After interphase-FISH procedure images from the identical areas of the slides (according to XY-coordinates) were taken.
Results and conclusions: Signal distribution characteristic for the above translocations could be detected in some of the nuclei of Pap-stained dysplastic cells. Both translocations were detected in three CIN2/3 and two CxCa. Furthermore in one CIN2/3 and in one CxCa only the der(10;14) was found. None of the cytologically normal Pap-smears or normal cells in CIN or CxCa showed one of the aberrations. The diagnostic/prognostic value of these translocations for cervical cancer screening will be assessed in further studies.

P114

Expression and prognostic relevance of hedgehog (HH) signaling pathway molecules SHH, GLI1 and FOXM1 in human breast cancer

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Background: HH signaling is thought to be active in human breast cancer stem cells and its activation in human mammary stem cells increases mammosphere-initiating cell number and mammosphere size (Liu et al., Cancer Res. 2006). Three important effectors within the HH pathway are the sonic hedgehog homolog (SHH), the glioma-associated oncogene homolog 1 (GLI1) and the transcription factor forkhead box M1 (FOXM1). Clear activation of these three molecules in human breast cancer in combination with progressive tumor development would give first hints that the HH pathway is generally involved in breast tumorigenesis and/or tumor progression. Thus, new avenues for the diagnosis and treatment of human breast cancer with aberrant HH signalling could be established.
Methods: Using semiquantitative realtime PCR (RT-PCR) and immunohistochemistry (IHC) we systematically analyzed SHH, GLI1 and FOXM1 expression in human invasive breast carcinomas (n=229) and normal breast tissues (n=58). SPSS version 14.0 was used for statistical analysis.
Results: SHH, GLI1 as well as FOXM1 were clearly overexpressed in human breast cancer in comparison to normal breast tissue both on the RNA and protein level. Univariate survival analysis revealed a significant association between GLI1 protein expression and overall survival (OS) as well as recurrence free survival (RFS) of breast cancer patients (p=0.019 for OS and p=0.055 for RFS). Furthermore increased expression of SHH, GLI and FOXM1 was significantly associated to a more aggressive breast cancer phenotype.
Conclusions: Overexpression of the HH members SHH, GLI1 and FOXM1 in breast cancer tissues in association with unfavourable patient prognosis supports a role for HH signaling in the development of human breast cancer. SHH, GLI1 and FOXM1 may represent novel breast tumor markers, especially in the growing field of breast cancer stem cell research.

P115

Chromosome fragility as part of the normal chromosome structure compared to Fanconi anemia patients

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A number of diseases are caused by DNA repair defects like Ataxia telangiectasia, Bloom syndrome, Nijmegen Breakage syndrome, Rothmund-Thompson syndrome, Xeroderma pigmentosa, Werner syndrome and Fanconi Anaemia (FA). All these syndromes show enhanced chromosome fragility and an increased risk for developing malignancies. That is why they are also designated chromosome breakage syndromes. The aim of the presented work was to clarify whether such disease associated breaks could be molecular cytogenetically co-localized to common fragile sites (FS), as both apparently appear in the same regions when comparing the breaks on a cytogenetic level. Here we analyzed chromosomal breaks induced by mitomycin C in lymphocytes of two FA patients with BAC clones from already exactly mapped common FS. The first result was that in over 90% of the FA-breaks a concordance on the molecular genetic co-localization was observed. Secondly, the distribution in individual common FS compared to FA associated chromosome breaks was completely different. Nonetheless, the results support a common basis for the break formation, a result also supported by the literature (e.g. Arlt et al., 2004; Howlett et al., 2005). Furthermore, FA could be used in future to serve as a model system for studying otherwise hardly to accessible rare FS regions in more detail.
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P116

cDNA analysis in patients suspected of HNPCC

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Germline mutations in the DNA mismatch repair genes MLH1 and MSH2, but also MSH6 or PMS2 predispose for hereditary nonpolyposis colorectal cancer (HNPCC). The disease is caused by truncating mutations, deletions, missense mutations or by the disruption of the normal mRNA splicing.
We performed cDNA analyses in 4 patient cohorts suspected of HNPCC:

1. In mismatch-repair deficient, MSI-H patients without disease-causing mutation or deletion we performed LONG-RANGE-PCR of the complete cDNA of the gene affected to identify aberrant splicing. Additionally, sequencing of genomically heterozygous SNPs was performed to ensure transcription of both alleles. In 3 of 15 patients, a monoallelic expression was identified in a coding SNP indicating a genetic defect.
2. In patients with missense mutations of unclear pathogenicity we analysed if the mutation causes aberrant splicing by PCRs spanning several exons neighbouring the missense mutation, searched for aberrant bands and performed sequencing the missense mutation to test biallelic expression. 5 patients with different missense mutations in MLH1 showed abnormal splicing of the respective exon or several exons and enhanced alternative splicing products as well. These sequence changes might be located in functionally important (exonic splice enhancer or splice silencer) sites, which are difficult to identify in silico.
   Further 19 patients with missense mutations in MLH1, MSH2, and

MSH6 showed normal splicing and biallelic expression.
3. Aberrant splicing was verified in all 13 patients with predicted splice site mutations in MLH1 or MSH2.
4. We analysed the extent of nonsense-mediated-mRNA-decay in 15 patients with early truncating mutations and found a complete decay in only 3 patients, a reduction in 4 patients and no decay of the mutated allele in 8 patients.

To discriminate alternative splicing from aberrant splicing due to genetic defects we analysed cDNA in a control population.

P117

Silencing of BRCA1 in fibroblasts with numerical gonosome aberrations and its effect on X-chromosomal genes

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It had been published by some groups that BRCA1 communicates with the inactive X chromosome, although a direct interaction with Xist has been disproven by other groups. Their studies were performed in cells of ovarian or breast cancer tissues and thus, might display a final stage of X chromosome regulation. Therefore, we used fibroblasts with numerical X chromosome aberrations to study epigenetic and genetic changes of active and inactive X chromosomes. By RNA interference we switched off the BRCA1 protein temporarily. DNA was extracted for methylation studies, RNA for realtime RT-PCR measurements. We will present data about expression levels of X-chromosomal genes. Some of the analyzed genes include genes that escape X inactivation usually and some genes undergoing X inactivation. Additionally, we will present data on the methylation status of the Xist promoter region.

P118

Modulations in the repair of gamma-irradiation induced DNA double strand breaks in individuals with childhood malignancy and second cancer

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Malignancies in children are unlikely to be due to environmental hazards or unhealthy lifestyle. Instead, genetic factors may play a predominant role. Highly efficient DNA repair systems are needed to protect the genome against continuous DNA damage from exogenous and endogenous sources. To elucidate the role of insufficient or limited DNA repair in the development of childhood malignancy and second cancer, we have analyzed the DNA repair capacity and kinetics in primary fibroblasts of 20 individuals who survived a childhood malignancy and then developed a second cancer as well as of 20 carefully matched persons with a childhood cancer who did not develop a second malignancy. DNA damage was induced by gamma-irradiation, UV-C, and mitomycin C treatment. DNA double strand breaks were quantified by immunofluoresent staining with anti-gammaH2AX antibodies (using the half-automated Metafer4 system) in at least 100 nuclei each at different time points (30 min, 4 h, and 24 h) after irradiation with a dose of 1 Gray. The foci numbers (mean plusminus two standard deviations) in normal fibroblast strains (from patients without cancer) served as controls. So far we identified four two-cancer patients who showed a significantly increased foci number and also differences in the foci distribution at 4 h after irradiation. Our results confirm the hypothesis that modulations in DNA repair contribute to the development of second cancer after childhood tumor therapy. Expression profiling of DNA repair genes (using a customized cDNA chip) and quantitative realtime RT PCR analysis of candidate genes in irradiated cells of the four conspicuous two-cancer patients (versus matched controls) are underway to identify the underlying DNA repair defect(s).

P119

Gene network and canonical pathway analysis in hematopoietic and soft tissue originated malignancies: A microarray experience of Medical Genetics Department of Kocaeli University in 2007–2008

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Background: We performed gene expression analysis in hematopoietic tissue, ovarian cancer, prostate cancer, cervical cancer; breast cancer, endothelial cell lines, preeclampsia and HELLP sydrome, using microarray technology in University of Kocaeli.
Materials and methods: ABI (Applied Biosystems, Foster City, CA, US) and Agilent (Agilent Technologies, Palo Alto, CA) platforms were used as microarray chips. Obtained data were analysed by using GeneSpring (GeneSpring 6.1,Silicon Genetics, Redwood City, CA) and Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Mountain View, CA, USA) software programmes for gene network and canonical pathway analysis. Array results were confirmed using Quantitative Real Time PCR (LightCycler, Roche Diagnostics GmbH, Mannheim, Germany) and TaqMan® Low Density Array Human Apoptosis Panel (TaqMan®, Applera, Norwalk, U.S.A).
Results: Our results represents the first gene network analysis in Turkey. Here we define the importance of bringing samples to the microarray laboratory in safe conditions and value of RNA integrity number.
Conclusion: This technology is very useful to suggest new pathognomonic- prognostic markers and new therapeutic targets.

P120

Mapping of 10p12 aberrations in Mantle Cell Lymphoma using custom high resolution microarrays

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Structural aberrations involving the short arm of chromosome 10 are recurrent secondary changes in t(11;14)(q13;q32)-positive Mantle Cell Lymphoma (MCL). The oncogene BMI1 has been proposed to be the molecular target of these aberrations. In order to further characterize the 10p aberrations in MCL, 70 primary MCL and 5 MCL cell lines were screened by interphase FISH with seven locus-specific probes. Chromosomal breakpoints in 10p14~12 were identified in 19 samples. GeneChip mapping (Affymetrix 100 K SNP Chip platform) in 34 MCL and 6 MCL cell lines identified 11 samples with copy number changes in 10p12. Overall, the molecular cytogenetic studies identified balanced translocations, deletions, gains and high level amplifications in 10p12. Because of the heterogeneous nature of aberrations, high resolution mapping of 10p12 aberrations in MCL was performed aiming at identifying the affected genes as well as underlying pathomechanisms. We designed a customized 44 K Agilent microarray with high definition oligonucleotide probes covering the critical region at 100 bp resolution. A panel of 12 t(11;14) positive MCL carrying 10p12 aberrations and the MCL cell line REC-1 were analyzed by CGH to these arrays. A total of 10 chromosomal breakpoints could be mapped within 1 Mb distal to BMI-1. Breakpoints were not recurrent, but clustered in two hot spot regions also harboring retroviral integration sites in mouse tumor

models. In five cases, combined cytogenetic and molecular cytogenetic analyses revealed translocations involving 10p with the chromosomal regions of 1p or q, 5q14, 14q11, 14q32 and 21q11. Identification of translocation partners in remaining cases and breakpoint cloning is ongoing.

P121

Colorectal carcinoma, colon-derived liver metastasis and hepatocellular carcinoma can be discriminated by the Ca2+-binding proteins S100A6 and S100A11

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It is unknown, on the proteomic level, whether the protein patterns of tumours change during metastasis or whether markers are present that allow metastases to be allocated to a specific tumour entity. The latter is of clinical interest if the primary tumour is not known.
In this study, tissue from colon-derived liver metastases (n = 17) were classified, laser-microdissected, and analysed by ProteinChip arrays (SELDI). The resulting spectra were compared with data for primary colorectal (CRC) and hepatocellular carcinomas (HCC) from former studies.
Of 49 signals differentially expressed in primary HCC, primary CRC, and liver metastases, two were identified by immunodepletion as S100A6 and S100A11. Both proteins were precisely localized immunohistochemically in cells. S100A6 and S100A11 can discriminate significantly between the two primary tumour entities, CRC and HCC, whereas S100A6 allows the discrimination of metastases and HCC.
Both identified proteins can be used to discriminate different tumour entities. Specific markers or proteomic patterns for the metastases of different primary cancers will allow us to determine the biological characteristics of metastasis in general. It is unknown how the protein patterns of tumours change during metastasis or whether markers are present that allow metastases to be allocated to a specific tumour entity. The latter is of clinical interest if the primary tumour is not known.
Melle et al., PLoS ONE. 2008;3(12):e3767. Epub 2008

P122

Array-based DNA methylation analysis of primary lymphomas of the central nervous system

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Primary lymphomas of the central nervous system (PCNSL) are diffuse large B-cell lymphomas (DLBCL) confined to the central nervous system and are associated with a poor prognosis. It is still a matter of debate whether they differ from systemic DLBCL with respect to their molecular features and pathogenesis. Using the Illumina GoldenGate Cancer Panel I array we have analysed the DNA methylation status of 1,505 individual CpGs in 5 PCNSL. The methylation profiles of the PCNSL were compared to those recently obtained from 48 systemic DLBCL and 10 normal hematopoietic controls (Martin-Subero et al., Blood, in press). Genes known to be imprinted or gender specific methylated (X-chromosomal genes) were excluded from further the evaluation. The analysis of the remaining 1,284 CpG loci (716 genes) identified 296 CpGs (194 genes) differentially methylated in PCNSL and normal controls. Remarkably, 157 of those 194 genes were also among the 174 genes differentially methylated between systemic DLBCL and controls. In the group of genes unmethylated in controls but methylated in PCNSL we observed a highly significant enrichment of polycomb (PcG) target genes ($p<0.0001$). A differentially methylation analysis resulted in only 4 CpG differentially methylated between PCNSL and DLBCL. Using either supervised or unsupervised cluster analysis we were not able to separate systemic DLBCL from PCNSL samples. Our results on 1,284 selected CpG loci of cancer related genes do not justify the delineation of PCNSL systemic DLBCL into two independent entities on the DNA methylation level. However, the analysis of a larger number of CpG loci of the genome might provide entity specific differences.

P123

Loss of imprinting is a recurrent phenomenon in malignant lymphoma cell lines

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To determine the extent of LOI in malignant lymphomas, we used a pyrosequencing-based approach to quantify the DNA methylation in DMRs of imprinted genes in a total of 13 cell lines from classical Hodgkin lymphoma, mantle cell lymphoma, anaplastic large cell lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma and primary mediastinal B-cell lymphoma. Pooled peripheral blood genomic DNA obtained from 20 healthy donors (10 males and 10 females) and enzymatically methylated DNA acted as controls. The median methylation levels of the peripheral blood samples were 25% (GRB10), 40% (NDN), 33% (PEG 3), 42% (PLAGL1), 30% (SNRPN), 50% (IGF2). Markedly higher methylation levels at the GRB10 (7p12, range: 77–90%), NDN (15q11, range: 65–81%) and PEG3 (19q13, range: 67–80%) loci were seen in all lymphoma cell lines analysed. The same holds true for the PLAGL1 (6q24, range 65–79%) and MEST (7q32, range 80–86%) loci with the notable exception of the both mantle cell lymphoma cell lines (range 25–55%), analysed. LOI at the SNRPN locus (15q11, range 33–38%) was seen in a subset of lymphoma cell lines. Loss of methylation at the IGF2 locus (11p15, range 12–35%) was detected in all lymphoma cell lines analysed. In summary, we show extensive aberrations in the DNA methylation pattern in DMRs of imprinted genes resulting in LOI in cell lines from malignant lymphomas.

P124

Mutation spectrum in Polish juvenile polyposis syndrome patients

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Juvenile polyposis (JP) is a rare condition in children characterized by the occurrence of multiple juvenile polyps in gastrointestinal tract and associated malformations such as: porphyria, psoriasis, mental retardation, congenital heart disease, cleft lip/palate, epilepsy, hereditary haemorrhagic telangiectasia, digital clubbing. Hypertrophic pulmonary osteoarthropathy and malrotation of the gut also could be observed. JS is inherited in an autosomal dominant manner syndrome affect 1 in 100000 people. The malignant potential of juvenile polyposis in JPS patient is increased and could be ranged more then 60% in alimentary tract. We performed mutation analysis in DNA isolated from 17 Polish patients with JPS. The entire coding sequence of the BMPR1A and SMAD4 genes were studied. We used methods SSCP and HA analysis for mutation screening. The DNA fragment presenting different migration patterns on the polyacrylamide gels were sequenced by direct PCR product sequencing using MEGABace500 (GE) DNA sequencer

according to manufacturer´s instruction. In result of molecular investigations we observed five mutations and ten exonic polymorphism and intronic variations. Moreover, using the Multiplex Ligation-dependent Probe Amplification (MLPA) - method with kit P158-A1 (MCR Holland) we were able to detected five additional large mutations. Detected the genomic deletions have size ranging from one exon to two whole genes. In our study in one case we observed deletion both PTEN and BMPR1A genes.
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P125

Activating somatic mutations in FGFR2 identified in breast cancer patients

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It is known that activation of receptor tyrosine kinase signaling is one of the mechanisms underlying tumor development and growth. Recently, genome-wide association studies identified SNPs in the FGFR2 gene as major risk factor for breast cancer susceptibility. FGFR2 encodes the fibroblast growth factor receptor 2, which is a receptor tyrosine kinase that activates FGF signaling upon autophosphorylation of the receptor. Overexpression of FGFR2 is present in approximately 5–10% of breast tumors.
In our study we examined the functionally most important regions of FGFR2 encoded by 8 exons in somatic tumor tissue of 140 sporadic breast cancer patients. Furthermore, we performed MLPA analysis to detect copy number variations in FGFR2 and FGF10, encoding the main ligand of FGFR2, in breast tissue. We did not identify any copy number changes in both genes. But we identified a somatic heterozygous missense mutation in exon 16 of FGFR2, c.1980G>C, in tumor tissue of a sporadic breast cancer patient. This mutation is predicted to lead to a substitution of the highly conserved lysine in the tyrosine kinase domain at position 660 to asparagine (p.K660 N). The mutation was absent in non-tumor breast tissue of the same patient and in blood-derived DNA from the patient and 200 control individuals. Due to the same quantity of wt and mutated allele, the mutation must have occurred at a very early stage of breast cancer development. We have compared the tyrosine kinase activity of p.K660 N and another recently described somatic breast cancer mutation in FGFR2, p.R203C, after expression in HEK294T cells. We clearly demonstrated that the intrinsic tyrosine kinase activity in both mutant proteins is highly increased as compared to the tyrosine kinase activity of wild type FGFR2. To our knowledge this is the first report of functional analysis of somatic breast cancer mutations in FGFR2 providing evidence for the activating nature of identified mutation.

P126

Transcriptional regulation of the neurofibromin 1 gene

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Introduction: Only a few studies investigated regulatory elements of the Neurofibromatosis Type 1 (NF1) gene promoter. Our previous experiments[1] pointed towards a role of methylation at a defined promoter region. Hajra et al.[2] and Zou et al.[3] reported a putative repressor element close to this region. Therefore, the aim of our study was to characterize several regulatory elements and putative transcription factors (e.g. AP2, SP1) binding sites by different approaches.
Methods: Using bioinformatic tools we localized putative transcription factor binding sites in the NF1 promoter and 5'UTR. Six overlapping promoter constructs were analyzed by reporter gene assays and co-transfected with AP2- and SP1- plasmid constructs.
Results: Luciferase assays indicated a positive regulating element between +230 and +283 and a negative regulating element between +284 and +354 downstream of the transcriptional start site. Constructs which include a region from +230 to +305 showed increased luciferase activity. Furthermore, increased activities were observed by co-transfection of a 553 bp promoter construct and AP2- and SP1-constructs compared to co-transfection of the same construct and empty vectors.
Conclusion: A new positive and a new negative regulatory element 230 bp downstream of the transcriptional start site of the NF1 gene were identified. Co-transfection experiments indicated the importance of AP2 for transcriptional regulation and pointed towards direct interaction of AP2 and SP1. Ongoing experiments, using mobility shift and Footprinting assays will be used to characterize certain binding sites and elements in detail.
1. A. Harder et al., Eur J Cancer 40, 2820 (2004)
2. A. Hajra et al., Science 21, 649 (1994)
3. M. X. Zou et al., Oncogene 23, 330 (2004)

P127

Identification of the first pathogenic mutation in OGG1 in combination with a MUTYH missense mutation in a patient with synchronous colorectal cancer

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The known genes predisposing for colorectal cancer (CRC) or attenuated forms of adenomatous polyposis coli can so far not explain the genetic basis of CRC-patients and families with microsatellite stable tumors. Biallelic mutations in the base excision repair gene Mut Y homologue (MUTYH) are responsible for variable polyposis phenotypes inherited recessively. Beside MUTYH, the proteins OGG1 and MTH1 (or NUDT1) act synergistically in the repair of 7,8-dihydro-8-oxoguanine (8-oxoG).
Several studies failed to detect pathogenic mutations in MTH1 or OGG1, but found missense mutations of unknown pathogenicity in either MTH1 (p.Arg31Gln) or OGG1 (p.Arg197Trp) in four patients with additional pathogenic or unclassified heterozygous mutations in MUTYH.
To investigate the role of a defective 8-oxo-G repair we performed a germline mutation screening in the genes MUTYH, OGG1 and MTH1 in 28 patients clinically categorised as atypical polyposis coli or HNPCC type X with no microsatellite instability in their tumors and no germline mutation detectable in APC and mismatch repair genes MLH1, MSH2 and MSH6.
We describe here the first pathogenic germline mutation in OGG1 in double heterozygote status with the MUTYH missense mutation in a female patient with synchronous colon cancer at age of 36 years pointing towards digenic inheritance for CRC predisposition. Furthermore, we identified in MTH1 two missense mutations, the novel p.Asp99Asn and p.Val83Met in monoallelic status in one patient each, and detected the common missense SNP p.Ser326Cys in OGG1 in 6 patients (21%). The incidence of MUTYH mutations was 0% for biallelic, 7% for monoallelic heterozygous pathogenic mutations (2) and 18% for monoallelic heterozygous missense mutations (5) in our cohort.
Conclusion: Our findings indicate that compound heterozygosity of germline mutations in two 8-oxo-G repair genes might be the tumor-predisposing genetic background in patients with CRC but were not causing adenomatous polyposis coli.

P128

Low level somatic mosaicism in a Tuberous Sclerosis patient diagnosed by combined DHPLC and sequence analysis

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Introduction: Tuberous Sclerosis (TSC) is an autosomal dominantly inherited tumor predisposition that can lead to tumors of multiple organs (skin, heart, kidneys, lung, eyes, brain). Skin manifestations may include adenoma sebaceum, white spots, periungual fibroma, and "Chagrin" type skin alterations. TSC is caused by point mutations and larger alterations in the TSC1 and TSC2 genes. TSC mutations occurr de novo in 70% of the cases, affecting all cells or occuring in different proportions in different cell types, due to somatic mosaicism. We present a TSC patient with apparently mild (currently skin only) phenotype, but also with a fraction of mutated germ cells.
Patient and methods: The 33 yr old patient presented with clinically mild TSC with a conspicious area of "Chagrin" skin type. DNA was isolated from whole blood, Chagrin skin fibroblasts, and sperm. Mutational screening was carried out by DHPLC and sequencing of conspicious fragments.
Results: Mutational screening had been performed on blood DNA by an external laboratory with a negative result. We then performed TSC2 deletion/ duplication screening by MLPA, again with a negative result as well. In contrast, analysis of DNA from the Chagrin skin area revealed a small proportion of a causative TSC2 missense mutation c.5042C>T (p.P1675L) that was hardly detectable by DNA sequencing. Mutational analysis of sperm DNA obtained the same result, thus detecting TSC2 mutation mosaicism.
Conclusion: This TSC patient with mild skin phenotype was diagnosed as a case of somatic mosaicism with a causative TSC2 missense mutation. This finding was not only demonstrated on extracted Chagrin skin DNA but also on sperm DNA. Detection of the small proportion of mutated cells was only successful by combined DHPLC and sequence analysis. Thus, this combined methodology is most recommendable in cases conspicious for mosaicism. Due to TSC2 germ cell mosaicism this result has profound impact for genetic counselling in this patient.

P129

High resolution analysis of amplified chromosomal regions on chromsome 2p24–25 of primary human neuroblastomas using Tiling-Array-CGH

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The amplification of so called oncogenes is a common finding in many human malignancies. Detailed molecular mechanisms of gene amplification are yet unknown. To exactly map the amplified chromosomal regions around the MYCN gene on chromosome 2p24–25 in primary neuroblastoma we performed a high resolution Tiling-Array-CGH analysis. We compared the genomic region from megabase 10.000 to 22.000 on the short arm of chromosome 2 of 40 MYCN amplified neuroblastomas. Basically, we found three different types of MYCN amplicons dependent on the absence (Type 1) or the existance of short or expanded non amplified regions within the amplicon boundaries (Type 2 or 3). We could show a clinical relevance for the different amplicon types. Using conventional PCR we could confirm the array based amplicon borders. The data allow us to describe the orientation of the consecutively arranged amplified regions and furthermore, the zones of fusion of amplified regions within the individual amplicons (Type 2 and 3) in detail. All amplicons are serially arranged in head-to-tail orientation. The edge fusions of core amplicons with more distantly amplified regions were mostly found arranged in the same direction but also inversely. Bioinformatical analyses revealed a nearly normal distribution of the core amplicon borders telomeric and centromeric to MYCN. Each tumor contains individual amplicon borders. Analyses of the sequences of 10.000 bases around the amplicon borders showed an increased existence of inverted repeat formations. Conspicuously, the mean core amplicon borders are associated with a high nucleosomal occupancy and an elevated Lamin-B1 binding giving hint for epigenetic phenomena playing a role in chromosomal amplification processes.
Taken together we could describe the amplified regions of 40 primary neuroblastomas in detail. Our investigations give hints for possible sequence embedded informations and possible epigenetic links important for the amplification process.

P130

14q deletions with breakpoints in the IGH locus in CLL and related diseases

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Interstitial deletions in the long arm of chromosome 14 are frequently observed in B-cell malignancies. Interphase FISH analyses of B-cell neoplasms with an IGH Break Apart-Probe (Abbott) identified a recurrent clonal loss of the proximal IGH signal. In a subset of these cases chromosome banding analysis revealed a deletion del(14)(q21~q24q32). Array CGH in six cases with loss of the proximal IGH signal demonstrated a recurrent breakpoint in 14q24 between 68.324 kb and 68.411 kb. Using BAC- and Fosmid clones we narrowed down the breakpoint region in 14q24 to the locus containing the early response gene ZFP36L1, which is involved in RNA decay. Further interphase FISH-screening of lymphomas and leukemias with loss of the proximal IGH signal or cytogenetic aberration in 14q2 identified another 39 cases with deletion breakpoint in the ZFP36L1-region. Of the 45 cases, 34 were diagnosed as chronic lymphocytic leukemias (CLL). IGHV somatic hypermutation (>2%) was detected in 9 of 24 (37,5%) CLLs and trisomy 12 in 17 of 45 cases (37,8%). By long distance PCR we amplified the genomic deletion junctions in 16 cases. Eleven breakpoints were located in the intron and five in exon 2 of ZFP36L1. In 14q32, one breakpoint mapped to the IGH joining region, all other breakpoints mapped to the IGH constant μ or g region. In the remaining cases, we confirmed a ZFP36L1-IGH-locus fusion by FISH. Reverse transcriptase PCR on 11 cases identified four fusion transcripts from the IGH germline transcript Iμ to exon 2 of ZFP36L1. Functional assays showed Exon 2 of ZFP36L1 to be sufficient to mediate RNA decay. Neither deregulated RNA expression of ZFP36L1, nor mutations or methylation of the remaining ZFP36L1-allele were detected. The recurrence of the deletion del(14)(q24q32), the Iμ-ZFP36L1 fusion transcripts and its predominance in CLL argue for a relevance in tumorgenesis, though the molecular mechanisms remain to be elucidated.

P131

Screening for t(11;22)(q23;q11) in 300 cases of familial breast cancer reveals no evidence for a significant involvement of this most common constitutional translocation in the pathogenesis of hereditary breast cancer

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Genetic susceptibility to breast cancer is caused by defects in an obviously large number of genes. Of the known breast cancer susceptibility genes, BRCA1 and BRCA2 are the most important as they confer the highest risk of developing breast and ovarian cancer. Altogether, mutations in BRCA1, BRCA2, ATM, TP53, CHK2 and PTEN account for only 20–30% of familial cases, suggesting the involvement of additional genetic factors. The failure in identifying further high penetrance genes despite BRCA1 and BRCA2 argues for a polygenic model, in which susceptibility to breast cancer is due to several loci, each conferring a modest independent risk.
Some findings suggest an association of constitutional translocations with an increased risk of developing breast cancer. Martin et al. (PNAS 2003; 100: 11517–11522) reported on a family with early-onset breast cancer and glioblastoma, in which an inherited constitutional translocation t(11;12)(q24;q23) segregated with the disease phenotypes. A similar combination was described by Wieland et al. (Genes Chrom Cancer 2006; 45: 945–949): in a family with numerous cases of postmenopausal breast cancer in three consecutive generations and a case of glioblastoma, the translocation t(11;22)(q23;q11) was found to co-segregate.
The breakpoints of t(11;22)(q23;q11) - the most common constitutional reciprocal translocation in human - have been characterized and the identification of translocation-positive individuals can easily be done using PCR for amplification of junction-specific fragments (Kurahashi et al, 2000. Am J Hum Genet 67 : 139–140). Using this approach we screened 300 individuals with a history of familial and/or early onset breast cancer and negative BRCA1-/BRCA2-testing for the presence of t(11;22)(q23;q11). No patient was found positive for either der(11) or der(22). These results do not suggest a remarkable association of t(11;22)(q23;q11) with common hereditary breast cancer as represented by this cohort of patients.

P132

Confirmation of the cytoplasmatic localization of the thyroid adenoma associated protein (THADA) by western-blot analyses

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In benign thyroid lesions translocations involving chromosomal band 2p21 cause rearrangements of the thyroid adenoma associated gene (THADA). This results in truncation of the 3'-part of THADA and from position 1352–1954 of the corresponding protein, respectively. On the basis of analyses of the genetic structure of THADA homologous genes in different vertebrates we have shown that the truncation at position 1352 is located within a most conserved part of THADA indicating a loss of function due to truncations of THADA as observed in benign thyroid lesions with 2p21 translocations and rearrangements of THADA, respectively. Nevertheless because there is nothing known about the function of the THADA protein we have presumed an involvement in the death receptor pathyway based on preliminary unpublished results as well as similarities to protein-protein binding proteins with receptor activities and to the superfamily ARM repeat. By the use of different GFP-fusion constructs we have previously shown that THADA-A3, an alternative splice form of THADA, as well as the corresponding truncated protein THADA-A3-Fus3p are located within the cytoplasma. To approve the cytoplasmatic localization of THADA we have performed western-blot analysis with a peptide derived antiserum against THADA. Using western-blot analyses on whole cell fractions from different human cell lines we have detected an approximately 216 kDa fragment confirming the predicted ORF by Drieschner et al. (2007). Further analyses on subcellular fractions have shown that THADA is located within the cytoplasma, thus confirming the cytoplasmatic localization of THADA as obtained by GFP-fusion proteins. These results corroborates our assumption that THADA is involved in the death receptor pathway.

P133

Conventional and molecular cytogenetic analyses of the chromosomal instability in cells of Fanconi anemia patients with complementation groups FA-A, FA-E, FA-D1, FA-D2 and FA-N

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Fanconi anemia (FA) is a rare monogenic chromosome instability disorder with congenital abnormalities, a high risk of acute myeloid leukemia (AML) and solid tumors. FA can be caused by pathogenic mutations in at least 13 different genes (FANCA, B, C, D1/BRCA2, D2, E, F, G, I, J/BRIP1, L, M and N/PALB2) interacting together with others (ATM, RAD51, NBS1 etc.) in the cellular DNA damage recognition and repair network. FA-cells are characterized by spontaneous chromosomal breakage and increased sensitivity to DNA cross-linking agents such as Mitomycin C (MMC). Genetic testing for FA is routinely based on conventional chromosome breakage analyses. Mainly chromatid breaks and radial figures are counted and compared to those of normal control cells. Subtle translocations are not detectable by this test. Here, we report the results of the conventional breakage test in comparison with molecular cytogenetic analyses in cells of FA patients with different complementation groups (FA-A, FA-E, FA-D1, FA-N) and one FA-D2 patient with a reversion. Our data show a high number of translocations in the cells of FA-D1 and FA-N patients while in the cells of the other FA complementation groups mainly chromatid breaks occur. Chromosomal translocations were detected by using a semi-automated "wcp-assay" for the analysis of chromosomal aberrations in FA patients established in our lab. This assay is based on the use of whole chromosome painting probes for single chromosomes. The analyses are carried out with an automated scanning microscope and the appropriate software. Additionally, the "wcp-assay" provides us with more detailed insights into the involvement of specific chromosomes in breakage events and with further information about the mechanisms of DNA repair. One purpose of our study is to correlate these detailed instability data with the clinical data, in order to define specific risk figures for hematological complications (e.g. AML) for each FA complementation group.

P134

Acquired imatinib resistance in CML is associated with TKD mutations of the BCR-ABL fusion gene and/or additional chromosomal aberrations: Certain TKD mutations but not TP53 mutations are associated with chromosome instability

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Imatinib resistance in CML was correlated to secondary mutations in the BCR-ABL tyrosine kinase domain (TKD) in 50% of all cases. Besides,additional chromosomal abnormalities (ACA) secondary to

the Philadelphia translocation have been implicated in resistance. In this study 88 CML pts with acquired imatinib resistance were analyzed in parallel for ACAs and TKD mutations. In 40 of the 88 pts (45.5%) at least one TKD mutation was detected. Of the 40 mutated cases 6 (15%) revealed two different TKD mutations whereas in 48 pts (54.5%) no mutation was detected with a sensitivity of 10–20%. In the group not affected by TKD mutations 15 of 48 (31.3%) cases showed ACAs. In the group with resistance mutations a nearly equal number of 15/40 (37.5%) cases revealed ACAs and the spectrum of aberrations was very similar. Thus the total amount of pts with ACAs, the spectrum of ACA as well as the number of ACAs per patient is nearly equal in the mutated and the unmutated cohort. In the 40 TKD mutated cases 16 different mutations were detected. Most of the recurrent mutations are distributed equally in the groups with or without ACAs. Solely in 12 cases with M244 V, G250E or Y253H we never observed ACAs indicating that mutations within this region are strong enough to cause high resistance. In comparison 5 of 11 cases with T315I and 3 of 4 with H396R mutations are associated with multiple or complex chromosomal aberrations (p=0.014). Five pts were analyzed at 2–4 times under dose escalation of imatinib and show that ACAs can preceed TDK mutations and vice versa. TP53 was sequenced in 16 TKD mutated cases (8 with and 8 w/o ACAs) but no TP53 mutation was detected. In conclusion,

1. ACAs are equally distributed between TKD mutated and unmutated cases.
2. AA exchanges in the region 244–253 are not observed together with ACA whereas T315I and F359C are associated with multiple aberrations.
3. TP53 mutations probably do not underly the genetic changes associated with imatinib resistance.

P135

Chromosome banding and spectral karyotyping in combination with the cytogenetic data analysis system CyDAS reveals regions of recurring genome changes in osteosarcoma cell lines

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Osteosarcoma (OS) is the most common primary malignant tumor of bone. It occurs with a peak incidence in children and with a second peak incidence in adults after age 50. Almost all OS are high-grade tumors and have a poor prognosis, with 10–20% having detectable metastases at diagnosis. Remarkable progress has been made in the treatment of affected patients, leading to an overall 5-year survival rate of 50%–70%. However, the genetic background of OS is poorly understood. Multiple clones with ploidies from haploid to near hexaploid can be found by conventional cytogenetics (CC). However, CC cannot resolve the complex rearrangements which are numerous in OS. Therefore, the combination of CC with multicolor karyotyping may help to detect aberrations and chromosome rearrangements in OS.
We analysed 12 OS cell lines using chromosome banding, spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH). The frequency of gains and losses of chromosomal material as well as the distribution of the breakpoints was evaluated by using a cytogenetic data analysis system (CyDAS).
All 12 cell lines showed very complex karyotypes. The overall evaluation of all cell lines by CyDAS exhibited gains in 2211 chromosome bands, and losses in 2788 chromosome bands. Gains were found most frequently in region 11q23-q24. Common regions affected by losses were 9q22-q32 and 8p23-p12. Overall, 590 breaks were observed. Most breakpoints affected bands 8q11, 20p11, 19q11, 18p11 and 1p11.
Although OS showed highly complex karyotypes and a multitude of genetic changes, a detailed analysis using SKY and CyDAS has helped to detect regions of recurring chromosome changes. Further analyses using techniques with a higher resolution, e.g. molecular karyotyping, may help to clarify the target genes of the chromosomally detectable genome changes.

P136

Gene expression and DNA methylation analyses in primary fibroblasts of patients with childhood cancer and second cancer

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Because children are usually not exposed to environmental hazards or an unhealthy lifestyle, it is plausible to assume that genetic and epigenetic changes are the primary cause for most malignancies in children. In particular, modulation or misregulation of DNA repair- and cell cycle-associated genes may contribute to cancer predisposition. With the help of the German Childhood Cancer Registry, we have recruited 20 persons who survived a childhood malignancy and then developed a second cancer as well as 20 carefully matched persons with a childhood cancer who did not develop a second malignancy. In order to identify candidate genes, we compared the DNA repair transcriptomes of primary fibroblasts from each one- and two-cancer patient with that of a pool of healthy controls, using a customized cDNA microarray with approximately 250 DNA repair-associated genes. Bioinformatic analysis did not provide statistically significant evidence for systematic gene expression differences between groups (one-cancer versus two-cancer patients). However, in many individual cancer patients we found genes that were significantly over- or underexpressed, compared to normal controls. By microarray analysis, 55 genes were similarly misregulated in at least two cancer patients. So far we performed quantitative real time RT PCR expression analyses of 16 of these genes in all patient and control cell lines (n=60) and confirmed 5 genes that were differentially regulated in many two-cancer patients. To identify epigenetic changes which may predispose to cancer we have started to analyze the DNA methylation patterns of differentially expressed genes in cancer patients. So far we found two genes, p16 and FBN1, which appeared to be differentially methylated in normal somatic cells (primary fibroblasts, blood cells) of two-cancer patients and controls. To identify copy number variations in tumor-relevant genes we have started SNP array analyes of all two- and one-cancer patients.

P137

High-dose chemotherapy with peripheral blood stem cell transplantation for patients with multiple myeloma: Prognostic impact of chromosomal aberrations and correlation with the ISS-score

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Multiple Myeloma (MM) is a malignant lymphoproliferative B-cell disease characterized by the accumulation of monoclonal plasma cells in the bone marrow. Genomic aberrations have been shown to significantly influence response to chemotherapy and survival in MM. The aim of this study was to evaluate the clinical relevance of specific chromosomal aberrations in 315 MM patients treated with high-dose chemotherapy (HDCT) and peripheral stem cell transplantation (PBSCT). According to the international staging system (ISS) patients were classified as MM stage I (50%), stage II (34%), and stage III (16%) at the onset of chemotherapy. nterphase FISH analysis on CD138-enriched plasma cells detected gains of chromosomes 1q21 (36%), 9q34 (62%), 11q23 (48%), 15q22 (55%), and 19q13 (55%), as well as deletions of chromosomes 6q21 (11%), 8p21 (19%), 13q14 (46%), 17p13 (10%), and 22q11

(15%). Furthermore, the IgH-translocations t(4;14) and t(11;14) were observed in a frequency of 13% and 19%, respectively. For the entire group, the median overall survival (OS) and progression-free survival (PFS) after HDCT was 6.4 and 2.2 years, respectively. First, we analyzed the prognostic impact of each individual chromosomal aberration on PFS and OS. After adjustment for the ISS-score deletions of 8p21, 13q14 and 17p13, translocation t(4;14) as well as gains of 1q21, 11q23 and 19q13 preserved significant impact on PFS, while deletion 17p13, translocation t(4;14) and gain of 1q21 were of statistical significance for OS. In the multivariate analysis only deletion 17p13 and ISS-score showed significant results on OS and deletion 13q14 and gain of 1q21 were significant for PFS. In conclusion, our results show that the heterogeneity seen in the clinical course of MM patients after HDCT can be correlated with distinct chromosomal aberrations. These results may have implications for the risk-adopted management of patients with MM.

P138

Mining of a large oncogenomic data collection shows recurrence of genomic imbalance events in heterogeneous neoplasias

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Anaysis of oncogenomic screening data by Comparative Genomic Hybridization (CGH) has been successful in target gene determination and identification of clinico-pathological and prognostic subgroups. However, the analysis of large CGH datasets from heterogeneous neoplasias may lead to insights into general oncogenomic features not limited to specific disease entities.
As the largest collection of molecular-cytogenetic tumor data from CGH experiments, the „Progenetix" database contains more than 19000 individual (a)CGH profiles. Here, approx. 6000 malignant epithelial tumors were selected for evaluation of data structuring methods. Data was processed using cluster analysis of disease- and histology specific imbalance frequencies, as well as with case based marker generation and subsequent partition and tree fitting approaches.
The findings of this pilot study indicate, that

a) a limited set of genomic hot spots recurrs in different combinations;
b) average imbalance profiles relate tumor entities with overall similar histological origin (e.g. various types of squamous cell or adenocarcinomas) and
c) highly complex cases, with a presumed lack of cytogenetic maintenance, have resembling imbalance patterns with low relation to clinico-pathological grouping.

The oncogenomic resemblance of medium-aberrant entity subgroups related by gross histologic appearance, versus the self-similar patterning of highly complex cases may point towards different maturation stages in the cells of origin. One could put the hypothesis forward, that highly aberrant and frequently aggressive tumor cell clones arise from immature "tumor stem cells", while the tumors more related to certain histological types represent descendants of already tissue-commited progenitors. Additionally, the preferential "recycling" of large genomic regions in tumor imbalances may point towards architectural structures in the genome, possibly related to evolutionary rearrangement events.

P139

Subsets of pilocytic astrocytomas showing genetic alterations of high grade gliomas

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Pilocytic astrocytomas are the most frequent gliomas of childhood. The majority of tumors were analyzed by conservative cytogenetic analyses and showed normal karyotypes. Our study investigating FISH and CGH analyses revealed more genetic alterations than cytogenetic analyses. Aim of our currentl study is to analyze if the for higher grade astrocytic tumors discussed prognostic alterations of the chromosomes 7 and 10 play also a role for the different clinical behaviour in a small subset of these normally benign brain tumors usually can be cured by surgery.
Using two-color fluorescence in situ hybridization (FISH), the distribution of aneuploidies for chromosomes 7 and 10 were assessed and compared with the clinical course of the patients.
However, gains of chromosomes 7 and 10 as well as losses of these chromosomes were detected alternately in the majority of pilocytic astrocytomas. Actually, in two patients with a tumor recurrence after 5 and 2 years, respectively, the primary tumors already had gains of chromosome 7 and losses of chromosome 10, which are well-known for high-grade gliomas.
In conclusion, using high resolution molecular cytogenetic methods, pilocytic astrocytomas show more genetic alterations than revealed by classical cytogenetic analyses. Moreover, our preliminary data show that patients with tumors harboring the prognostic unfavourable genetic alterations of high grade astrocytomas may also have a more unfavourable clinical course.

P140

Differences in DNA damage repair between monozygotic twins, one with childhood malignancy and second cancer and one without cancer

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We have compared the DNA repair transcriptomes and the DNA repair kinetics in a pair of monozygotic twins; the one twin survived a childhood acute lymphatic leukemia and then developed a thyroid carcinoma, whereas the other one did not develop cancer. Because twins are endowed with the same genetic information and as children also shared the same environment, it is plausible to assume an important role of epigenetic factors for cancer development. To identify constitutively misregulated genes predisposing the one twin to cancer, we have compared the expression patterns of approximately 250 DNA repair and cell cycle associated genes in untreated primary fibroblasts, using a customized cDNA microarray. Bioinformatic analyses revealed 32 genes that were differentially expressed between the two twins. By quantitative realtime RT PCR 9 of 16 analyzed genes showed increased mRNA levels in the cancer twin compared to the control twin. To study modulations in DNA repair between twins, primary fibroblasts were exposed to gamma irradiation, UV-C and mitomycin C, respectively. Double-strand breaks were quantified at 30 min, 4 h and 24 h after induction of DNA damage by half-automated counting of the number of gamma H2AX foci. At 4 h after irradiation with a dose of 1 Gray, the cancer twin exhibited an increased foci number, compared to the unaffected twin and six health controls. Expression profiling of cells after DNA damage induction and promoter DNA methylation analyses of the misregulated genes are underway.

P141

HDAC class I, II and III inhibitors induce cellular senescence in neuroblastoma and prostate cancer cells

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Cellular senescence is a phenomenon that leads to an irreversible block of cellular division capacity and appears in both cell culture and in vivo.

The induction of an irreversible cell cycle arrest would be very useful in treatment of cancer. Thus, this mechanism could potentially be a new approach for cancer therapy. Histone deacetylases (HDACs) are considered as therapeutic targets to treat cancer patients. They are of therapeutic interest because they inhibit cancer growth and are used in various clinical trials. Reactive oxygen species (ROS) induces an irreversible senescent-like cell cycle arrest similar to senescence. Here, we analyzed whether specific inhibitors of HDAC class I, II and III induce senescence in neuroblastoma and two different human prostate cancer cell lines, an androgen-dependent and an androgen-independent. The cellular senescence status was confirmed by the measurement of the SA-β-galactosidase activity, a well-known marker for cellular senescence. Further, we characterized Senescence Associated Heterochromatin Foci (SAHFs) as a further proof for cellular senescence. We find that HDAC class I, II and III inhibitors induce cellular senescence in neuroblastoma and in both used human prostate cancer cell lines. We also show that the cell cycle arrest is not reversible. Furthermore, we find by western blot analysis that in neuroblastoma cells HDAC class I and II inhibitors down-regulate the E2F1 protein, which has a role in the control of cell cycle. However, the cell cycle inhibitor p21 protein levels seemed to be unchanged, suggesting a p53-independent pathway for cellular senescence. Interestingly, the HDAC class III Inhibitor induce cellular senescence and also ROS levels. These results indicate an association of ROS production and cellular senescence in neuroblastoma cells. The data underline that tumor cells undergo cellular senescence and irreversible cell cycle arrest which could be a new possibility for tumor suppression.

P142

Characterization of anaplastic astrocytoma by conventional cytogenetics, FISH-techniques, SNP-A karyotyping and gene expression analysis

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Astrocytic tumors comprise the largest and most common group of brain tumors. Anaplastic astrocytomas (WHO grade III), also known as malignant and high grade astrocytomas, may arise from a diffuse astrocytoma or de novo with no indication of lower-grade precursors. Data on combined cytogenetic and molecularcytogenetic analyses of anaplastic astrocytomas are rare. Therefore, we analyzed genomic alterations of five de novo anaplastic astrocytoma using high-density single nucleotide polymorphism arrays (SNP-A) combined with GTG-banding, FISH-techniques, and gene expression analysis. We found 169 structural chromosomal aberrations with highest frequency on chromosomes 1, 2, 3, 4, 10, and 12. Additionally, we unveiled loss of heterozygosity (LOH) without copy number changes in 4/5 anaplastic astrocytomas on chromosome regions 5q11.2, 5q22.1, 6q21, 7q21.11, 7q31.33, 8q11.22, 14q21.1, 17q21.31, and 17q22, suggesting partial uniparental disomy (UPD). To our knowledge, these aberrations have not been described by previous studies. UPD´s are currently considered to play an important role in the initiation and progression of different malignancies. Gene expression analyses reveal dysregulation of molecular pathways commonly observed for astrocytoma.The significance of novel genetic alterations in anaplastic astrocytoma presented here needs to be confirmed in a larger series.

P143

Allel-specific expression of the APC gene in mutation-negative patients with adenomatous polyposis

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Background: Adenomatous polyposis syndromes are characterised by multiple colorectal adenomas and a high lifetime risk of colorectal cancer. Germline mutations in two genes are involved in different forms: the autosomal dominant familial adenomatous polyposis (FAP) is caused by mutations in the APC gene and the autosomal recessive MUTYH-associated polyposis (MAP) by mutations in the MUTYH gene. However, in a substantial number of patients the genetic basis of the disease is unknown. Recent studies indicate that reduced expression of tumour suppressor genes may have pathogenic relevance.
Methods: We examined the allele-specific expression of the APC gene in unselected patients with >10 synchronous colorectal adenomas, in whom no APC or MUTYH mutation was identified, by use of a SNaPshot analysis, a primer extension method. In 31 unrelated patients and 10 normal controls who were informative for two APC polymorphisms the ratio of the peak areas of the different alleles (nucleotide "C"/"T" in codon 486 and "G"/"A" in codon 535) in cDNA relative to genomic DNA (gDNA) extracted from blood samples was determined.
Results: In controls, the median cDNA/gDNA peak ratio was 0.89 (SD ±0.13). 8/31 patients (26%) showed reduced (peak ratio ≤0.6) allelic mRNA expression, the degree of reduction ranged from 40% to 72%. In 3 patients results were inconsistent; the remaining cases had balanced expression. 6/8 cases showing reduced expression had an attenuated, 2 had a classical colorectal phenotype.
Conclusions: These findings suggest that reduced mRNA expression may be causative for the development of a polyposis in a subset of mutation-negative patients. Unbalanced allelic mRNA expression may point to cryptic germline mutations in the APC gene or associated regulatory regions that are not detectable by standard methods of mutation analysis. However, the underlying genetic mechanisms remain to be uncovered, yet.
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P144

Array-based comparative genomic hybridization profiling of diffuse astrocytomas for genomic aberrations linked to prognosis

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The clinical course of patients with diffuse astrocytomas of WHO grade II (AII) is highly variable. To identify genomic alterations possibly linked to prognosis, we screened 11 AII from patients with a long recurrence free interval (RFI) of 60–144 months (AII-long), 9 AII from patients with a short RFI of 2–25 months (AII-short), and 12 anaplastic astrocytomas (WHO grade III, AAIII) for genomic imbalances by

genome-wide array-based comparative genomic hybridization. The number of genomic imbalances was higher in AII-short as compared to AII-long, with an average of 9.7±1.6 vs. 7.5±2.4 (mean±SEM) alterations per tumor (range: 3–19 vs. 2–31), and highest in AAIII (mean±SEM: 14.3±3.3, range: 2–47). The following imbalances were identified to be frequent in AAIII and more frequent in AII-short as compared to AII-long: Gain on 7q (AAIII: 67%, AII-short: 67%, AII-long: 45%), loss on 10q (AAIII: 50%, AII-short: 44%, AII-long: 18%), loss on 14q (AAIII: 50%, AII-short: 33%, AII-long: 18%), loss on 19q (AAIII: 58%, AII-short: 44%, AII-long: 27%), gain on 20p (AAIII: 42%, AII-short: 33%, AII-long: 18%), gain on 20q (AAIII: 42%, AII-short: 22%, AII-long: 9%), and loss on 22q (AAIII: 33%, AII-short: 22%, AII-long: 0). In summary, AII from patients with short RFI seem to contain a higher average number of genomic imbalances per tumor than AII from patients with a long RFI. Furthermore, our data suggest that the presence of gains on 7q, 20p and 20q as well as losses on 10q, 14q, 19q and 22q may be linked to poor prognosis in AII. The regions of minimal gain or loss on these chromosomes will be further delineated to identify candidate genes associated with the outcome of diffuse astrocytoma patients.

P145

Variability in DNA repair – an explanation for the variable expressivity of NF 1?

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Neurofibromatosis type 1 (NF 1) is an autosomal dominantly-inherited disorder, affecting 1:3500 worldwide. NF 1 is characterized by the occurrence of multiple dermal neurofibromas, café au lait spots and lisch nodules. The tumor burden increases throughout the life of NF 1 patients and, up to now, it is impossible to give any prognosis at the time of diagnosis on the number of neurofibromas a patient will develop. NF 1 is caused by mutations in the NF 1 gene, a tumor suppressor gene located on chromosome 17. Each neurofibroma results from an individual somatic mutation in the wild type copy of the NF 1 gene in a schwann cell. The variability in the numbers of neurofibromas between different patients is enormous and reaches from only a few to thousands of neurofibromas. Therefore the interindividual variability in the number of neurofibromas may reflect interindividual variability of somatic mutation rates which may result from interindividual differences in the DNA repair capacity. As a first step, to verify this hypothesis DNA repair efficiency was tested by Comet Assay (single-cell gel electrophoresis) with fibroblasts from a small number of patients with a few and a lot of neurofibromas. Fibroblasts from NF 1 patients were irradiated with 2 Gy of Caesium 137, suspended in a thin agarose gel on a microscope slide, lysed, electrophoresed and stained with Ethidium bromide (EtBr). The increased DNA damage of the cells is indicated by the increased migration of chromosomal DNA from the nucleus. In this first series of experiments no differences in the DNA repair efficiency between the two groups of patients were found. However, to confirm these preliminary results more fibroblast cell lines of different patients will be tested in a second step.

P146

Gene blocking approach of the oncogene activator TMPRSS2 by application of peptide nucleic acids (PNA) in prostate cancer cell lines

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A high fraction of prostate cancers harbor a gene fusion between the 5‘ part of the androgen-dependent transmembrane protease TMPRSS2 and an ETS transcription factor like ERG or ETV1. Blocking the oncogenic fusion transcript could be a chance to inhibit tumour growth. In this study we chose peptide nucleid acids (PNA) for a stable inhibition of the activating fusion partner TMPRSS2. PNAs are a class of synthetic nucleic acid analogues in which the sugar phosphate backbone has been replaced by uncharged repetitive units of N-(2-amino-ethyl)-glycine.They are capable for sequence specific recognition of DNA or RNA and form thermal stable double or triple helical complexes with their target nucleic acids. Furthermore they are highly resistant to enzymatic degradation and thus are believed to degrade only very slow inside a living cell. Recent studies suggest that the gene blocking effect is based on strand invasion of genomic DNA and could be best achieved at target sites close to the transcription start. For the gene blocking approach we characterized the 5‘end of the TMPRSS2 gene by 5‘race and deletion experiments of the promoter region in a luciferase reporter gene assay. In addition to the known transcription start site (TSS_1) several alternative initiation sites were identified downstream of TSS_1. The TMPRSS2 promoter exhibited weak expression in our luciferase reporter gene assay and may depend on androgen response elements which are known to be localized ~13.5 kb upstream of TSS_1. The first two tested PNA molecules, one at TSS_1 and one ~200 bp downstream, failed gene blocking in vitro (luciferase assay) and in vivo, as measured by real time PCR of the endogeneous TMPRSS2 expression. These experiments do not exclude that further PNA target sites, or a combination of PNAs towards various transcription sites could be able to block TMPRSS2 expression.

P147

Detailed molecular cytogenetic characterization of head and neck cancer cell lines from Fanconi anemia patients using high resolution array CGH

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Fanconi anaemia (FA) is a rare inherited chromosome instability disorder with congenital abnormalities and a high risk for acute myeloid leukemia (AML) but also solid tumors. Head and neck cancers are among the most common tumors to develop in FA patients (relative risk 700-fold). The frequency, age of onset, and clinical course of these tumors are different from non-FA patients. Open questions are why FA patients develop HNSCC preferentially and so early and which role the FA/BRCA pathway plays in the initiation of HNSCC. Up to now, molecular cytogenetic and array based data for FA-HNSCC are not available in the literature.

To get deeper insights in chromosomal rearrangements in FA-HNSCC, we investigated three rare FA-HNSCC cell lines published by van Zeeburg et al. (2007) using different molecular cytogenetic and array based techniques including SKY-FISH, locus-specific interphase and metaphase FISH, conventional CGH and BAC-, SNP- and high resolution oligo arrays (NimbleGen 385 k, Agilent 244 k). Additionally, we addressed the question of gene silencing by hypermethylation using bisulfite pyrosequencing of specific CpG islands.

Our data show that most (large) chromosomal imbalances found in FA-HNSCCs are comparable to non-FA HNSCC (e.g. gain of 3q and loss of 8p). Additionally, we detected small hetero- and homozygous deletions of CSMD1 and SMAD4, of which the last gene is presumably affecting the TGF-beta-Smad signaling pathway. Two new high-level amplifications in chromosomal bands 4p13 and 11q22.3 next to breakpoints of gained and deleted chromosomal segments could be detected for the first time in FA-HNSCC. Hypermethylated regulatory sequences presumably leading to reduced gene expression or silencing could be detected for MGMT, CCND2, and p16 in FA-HNSCC.

Main goal of these analyses is to establish sensitive single cell approaches for the early detection of primary lesions in the tumorigenesis of HNSCC in Fanconi anemia patients.

P148

Amplification of the CCND2 gene in a low-grade B-cell lymphoma

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The translocation t(11;14)(q13;q32), which juxtaposes the CCND1 gene nexto to IGH locus resulting in aberrant cyclin D1 expression, is the characteristic chromosomal aberration of mantle cell lymphoma (MCL). We have recently shown, that in a minority of MCL deregulation of cyclin D2 through translocation of the CCND2 gene nexto one of the immunoglobulin (IG) gene loci can substitute for the t(11;14) (Fu et al., Blood, 2005; Gesk et al., Blood 2006). Here we provide evidence that amplification of the CCND2 might constitute a novel pathogenetic mechanism involved in lymphomagenesis. Cytogenetic reference diagnostics was performed on a peripheral blood sample in the framework of the European MCL Study in a 67-year-old patient with bone marrow infiltration by medium sized lymphoid cells with a diffuse growth pattern. By FACS, the tumor cells displayed an immunophenotype (IgM^+, $CD5^+$, $CD19^+$, $CD10^-$ and $CD23^-$) compatible with the diagnosis for MCL. Nevertheless, they lacked cyclin D1 expression by immunohistochemistry as well as the IGH-CCND1 fusion indicating t(11;14) by FISH rendering a classical MCL unlikely.Conventional R-banding analysis revealed a complexly aberrant tumor cell clone with the karyotype 45,X Y,t(1;12)(p36;q24),der(3)t(3;3)(p22;q13),der(6;8)(p10;q10)inv(6)(p12p22)del(8)(q21q23),+9,+12,dic(9;12)(p11;p13)amp(12)(p13),der(12;14)(q10;q 10)[cp20]. Subsequent FISH analyses using recently established probes for the CCND2 locus in 12p13 showed an amplification of the CCND2 gene. These findings suggest that amplification of CCND2 locus might be a rare oncogenic mechanism in lymphomagenesis.

P149

Which role plays the pseudogene POU5F1P1 on 8q24 in prostate cancer development?

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Three regions of linkage disequilibrium on 8q24 show association with an increased prostate cancer risk. The responsible variants are not yet known. Neither known is the function of the risk alleles because they are located in a so-called „gene desert". The only expressed coding sequence in this genomic area located within one of these associated regions corresponds to a pseudogene of POU5F1. Together with Sox2, Nanog, Klf4 and Myc the transcription factor POU5F1, alias Oct-4, one of the master regulators of pluripotency and self-renewal of stem cells, is proposed to have the capability to reprogram human somatic cells into pluripotent stem cells. Its pseudogene POU5F1P1 on 8q24 contains the whole coding sequence of the original gene showing a homology of 97% in the nucleotide sequence and 95% in the coded amino acid sequence missing just one amino acid.
To answer the question if the expression of POU5F1P1 has an effect for the development of prostate cancer we performed a quantitative RT-PCR. We could show a significant overexpression of POU5F1P1 in prostate cancer tissue compared to normal prostate tissue in sporadic prostate cancer patients.
To characterize the structure of the POU5F1P1 mRNA and to identify the promotors driving its expression we performed 5'RACE-experiments. The analysis resulted in the identification of a number of exons and eight different transcription start points. None of these sequences harboured a known variant associated with prostate cancer. Therefore, we started to resequence the promotor and the exonic sequence of POU5F1P1 in 95 prostate cancer patients to identify possibly functional variants.
Our results show that the pseudogene POU5F1P1 on 8q24 is expressed in prostate cancer tissue and according to the function of its mother gene may play a role in reprogramming prostate cells into prostate cancer stem cells. This could give these cells the capability of self-renewal and therefore ongoing growth for the resulting tumour.

P150

Extent of chromosomal instability in BRCA1-related breast cancer

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Introduction: BRCA1 seems to play an important role in maintaining centrosome integrity, thus triggering regular distribution of chromosomes during mitosis. The aim of this study was to determine whether there is an increased chromosomal instability in BRCA1-related compared to sporadic breast cancer that may help to identify BRCA1 mutation carriers.
Methods: Breast cancer tissues from 65 unselected sporadic tumors and from 35 tumors of BRCA1 mutation carriers were analysed by fluorescence in situ hybridization (FISH) using probes for centromeres of chromosomes 1, 7, 8 and X. Per tumor, signals from an average of 50 interphase nuclei were counted. Chromosomal instability (CIN) values were determined as percentages of cells with a nonmodal chromosome count and compared to grading, p53 and Ki67 expression (threshold >10% cells). The Wilcoxon-Mann-Whitney test was applied for statistical analysis.
Results: No difference in the extent of chromosomal instability was observed between sporadic and BRCA1-related breast cancers. The mean CIN value for all four chromosomes was 43.31 for controls and 40.97 for BRCA1 tumors (p=0.625). Regarding the distinct chromosomes, the most significant difference between BRCA1-related (CIN mean value 37.30) and sporadic breast cancer (CIN mean value 47.67) was seen for chromosome 1 (p=0.021). Comparison of G1/G2 (CIN mean value 38.78) with G3 (CIN mean value 45.48) tumors from both groups showed a significantly higher degree of CIN in high-grade G3 tumors (p=0.022), which was mainly due to chromosome 7 (CIN mean 31.09 versus 46.37, p= 0.001) and chromosome 8 (CIN mean 37.47 versus 48.28, p=0.01). No association was observed between CIN and p53 (p=0.252) or Ki67 (p=0.459) expression.
Conclusions: Unexpectedly, BRCA1-related breast cancer does not show a higher extent of chromosomal instability compared with sporadic breast cancer. We speculate that BRCA1-deficiency leads to highly instable clones that are rapidly eliminated by apoptosis.

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Heritability of DNA repair capacity in twins

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The cellular capacity of DNA damage repair can be assessed with a variety of tests which are mostly derived from mutagenicity assays. However, the relation by which genetic and environmental factors each contribute to the observed test results is hardly known. We compared DNA repair capacity of monozygotic (MZ) and dizygotic (DZ) twins to address this question. Whereas the micronucleus test (MNT) visualizes unrepaired DNA double strand breaks, the mitotic delay assay (MD)

shows changes in cell cycle arrest after DNA damage. Peripheral blood samples were obtained from 64 probands, consisting of 24 MZ and 8 DZ twin pairs.
Cytokinesis-blocked MNT was performed with spontaneous (MNT-s) or irradiated lymphocyte cultures (2 Gy of ionizing radiation at culture setup). Cells were binucleated with Cytochalasin-B after 44 h and harvested 24 h later. MN frequencies were counted with an automated system. Lymphocyte cultures for MD were irradiated after 54 hours, 18 h prior to measurement, and compared to untreated cultures for MD index calculation (G2/S ratios).
From these data, heritability could be calculated. To obtain an estimate of the upper limit we also used variance of the entire sample in addition to that of DZ. (1) For MD we found h^2=0.82 (DZ) and 0.93 (whole sample). (2) Spontaneous MN frequencies are considered to be strongly influenced by environmental factors and revealed h^2=0.26 (DZ) and h^2=0.65 (WS). (3) Induced MN frequencies are believed to be more robust towards exogenic influences, and yielded h^2=0.87 when referencing to the whole sample. In contrast, h^2=0.015 when referenced to DZ. This is caused by the unexpected low variance in 3 DZ pairs, which is presently unexplained. Albeit the yet small number of DZ pairs, our data provide strong evidence that DNA repair is genetically determined to a major part and largely independent from modifying environmental factors. This suggests that cellular DNA repair capacity may be of use as a marker of cancer risk.

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The results of different DNA repair assays are independent

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The reduced DNA repair capacity of breast cancer patients compared to healthy controls has been shown with a variety of tests. Most common were chromosome aberrations, the micronucleus (MN) test with and without induction of DNA double strand breaks by irradiation, and the „mitotic delay" (MD) which measures the accumulation of cells in G2-phase during DNA repair. Sister chromatid exchanges (SCE) imply DSB repair events and may represent a different repair pathway. They occur spontaneously and can be induced by crosslinking agents and adducts. The correlation between these measures on an individual basis is still unknown, though an association to increased cancer risk has clearly been shown for each of these repair endpoints. Thus, we performed all of these tests in parallel on the same probands.
Peripheral blood samples were obtained from 130 probands, breast/ovarian cancer patients and controls. MN test was performed with spontaneous and irradiated lymphocyte cultures (2 Gy). Cells were binucleated with Cytochalasin-B after 44 h and harvested 24 h later. Counting of MN frequencies was performed with an automated system. Samples for MD were irradiated after 54 hours, 18 hours prior to measurement, and MD index was calculated by comparision to not irradiated controls. SCE test was performed on a subsample of 35 probands. The samples were treated with Bromodeoxyuridine for SCE visualization. Induced cultures were incubated with Benzo[a]pyrenediepoxide for 30 min before culture setup.
None of the tests we applied produces correlated results, (R^2< 0.11), with the exception of basal and induced SCEs. This correlation was weak but significant (R^2=0.41 p<0.0001).
There is a strong indication that the major part of the applied DNA repair tests does indeed produce independent results. As each test has successfully been shown to discriminate between cancer patients and controls, the creation of a joint general risk index may be possible.

P153

Analysis of frequency CHEK2, P53, NOD2/CARD15 and RET gene polymorphisms in polish patients with differentiated thyroid cancer

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Thyroid carcinomas are the most often carcinomas of endocrine system with still growing up frequency. The most often occurs papillary and follicular thyroid cancer (80–90%), which belong to group of tumors well prognoses and slowly progress and benignity. Very serious problem are recurrences and regional or remote metastasis. There were observed numerous cases of osteolytic, cerebral and pulmonary metastasis. Progression from well differentiated thyroid cancer to malignant anaplastic carcinoma is possible also.
In this focus, very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment as well. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow to better diagnosing.
The dependence of differential thyroid cancer occurrence on DNA variation: I157T in CHEK2 gene, R72P in P53 gene, 1007 fs in NOD2/CARD15 gene and synonymic G2497T substitution in RET protooncogene was examined. 296 patients with differentiated thyroid cancer and 200 individuals from population group was examined. I157T and R72P variants were analyzed by pyrosequencing and 1007 fs by PCR-SSCP and DNA sequencing.
There were no significant differences in allele or genotype frequencies in analysis of RET G2497T substitution and R72P in P53 gene but mutated allele frequencies of 1007 fs and I157T was 8,95% and 4,9% in patients with thyroid cancer, compared with 2,92% and 2,1% in control individuals respectively. Our findings indicates that particular characteristics of cancer risk genes on RNA level as well as DNA changes, which may influence on transcription is necessary. Additionally a summary effect of different SNP changes as a cancer predisposing factor is possible, so further analysis will be performed.

P154–P255 Molecular and biochemical basis of disease, developmental genetics, neurogenetics

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Complete inactivation of CCM1, CCM2 or CCM3 in endothelial cells causes cerebral cavernous malformations

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Cavernous vascular malformations occur with a frequency of 1:200 and can cause recurrent headaches, seizures, and hemorrhagic stroke if located in the brain. Familial cerebral cavernous malformations (CCM) have been associated with germline mutations in CCM1/KRIT1, CCM2 or CCM3/PDCD10. For each of the three CCM genes, we here show complete localized loss of either CCM1, CCM2 or CCM3 protein expression depending on the inherited mutation. Cavernous but not adjacent normal or reactive endothelial cells of known germline mu-

tation carriers displayed immunohistochemical negativity only for the corresponding CCM protein but not for the two others. Thus, we have proven loss of function at the protein level with newly generated antibodies optimized after application of the PepSpotsTM epitope-mapping technique. Our data are also the first to demonstrate endothelial cell mosaicism within cavernous tissues and provide clear pathogenetic evidence that the endothelial cell is the cell of disease origin.
Endothelial cells lining pathological caverns only represent a small subset of cells within CCM tissue. A further challenge for molecular analyses is the fact that CCM tissue is often thrombosed, reorganized, calcified, and artificially altered by electrosurgical coagulation of the cavernous lesion itself as well as the feeding and draining vessels. To enrich for mutant cells, DNA is purified from small amounts of laser-microdissected starting material and preamplified by whole genome amplification. We will report on our progress using archival paraffin-embedded as well as frozen clinical samples.

P155

In-frame deletion paves the way for domain mapping of the cerebral cavernous malformation 3 (CCM3) protein

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Cerebral cavernous malformations (CCM) may cause recurrent headaches, seizures, and hemorrhagic stroke and have been associated with loss-of-function mutations in CCM1/KRIT1, CCM2, and CCM3/PDCD10. The CCM3/PDCD10 amino acid sequence does not reveal significant homologies to protein domains with known structure. With the help of the only published human in-frame deletion of the CCM3 gene, CCM3:p.L33_K50del, we have identified the interaction domain of CCM3 with the oxidant stress response serine/threonine kinase 25 (STK25, YSK1, SOK1) and with the mammalian Ste20-like kinase 4 (MST4, MASK). Consistently, nano-LC-MS/MS analyses revealed two STK25 phosphorylation sites at serine 39 and threonine 43. Using a novel zebrafish model of CCM, we could recapitulate the data biochemically and demonstrate that the newly mapped STK25 and MST4 interaction domain within the CCM3 protein plays a crucial role for vascular development in zebrafish.

P156

A 899 bp copy number polymorphism affects mutation detection in the human PTEN gene

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Performing MLPA-based mutation detection in the tumor suppressor gene PTEN (phosphatase and tensin homolog), we discovered an apparent deletion of exon 2 in two unrelated patients with asymmetric overgrowth, not consistent with the diagnosis Proteus syndrome. However, only the two MLPA-probes upstream of exon 2 showed a deletion, whereas the probe in the downstream intron appeared to be normal. To figure out this data inconsistency, we cloned and sequenced the deletion breakpoints from these individuals. Although unrelated, they carry the very same intronic deletion of 899 bp, 57 bp downstream of exon 2 (c.80–956_del899). Since the deleted region does not involve exon 2, nor does it contain any known features (like enhancer, small RNA coding region, splice branch site, evolutionary conserved sequence etc.) it is likely that it represents a micro-CNV without significant phenotypic effect. This assumption is supported by the fact, that each of these two individuals has inherited the deletion from a healthy parent. In order to figure out the allele frequency of this CNV, we screened 200 unrelated individuals by PCR (N = 100) or MLPA (N = 100) and found 6 additional carriers with exactly the same c.80–956_del899 variation. Thus, the estimated allele frequency is approximately 3%, at least in the German population, which has been investigated so far. The estimation of allele frequency in other populations is in progress, as well as investigations concerning the origin of this micro-CNV (founder effect vs. de-novo formation).
Besides the population genetic aspect, this CNV is clinically relevant in so far, that it may lead to incorrect results:
- false-positive results in MLPA due to the absence of the genomic region, to which two MLPA probes have been directed.
- false-negative results in exon 2 sequencing, due to "allele dropout", when the intron-based primer for exon 2 amplification does not find its binding site on the c.80–956_del899 allele of PTEN.

P157

SPG15 is a rare cause of autosomal recessive spastic paraplegia

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Background: Hereditary spastic paraplegias (HSP) are clinically and genetically highly heterogeneous. Recently two novel genes, SPG11 and SPG15, associated with autosomal recessive complicated HSP were identified. Clinically, both are characterized by a complicated and rather similar phenotype consisting of early onset HSP with cognitive deficits, thin corpus callosum (TCC), peripheral neuropathy and mild cerebellar ataxia.
Objective: To determine the frequency of SPG11 and SPG15 in patients with early onset complicated HSP and to further characterize the phenotype of SPG11 and SPG15.
Results: A sample of 36 index patients with early onset complicated HSP and a family history compatible with autosomal recessive disease was collected and screend for mutations in the spatacsin and spastizin gene. Overall frequency of SPG11 was 14% (5 out of 36) but was considerably higher in patients with TCC (42%). One single patient with mental retardation and thinning of the corpus callosum was compound heterozygous for two novel SPG15 mutations. Additionally, several new polymorphisms and sequence variants of unknown significance have been identified in the SPG15 gene.
Conclusions: At present no phenotypic features are known that allow phenotypic discrimination of SPG11 and SPG15. Therefore priority of genetic testing should be driven by mutation frequency that appears to be substantially higher in SPG11 than in SPG15. TCC seems to be the best predictor for SPG11 as well as SPG15.

P158

A distinct DNA methylation boundary in the 5´-upstream sequence of the FMR1 promoter binds nuclear proteins and is lost in fragile X syndrome

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Epigenetic mechanisms have assumed an important role in molecular biology and medicine. Current work on epigenetics is based on the findings that sequence-specific modifications of DNA by 5-methyldeoxy-cytidine and of H3 and H4 histones by acetylation or methylation lead to long-term gene silencing. Mammalian genomes carry unique patterns of DNA methylation that can be cell-type specific and inter-individually conserved. The mechanism of expansion of the naturally occurring 5´-$(CGG)_n$-3´ repeat on human chromosome Xq27.3, associated with the fragile X syndrome (FRAXA), remains unknown. In the present study, bisulfite sequencing analyses have revealed a zone of demarcation between a completely CpG-methylated and an unmethylated human genome segment in the FMR1 (fragile X mental retardation gene 1) promoter and its 5´-upstream region. This boundary lies about 650 to 800 nucleotides and 65 to 70 CpG pairs upstream of the 5´-$(CGG)_n$-3´ repeat and has been identified in human DNA of males from cell lines, primary cells, and several organs, both fetal and adult. In DNA from females and pre-mutation individuals, one chromosome carries the methylation boundary, whereas the other allele is more completely methylated, reflecting the hyper-methylated state of an inactive X chromosome. The boundary is also present in the equivalent DNA sequence from mouse brain and liver. A 630 bp human sequence spanning the methylation boundary binds specifically to nuclear protein(s) as detected in EMSA experiments. The methylation divide is lost in DNA from fibroblasts and peripheral blood mononuclear cells of FRAXA patients. This loss correlates with hyper-methylation of the FMR1 promoter in FRAXA individuals and is thought to arise through an altered chromatin structure at the boundary. This structure might be involved in the regulation of the FMR1 promoter and the stability of the CGG repeat.
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P159

Caveolin-3 mutations and their effects on crucial signaling and trafficking pathways

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Caveolins are structural and functional proteins localized in flask-shaped vesicular invaginations of the plasma membrane called Caveolae. To date the physiological functions of caveolins are poorly understood. However, they have been implicated in the internalization of receptor tyrosine kinases/growth factor receptors. Specific point mutations in caveolin-3, the isoform which is predominantly expressed in skeletal and cardiac muscle fibers, cause different muscle diseases including rippling muscle disease, limb-girdle muscular dystrophy (LGMD) 1C or hyperCKemia. Until now the pathomechanisms of these disoders are largely unknown. Therefore, we investigated how different pathogenic point mutations of caveolin-3 affected the subcellular localization and function of the protein after transient transfection into several cell lines. As has already been shown for the mutation R26Q [Carozzi et al.; (J Biol Chem., 277, 17944–9, 2002)], the amino acid changes P28L, A45T and G55S lead to an accumulation of the mutated protein in the Golgi complex. Using immunofluorescence, immunoblotting and surface biotinylation methods, we found that caveolin-3 mutations differentially alter signaling and intracellular trafficking patterns of Trk neurotrophin receptors and of the epidermal growth factor receptor. They have no apparent effect on STAT-3 signaling, which constitutes a major pathway downstream of the neurocytokines IL-6 and LIF. These data indicate that caveolin-3 mutations differentially affect the trafficking and function of receptor tyrosine kinases. These alterations might contribute to the pathogenesis of caveolinopathies.

P160

Andermann syndrome can present as hereditary motor and sensory neuropathy – clinical variability of two sibs with compound heterozygous KCC3 mutations

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Andermann syndrome (OMIM 218000) or Charlevoix disease is a rare autosomal recessive disorder characterized by agenesis of the corpus callosum, progressive motor-sensory neuropathy, mental retardation and facial features. After identification of the KCC3 (SLC12A6) gene as the underlying gene defect, mostly truncating mutations have been described. In a consanguineous Turkish family with a mild phenotype, a homozygous missense mutation was found (Uyanik et al. Neurology 2006;66:1044).
We report two sibs born to healthy unrelated parents with the initial diagnosis of demyelinating hereditary motor and sensory neuropathy (HMSN). The index patient developed distally pronounced pareses during childhood. She had a finger tremor, marked muscular hypotonia and a very shy behavior. After a slow speech development she attended a normal secondary school. There was no history of seizures, cerebral MRI was normal at the age of 7, as were hearing and vision. At 9.5 years nerve conduction studies revealed a mixed axonal-demyelinating neuropathy, sural nerve biopsy showed segmental demyelination. Extensive genetic testing for HMSN was negative.
The younger brother had a prenatally diagnosed corpus callosum agenesis, which was confirmed by MRI at 2 years. His psychomotor development was slightly retarded, muscle tone was reduced, he had hand tremor and joint laxity similar to his sister. Distal muscle atrophy was compatible with HMSN, at 5 years he developed bilateral foot deformity.
Mutation analysis of the KCC3 gene showed a compound heterozygous mutation, a paternal splice mutation c.1118+1G>A and a maternal missense mutation c.1616G>A (p.Gly539Asp) in both sibs, which were not detected in 50 controls. Our observation further broadens the phenotype of Andermann syndrome. The clinical picture in the index patient was well compatible with non-syndromic HMSN, and the correct diagnosis would not have been established without the corpus callosum agenesis in the younger brother.

P161

Do KCNQ1 and KCNE1 play a role for the etiology of Silver-Russell syndrome?

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Silver-Russell syndrome (SRS) is a heterogeneous disease which is mainly associated with intrauterine and postnatal growth retardation. The clinical diagnosis can be confirmed by detection of (epi)genetic

mutations in 11p15 (>38% of patients) as well as on chromosome 7 (~10%). The observed aberrations affect epigenetically regulated regions, supporting the relevance of imprinted genes in the etiology of SRS.
11p15 is regulated by the two imprinting control regions ICR1 and ICR2. Most of the patients show methylation defects in the ICR1 and in some cases a maternal duplication of both ICRs can be found. Recently, the first case of SRS with a maternal duplication restricted to the ICR2 was reported. Therefore, the imprinted and growth regulating genes in the ICR2 are now also discussed as SRS candidate genes. So far, other ICR2 regulated genes not involved in growth, e.g. KCNQ1 (KvLQT1), have not yet been considered to be involved in SRS.
By microarray analyses we now detected one SRS patient with a duplication in 21q22 including the complete coding region of KCNE1. This gene encodes ISK which co-assembles with KvLQT1 to form the cardiac I(Ks) channel. Loss- or gain-of-function mutations in KCNQ1 or KCNE1 can either lead to the long- or to the short-QT syndrome. Due to the identification of the two SRS patients with genomic duplications of the I(Ks) subunits we sequenced the KCNQ1 and KCNE1 genes in 10 additional SRS patients. Nevertheless, we did not detect any pathogenic variant.
Since SRS patients with 11p15 duplication do not show any obvious cardiac problems a copy number variation of KCNE1 and KCNQ1 does not seem to influence cardiac function. However, the fact that cardiac defects often become manifest in later life makes our observations relevant for future functional analyses of KCNQ1 and KCNE1. Furthermore, the investigations should be extended to genes in 11p15 that are not associated with growth to estimate their relevance for SRS.

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Spinocerebellar ataxia type 11 (SCA11) is a rare cause of dominant ataxia in European patients

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Background: At least 28 loci have been linked to autosomal dominant spinocerebellar ataxia. Causative genes have been cloned for nine nucleotide repeat expansions (SCA1,2,3,6,7,8,10,12&17) and eight genes with missense mutations (SCA4,5,11,13,14,15(16),27&28). Recently, a large British pedigree has been reported to carry mutations in the TTBK2-gene in SCA11. In order to assess the prevalence and clinical phenotypes of SCA11, we screened 148 patients with autosomal-dominant ataxias for mutations in TTBK2.
Methods: In 69 ADCA patients the complete coding sequence of the TTRK2-gene has been PCR-amplified and screened for mutations by high-resolution-melting (HRM) analysis. In a second cohort of 79 ADCA patients, functional relevant exons have been directly sequenced.
Results: In a total of 148 ADCA families we identified only one potentially disease-causing SCA11 mutation: A two basepair deletion (c.1306_1307delGA, p.D435fs448X in exon 12, leading to a premature stop codon) segregating with the cerebellar phenotype in a 3-generation family from France.
Conclusion: SCA11 is a rare cause of spinocerebellar ataxia in Caucasians accounting for less than 1% of dominant ataxias.

P163

Identification and characterization of a 6,5 kb genomic deletion in the TGFBR2 gene in a patient with Loeys-Dietz syndrome applying MLPA and oligo array-CGH

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Loeys-Dietz syndrome (LDS) is an autosomal dominant aortic-aneurysm syndrome characterized by arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate. It is caused by heterozygous mutations in the genes encoding transforming growth factor receptor β receptors 1 and 2, respectively.
We describe the molecular genetic analysis in a 32 year old patient presenting LDS typical skeletal, craniofacial and cutaneous features including bivid uvula and patent ductus arteriosus. Direct sequencing of all coding exons of the TGFBR1 and TGFBR2 gene in genomic DNA revealed no mutation. Subsequent MLPA analysis of all seven exons of the TGFBR2 gene identified a heterozygous deletion of exon 7. To verify the result and to characterize the extent of the deletion we designed a custom microarray containing more than 1500 oligonucleotides specific for the TGFBR2 gene and the adjacent region on chromosome 3p22 as well as about 14.000 oligonucleotides spread allover the human genome (8×15 k format, Agilent Technologies). Hybridization could confirm a deletion of 6,5 kb including part of intron 6 and exon 7 of the TGFBR2 gene as well as 2,5 kb of the adjacent proximal region of chromosome 3p22. To our surprise, the mother of the patient is also carrier of the deletion. She presents only slight skeletal features and kinking of the left arteria carotis and has never been diagnosed before with Loeys-Dietz syndrome. All known TGFBR2 mutations associated with connective tissue disorders are essentially missense and rarely nonsense, splice or frameshift mutations all affecting the serine-threonine kinase domain (S/T kinase D). To our knowledge this is the first report of a genomic deletion in the TGFBR2 gene in a patient with LDS affecting the S/T kinase DXI, the C-terminal cytoplasmic domain and the adjacent region. This finding emphasizes the involvement of deletion screening of the TGFBR2 gene in LDS.

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Expression analysis of C15orf2, an intronless gene in the Prader-Willi / Angelman syndrome region

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The Prader-Willi syndrome (PWS) 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. In 2000, we identified an intronless gene in this region (C15orf2), which maps between NDN and SNURF-SNRPN and encodes an 1156-amino-acid protein of unknown function. By Northern-Blot analysis we detected expression only in adult testis. Based on these findings we had suggested that C15orf2 might play a role in spermatogenesis. This notion has been substantiated by the finding that C15orf2 harbors several genes for PIWI interacting RNAs (piRNAs), which are believed to regulate spermatogenesis. Using RT-PCR, we have now detected expression of C15orf2 in a number of different tissues including fetal brain. By analysing expressed fragment-length polymorphisms in C15orf2, we found biallelic expression in adult testis but paternal only expression in fetal brain. The latter finding is compatible with a role of this gene in PWS.
So far, next to nothing is known about the protein of C15orf2. Analysis of the amino acid sequence by PSORTII, a software for the prediction of eukaryotic protein subcellular localization, revealed a possible localization of C15orf2 in the nucleus. We could confirm this prediction by

transient overexpression of FLAG-tagged C15orf2 in Hela and HEK293 cells. The nuclear localization of C15orf2 suggests that the protein may have a direct or indirect function in the regulation of gene expression.

P165

Charcot-Marie-Tooth neuropathy due to NEFL mutation: A family with mixed axonal and demyelinating neuropathy and marked myopathic changes

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Mutations in the NEFL gene encoding the neurofilament light chain (NF-L) protein cause autosomal dominant Charcot-Marie-Tooth neuropathy (CMT), which is usually classified as an axonal type (CMT2E). Here we describe a large German family with autosomal dominant CMT caused by the NEFL Glu396Lys mutation in three generations. Clinical data of eight affected family members were available. Age at onset was during childhood (1–10 years); loss of ambulation was reported in two patients at age 55 years. Clinically, proximal and distal muscles were affected and CK levels were markedly elevated with increasing age. Electrophysiology revealed a mixed axonal and demyelinating type of neuropathy. In summary, the clinical presentation in this family is relatively severe and progressive with age compared to other CMT2E families. Sural nerve biopsy analysis confirmed the mixed axonal and demyelinating type of neuropathy. There were prominent focal organelle accumulations in axons as well as foci of myelin folds. Schwann cells and perineurial cells displayed peculiar vacuolar inclusions that contained autophagic material and protruded into the nuclei. Muscle biopsies of two patients revealed a considerable number of necrotic muscle fibers and markedly increased central muscle fiber nuclei. Our data extend the phenotypes associated with NEFL mutations and suggest that not only axons, but also Schwann cells and skeletal muscle fibers are primary disease targets. These observations are in line with previous experimental studies showing that the NF-L protein is involved in Schwann cell and skeletal muscle fiber development.

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Cleavage of htt by caspase-3 and -6 in the Huntington's disease transgenic rat model

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Huntington's disease (HD) is a polyglutamine disorder based on an expanded CAG triplet repeat leading to cerebral and striatal neurodegeneration. The clinical triad of motor dysfunction, cognitive decline, and psychiatric manifestations characterizes HD.We have recently generated a transgenic rat model of HD expressing a truncated htt (exons 1–14) with 51 CAG repeats. This rat model closely resembles the human HD phenotype exhibiting emotional disturbance, motor deficits and cognitive decline. Proteolysis of mutant huntingtin is crucial to the development of Huntington disease. The group of Michael Hayden generated YAC mice expressing caspase-3- and caspase-6-resistant mutant htt (Graham et al. (2006), Cell 125:1179). Mice expressing mutant caspase-6-resistant htt maintain normal neuronal function and do not develop striatal neurodegeneration. In contrast, preventing caspase-3 cleavage of mhtt provides no protection from striatal atrophy in vivo.
To investigate the cleavage of htt in the transgenic rat model we performed western blot of brain lysates using N-terminal anti-htt and anti-polyglutamine antibodies. As expected, endogenous and transgenic (mutant) rat htt is cleaved by both recombinant caspase-3 and caspase-6. However, the detection of mutant htt is difficult due of its low expression and high background using N-terminal anti-htt antibodies. We just started to use the neoepitope antibodies recognizing htt cleaved by caspase-3 (htt552) or caspase-6 (htt586).
Currently we investigate the toxicity of mutant rat htt resistant to cleavage by caspase-3 and caspase-6, the subcellular distribution of caspase-3 and –6 cleaved rat htt and the age-dependent expression of apoptosis-related genes in brains of tgHD rats.
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In-vitro analysis of LIPH mutations causing hypotrichosis simplex: Evidence confirming the role of lipase H and lysophosphatidic acid in hair growth

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Hypotrichosis simplex (HS, MIM 146520, MIM 605389 and MIM 611452) is a rare form of isolated hypotrichosis that can be inherited in either an autosomal dominant or autosomal recessive mode. Hair loss usually begins in early childhood, and is diffuse and progressive. The extent of hair loss on the scalp and body is variable and ranges from slight hair loss to complete baldness. HS affects men and women equally. Mutations in LIPH, which encodes lipase member H, have recently been shown to cause an autosomal recessive form of HS (Kazantseva et al. Science 2006). Further genes identified to cause autosomal recessive HS include DSG4 (Kljuic et al. Cell 2003) and P2RY5 (Pasternack et al. Nature Genet 2008).
Here we describe the first Central European (Austrian) patient with HS to be found to carry compound heterozygous mutations in the LIPH gene: a 7-base pair frameshift duplication (c.403_409dup; p.Gln137HisfsX1), and a recently reported 30-amino acid in-frame duplication (c.280_369dup; p.Gly94_Lys123dup).
We recently suggested that LIPH, its product lysophosphatidic acid (LPA) or a closely related analogue, formed from phosphatidic acid (PA), and the newly identified G protein-coupled receptor for LPA, p2y5, act in the same pathway to control hair growth in humans (Pasternack et al. 2008). On the basis of this finding, we designed the first bio-assay to measure LPA production by LIPH via cells that express the recombinant p2y5 receptor.
Both the 7-basepair duplication frameshift mutation and all known in frame mutations were observed to reduce the in-vitro activity of the lipase in response to the addition of PA, the substrate of lipase H. The reduced production of LPA led to a reduced response of cells expressing the human p2y5 receptor. Our study increases the spectrum of known LIPH mutations and provides biochemical evidence for the important role of lipase H and its product LPA in human hair growth.

P168

Mutation analysis of the heme-oxygenase-1 gene in pre-eclampsia patients with a family history of hypertension in pregnancy

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Heme oxygenase (HO) is the rate-limiting enzyme in the degradation of heme to biliverdin, releasing free iron and carbon monoxide. It is important for placental angiogenesis and the utero-placental hemodynamic control. Recently, it was demonstrated that HO-1 is able to reduce the release of the two anti-angiogenic factors soluble Endoglin (sEng) and soluble Flt-1 (sFlt-1) which are both elevated in the serum of women with pre-eclampsia, from endothelial cells and pre-clamptic placental villous explants. A possible regulatory function of HO-1 in the pathogenesis of pre-eclampsia can therefore be assumed. As pre-eclamptic disorders have a clear genetic component we performed a mutation analysis of the HO-1 gene in pre-eclampsia patients with a family history of hypertension in pregnancy. In 38 index patients, the promoter region, the whole coding region and the intron/ exon boundaries of the HO-1 gene were screened for mutations by direct sequencing. No pathogenic variants were detected but we observed seven single nucelotide polymorphisms, three of them were novel: two (-156T>C and-413A>T) are located in the promoter region of the HO-1 gene, the third nucleotide exchange (99G>C) was the only variant observed in the coding region leading to an amino acid exchange of Histidine for Aspartate. The other previously described polymorphisms we observed were intronic. Allelic frequencies in pre-eclampsia patients were not significantly different compared to healthy controls for any of the polymorphisms. Based on our results we conclude that variants in the HO-1 gene do not play a relevant role in the etiology of pre-eclampsia.

P169

Analyises of the BORIS gene in Silver-Russell syndrome patients

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Silver-Russell Syndrome (SRS) is a heterogeneous disease associated with intrauterine and postnatal growth retardation, skeletal asymmetry and facial dysmorphisms. In 7–10% of patients maternal uniparental disomy for chromosome 7 can be observed, nearly 40% of patients carry epimutation resulting in hypomethylation of the imprinting center region 1 (ICR1) in 11p15.5. This still leaves 40% patients with unknown genetic aetiology. Based on the observation that the CTCF homologue BORIS is involved in imprinted genes expression and that it binds to methylated alleles we assumed that loss-of-function mutations in BORIS might have similar functional consequences as a ICR1 hypomethylation. Aim of our study was therefore to identify BORIS gene mutations in 10 patients with SRS features but unknown genetic aetiology. Mutation analyses revealed five genomic variants but pathogenic mutations were not observed. Thus we conclude that alterations of the BORIS gene are probably not associated with SRS.

P170

Six novel mutations identified in a cohort of 21 patients by direct sequencing of the SLC3A1 and SLC7A9 cystinuria genes

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Cystinuria (OMIM 220100) is an disorder of transport of the dibasic amino acids and cystine in the proximal renal tubule and in the epithelial cells of the gastrointestinal tract. We analysed a cohort of 21 unrelated subjects clinically diagnosed as affected by cystinuria on the basis of stone formation, prevalently of German origin. Analysis of all coding regions and exon-intron boundaries of the SLC3A1 and SLC7A9 genes by direct sequencing allowed us to identify 25 different mutations in 19 of the 21 patients accounting for 34 of all affected chromosomes. Six of the 25 are novel mutations, four in SLC3A1 and two in SLC7A9. Interestingly, two of our patients carried three mutations each, two patients were mixed heterozygous for SLC3A1 and SLC7A9 variants. In summary, these findings expand the spectrum of SLC3A1 and SLC7A9 mutations and confirm the heterogeneity and complexity of cystinuria. Assuming an autosomal recessive inheritance of the disease, our detection rate was 81% and thereby relatively high in comparison to other studies; nevertheless we have to consider that at least SLC7A9 mutations are often dominant, we therefore think that our effective detection rate is higher.

P171

Nonsense may make sense: Modifying effect of nonsense-mediated decay in recessive polycystic kidney disease (ARPKD)

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Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in the PKHD1 gene. The longest open reading frame comprises 66 exons encoding a 4,074 aa type I membrane protein called Polyductin or Fibrocystin. Functional investigations in PKHD1 are considerably hampered by the large size and the restricted expression pattern of PKHD1/Polyductin, e.g., it is not expressed in tissues that are usually available for analysis (lymphocytes and/or fibroblasts). In a large number of more than 300 analysed ARPKD families, strong and clear-cut genotype-phenotype correlations have been drawn for the type of PKHD1 mutation. So far, all patients carrying two truncating mutations showed peri- or neonatal demise. Interestingly and in contrast to this genotype-phenotype correlation, we could recently identify four unrelated, only moderately affected ARPKD patients with homozygous PKHD1 mutations expected to lead to premature termination of translation. To unravel the mechanisms that underlie this obvious contradiction, we analysed these patients by bioinformatics, minigene-experiments, Real-Time PCR and Western blotting on transcript and protein level. In all cases, we could detect alterations on transcript level which may indicate that nonsense-mediated mRNA decay (NMD) play a crucial role in defining the phenotype of ARPKD patients. A plausible explanation for the surprisingly mild/moderate phenotype of these patients might be circumvention of NMD. In line, it can be hypothesized that a shortened Polyductin/Fibrocystin isoform with residual protein function is translated.

P172

Disruption of the CNTNAP2 gene and additional structural genome changes in a boy with autism spectrum disorder

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In a boy with mild facial dysmorphisms and autism spectrum disorder (ASD) we elucidated a very complex chromosomal rearrangement involving at least three breaks on chromosome 1 and 7 breaks on chromosome 7. Chromosome banding revealed an inversion of region 7q31.33–7q35 on the derivative chromosome 7. FISH with region-specific BACs mapped the proximal inversion breakpoint to 125.5–125.7 Mb and the distal inversion breakpoint to 144.2–144.4 Mb. Additional breaks and structural changes were observed in the immediately adjacent CNTNAP2 gene. This is one of the largest genes (2.3 Mb, 24 exons) in the human genome that extends from nt 145,444,902 to nt 147.749,019. Two gene segments, the first one containing exon 1 and intron 1 and the second one containing intron 8 to intron 12 were transposed and inserted into a gene-poor region (191–194 Mb) on chromosome 1q31.2. The CNTNAP2 sequence between the two transposed segment as well as intron 13 to the 5-UTR were retained on the der(7). SNP array analysis revealed an additional de novo deletion on chromosome 7q35 containing exon 2 of CNTNAP2, another de novo deletion on chromosome 1q44 (213–219 Mb, containing 16 annotated genes, including KCNK2 and USH2A), and a maternally inherited duplication on chromosome 1p32.3. By FISH, the 1q31.2 insertion and the 1q44 deletion mapped to the same chromosome. Since in both de novo deletions the maternal SNPs were retained, we presume that this complex rearrangement, disrupting CNTNAP2, has occurred in the paternal germline. The CNTNAP2 gene has been associated with ASD and the Gilles de la Tourette syndrome (GTS). We compare the disruption of the CNTNAP2 gene in our patient with the one reported for a patient with GTS, with the one in a patient with ASD, and with those in healthy individuals and suggest a mechanism to explain the phenotypic differences among these carriers of CNTNAP2 disruptions.

P173

Novel mutations in the smooth muscle cell alpha actin gene associated with thoracic aortic aneurysms/dissections

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Objective: Mutations in the gene encoding smooth muscle cell alpha actin (ACTA2) have recently been shown to cause familial thoracic aortic aneurysms leading to type A dissections (TAAD). Today, only 8 missense mutations are listed in the human gene mutation database (professional release 2008.4). The present study was aimed at exploring further genetic variations causing this life-threatening disease.

Subjects and methods: Resequencing of the ACTA2 gene was performed for 40 patients with TAAD (with [21] or without [19] suspected Marfan Syndrome, MFS) that had previously tested negative for mutations in the genes FBN1 (fibrillin-1) and TGFBR2 (transforming growth factor beta receptor type II). Peripheral blood DNA served as the template for PCR amplification of all 9 exons and intron flanks prior to conventional capillary sequencing of both DNA strands using the Beckman platform.

Results: Three previously unknown heterozygous missense mutations were detected, c.115C>T (p.R39C) in exon 2, c.145A>G (p.M49 V) in exon 3 and c.910G>C (p.G304R) in exon 8 (referring to NM_001613.1 and NP_001604.1, resp.). The first two mutations affect residues within (M49) or adjacent to (R39) the DNAse-I-binding loop within subdomain 2 of alpha actin. The carrier of the R39C substitution was suspected of having MFS. The third mutation, G304R, which was found in a patient thought to have isolated TAAD, directly affects the ATP binding site. Viewing the positions of the affected amino acids, it is tempting to speculate that all three mutations cause altered polymerization of actin monomers. Online prediction programs, such as Panther and PolyPhen, further underpin a pathogenic effect of the novel variations.

Conclusion: TAAD patients with or without additional signs of MFS were found to carry previously unknown ACTA2 mutations that warrant further investigation of their consequences in the structure and function of smooth muscle cell alpha actin.

P174

MLPA in SMA diagnostics: Experience after more than 500 cases

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Infantile spinal muscular atrophy (SMA types I-III) is a common autosomal recessive neuromuscular disorder. More than 95% of 5q13-associated SMA patients show a homozygous SMN1 exons 7 (and 8) deletion, the remaining 2–5% are usually compound heterozygous for the deletion and a point mutation on the other allele. In patients without functional SMN1, the copy number of the highly homologous SMN2 gene influences the clinical course of the disease.Since 2006, we have analysed more than 500 requests of SMA diagnostics by MLPA. Patients were referred for molecular genetic investigation due to suspected SMA or for carrier testing.In comparison to previously used methods (PCR-based homozygosity screening, quantitative analysis of SMN copies) additional information is gained by MLPA:a) Simultaneous determination of SMN1/SMN2 exons 7 and 8 copy numbers.b) Analysis of the total number of SMN exons 1, 4 and 6 indicates deletions other than the classic SMN1 exon 7 deletion. We identified two multi-exon deletions.c) MLPA unequivocally reveals hybrid genes which are common in the complex SMN region. However, we also identified unusual genotypes. Two patients with deletions restricted to exon 7 in the classic homozygosity screening and initially thought to be carriers of hybrid genes were indeed carriers of a mutation in the primer region. This of course had consequences for genetic counselling of family members.d) The polymorphism described by Arkblad et al. (2006) of an extra SMNex1–6 fragment was almost exclusively found in connection with an SMN2 deletion among our patients. Only three patients with an SMN1 deletion presented with this polymorphism.In conclusion, our data show that MLPA has several advantages compared with other methods and thereby improves SMA diagnosis.

P175

Large deletions at the KCNV2 gene locus are common in patients with cone dystrophy with supernormal rod response

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Cone Dystrophy with Supernormal Rod Response (CDSRR) is a rare, autosomal recessively inherited subform of retinal dystrophies characterized by an early severe loss of cone photoreceptor function associat-

ed with reduced visual acuity and color vision defects and a pathognomonic elevated rod b-wave response in electroretinogram recordings. It has been recently shown that CDSRR is specifically caused by mutations in KCNV2, encoding an accessory subunit of a photoreceptor voltage-gated potassium channel. Up to now only point mutations or small indel mutations has been described by other groups. In a screen for KCNV2 mutations in patients with CDSRR and similar type of retinal disorders we found two unrelated subjects which failed to amplify exon 1 sequences and four additional independent patients with apparently homozygous point mutations that failed to segregate concordantly in the respective nuclear family. Applying a TaqMan-based realtime PCR we could demonstrate reduced copy number for exon 1 of KCNV2 in all these patients and heterozygous parents. In order to map the extent of the deletions we used a combination of techniques including LOH analyses of flanking STR and SNP markers, Nimblegen Array-CGH and long distance PCRs for breakpoint coverage and breakpoint sequencing. We found that both patients with homozygous mutations share an identical ~9 kb deletion including almost the entire exon 1 and large parts of intron 1 of the KCNV2 gene. The remaining four patients had other non-identical heterozygous deletions. Two of them, one with a ~11.5 kb deletion covering large parts of exon 1 and the coding sequence of the terminal exon 2 and one with a ~80 kb deletion of the entire KCNV2 locus have already been fully resolved. In conclusion, we found larger deletions in 6 of 27 patients with KCNV2 mutations indicating that such genomic rearrangements are common and have to be taken into consideration in the genetic diagnostics of CDSRR.

P176

Combined homology modeling and evolutionary significance evaluation of missense mutations in blood clotting factor VIII to highlight aspects of structure and function

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Most small lesions in the factor VIII (F8) gene that cause Hemophilia A are single nucleotide substitutions resulting in amino acid replacing (missense) mutations and leading to various phenotypes, ranging from mild to severe. We took a combined approach of homology modeling and quantitative evaluation of evolutionary significance of amino acid replacing alterations using the Grantham Matrix Score (GMS) to assess their structural effects and significance of pathological expression. Comparative homology models of all amino acid substitutions summarized in the F8 mutations database plus these identified and reported lately by us or by our collaborators were evaluated. Altogether 640 amino acid replacing mutations were scored for potential distant or local conformation changes, influence on the molecular stability and predicted contact residues, using available F8 domain models. The average propensity to substitute amino acid residues by mutation was found comparable to the overall probability of de novo mutations. Missense changes reported with various Hemophilia A phenotypes were all confirmed significant using GMS. The fraction of these, comprising residues apparently involved in intermolecular interactions, exceeds the average proportion of such residues for F8. Predicted contact residues changed through mutation were visualized on the surface of F8 domains and their possible functional implications were verified from the literature and are discussed considering available structural information. Our predictive modeling adds on the current view of domain interface molecular contacts. This structural insight could aid in part to the design of engineered F8 constructs for therapy, to possibly enhance their stability and prolong circulating lifetime.

P177

Screening for genomic imbalances in idiopathic Silver-Russell syndrome patients

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Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous disorder. The phenotype is characterized by intrauterine and postnatal growth restriction as well as additional morphologic abnormalities. These include a triangular shaped face with a high forehead and downsloping corners of the mouth, relative macrocephaly and skeletal asymmetries as hemihypertrophy or clinodactyly of the fifth digits.
In about 50% of SRS cases (epi)genetic alterations can be detected: >38% show a hypomethylation of the ICR1 in 11p15, a further 10% carry maternal UPD of chromosome 7 (matUPD(7)). In single cases, chromosomal aberrations have been reported. Nevertheless there still remain 50% of SRS patients without known (epi)genetic alterations.
To find out whether submicroscopic imbalances contribute to the aetiology of the disease, we subjected 20 SRS patients without (epi)mutations in 11p15 and matUPD(7) to genomic microarray analysis using the Affymetrix GeneChip® Human Mapping 500 K Array Set. The detected imbalances were surveyed in respect to their gene coverage and possible overlaps with registered copy number variations (CNVs) by online databases query (UCSC, DECIPHER, DGV).
Thereby we found two published CNVs per patient on average. In 10 of the 20 analysed SRS patients we additionally identified altogether 13 so far unregistered copy number alterations (CNAs) which did not show any overlap. Within seven of these CNAs complete coding regions of one ore more genes were localized. By characterising the parents of the SRS patients by microarray and microsatellite analyses a de-novo origin could be determined for three of these CNAs. These variants are currently under investigation. For two CNAs a parental inheritance could be confirmed: these were regarded as apathogenic. In conclusion, our study did not provide evidence for a common submicroscopic imbalance in SRS.

P178

Ubiquitous expression of multiple splice isoforms for FAM58A, the gene mutated in STAR syndrome

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STAR syndrome (OMIM #300707) is an X-linked dominant disorder characterized by a set of malformations affecting the distal extremities (toe syndactyly), facial features (telecanthus), the anogenital region and kidney (renal) function. The syndrome is caused by mutations in the FAM58A gene on Xq28. The FAM58A coding region is partitioned into 5 exons and encodes a putative nuclear protein sharing a cyclin fold motif with the cell-cycle regulating cyclins.
Using RNA isolated from the human kidney cell line HEK293 for RT-PCR, cDNA cloning and sequencing, we identified two alternative splice acceptor sites: one in exon 4 and one in exon 5, 30 bp and 60 bp, respectively, from their upstream acceptor sites. In addition, we detected splice isoforms missing exon 4. All six possible splice isoforms that can be generated by combinatorial use of the four alternative splice acceptor sites and by exon 4 skipping exist in HEK293 cells. BLAST analysis against the human EST data base confirmed the validity of the variants. All alternative splices are in-frame and generate mRNAs encoding protein isoforms that retain the cyclin fold, which is encoded by exons 1 through 3.

Employing semi-quantitative RT-PCR, we investigated the expression of these FAM58A splice variants in 20 different human tissues, using a commercially available mRNA panel. All splice isoforms showed solid and about equal expression in all tissues investigated, except for one splice variant that was more weakly expressed in some tissues.
The wide-spread expression indicates an essential role of the FAM58A variants. Little is known concerning the functions of these isoforms, but knock-down experiments with siRNA have indicated that splice forms with a full-length exon 5 are important for normal cell proliferation. The cellular distribution and function of selected splice variants is now under investigation by transfection of appropriate constructs into cell lines.

P179

Dicer is required for Sertoli cell function and for spermatogenesis

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Dicer is a key enzyme that processes microRNA precursors into their mature form, enabling them to regulate gene expression. Dicer null mutant mice die before gastrulation. To study Dicer function in testis development, we crossed mice carrying a conditional Dicer allele with an AMH-Cre transgenic line, thereby inactivating Dicer in Sertoli cells around embryonic day 13.5 (E13.5), shortly after the formation of testis cords. The resulting testes with Dicer null Sertoli cells show normal embryonic development, and at postnatal day 0 (P0), testis cords are normal in number and histologically undistinguishable from controls. Subsequently, Dicer mutant testes show a progressively aberrant development, so that at P6, they contain a reduced number of disorganized testis cords, leading to primary infertility. Apoptosis assays show that mutant gonads undergo a massive wave of apoptosis starting at P_3, causing progressive loss of Sertoli cells, but also of germ cells, resulting in drastically reduced testis size. Real-time RT-PCR analyses reveal downregulation of markers playing crucial roles in testis development, structural integrity of testis cords and spermatogenesis at P0, indicating that Dicer mutant testes are transcriptionally compromised already at this stage. These findings show that Dicer is required for Sertoli cell function and survival as well as for spermatogenesis in mice, thereby suggesting an essential function of Dicer for fertility also in humans.

P180

Alu-Alu recombination underlies the vast majority of large VHL germline deletions: Molecular characterization and genotype-phenotype correlations in VHL patients

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Von Hippel-Lindau disease (VHL) is an autosomal dominant cancer syndrome. Affected individuals are predisposed to multiple tumors, primarily the central nervous system (CNS), eyes, adrenals and kidneys. The VHL tumor suppressor gene on chromosome 3p25-p26 is partially or completely deleted in 20–30% of families with VHL. We identified deletions ranging from 0.5 kb to 250 kb affecting part of or the entire VHL and flanking genes in 54 families. In 33 of the index patients, the breakpoints were precisely characterized by DNA sequencing. Of the 66 breakpoints, 60 (90%) were located in Alu elements, revealing Alu-mediated recombination as the major mechanism for large germline deletions of the VHL gene, which lies in a region of high Alu density. Interestingly, an AluYa5 element in VHL intron 2, the evolutionarily youngest Alu element and the only such element in the entire region, was found to be the most recombinogenic, involved in 7 of the 33 deletions. In comparison to VHL patients in general, the 54 index cases and their affected relatives showed a higher occurrence of renal cell carcinomas (RCC) and of CNS hemangioblastomas. We not only noted the association of RCC with retention of the HSPC300 gene, but also observed a significant correlation between retention of HSPC300 and the development of retinal angiomas (AR). This study reveals that germline VHL deletions provide a particularly rich source for the study of Alu-mediated unequal crossover events, and provides evidence for a protective role of the loss of the actin-regulator gene HSPC300 for the development of both RCC and AR.

P181

Mutation and SNP analysis in the DNA damage mediator checkpoint 1 (MDC1) gene in 28 patients with systemic sclerosis

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Systemic sclerosis (SSc) is a disease with dysfunction of the immune system, endothelium, and fibroblasts and a heterogeneous phenotype characterized by fibrosis (Tan FK, Rheum Dis Clin N Am 2003, 29:211–223). The disease is the end result of a complex interaction of genetic factors and unknown environmental influences. In 1988, a linkage of increased chromosomal breakage rates, suggestive of a DNA repair defect in cultured lymphocytes of SSc patients with the Major Histocompatibility Complex (MHC) on the short arm of chromosome 6 was described (Rittner G et al., Hum Genet 1988, 81:64–70). One candidate DNA repair gene within the MHC is MDC1. This gene has an amino-terminal forkhead-associated (FHA) domain and a tandem repeat of breast cancer susceptibility gene 1 carboxyl terminal (BRCT) domains (Xu X & Stern DF, FASEB J 2003, 17:1842–1848). After DNA damage resulting in chromosomal double strand breaks MDC1 mediates the formation of gammaH2AX foci as a first step of DNA repair. We have studied blood samples of 28 SSc patients from the Bonn and Mainz area. Chromosomal breakage rates were counted in metaphases from lymphocyte cultures. PCR amplification and sequencing of all exons of MDC1 identified at least one SNP in 11 of the 28 patients. Compared with the NCBI database, two different SNPs in exon 9 appeared to be overrepresented in our patient population. One non-synonymous mutation was found in exon 4, the same non-synonymous mutation twice in exon 6, and one mutation in the untranslated region of exon 14. In conclusion, we found evidence for an involvement of the DNA repair gene MDC1 in the pathogenesis of some, but not all patients with systemic sclerosis. The significance of the occurrence of SNPs and mutations in this gene remains to be shown in larger cohorts of SSc patients, as well as by functional studies of the formation of DNA repair foci, the cell cycle arrest and apoptosis rates in these patients.

P182

A synonymous C>T substitution within a methylated exonic CpG-island of UBE1 causes reduced expression in XL-SMA patients and carrier females: A new mutation mechanism?

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Recently, we have associated two missense mutations and four times the same synonymous C>T substitution (c.1731C>T, p.Asn577Asn), all located in exon 15 of the UBE1 gene, with X-linked infantile spinal-muscular atrophy (XL-SMA; MIM301830; Ramser et al., 2008). XL-SMA is an X-linked motor neuron disorder that presents with hypotonia, areflexia and multiple congenital contractures (arthrogryposis) associated with anterior horn cell loss and infantile death. UBE1 codes for the Ubiquitin-Activating Enzyme E1 that catalyzes the first step in the ubiquitin-proteasome system (UPS) which is responsible for intracellular degradation of proteins. Here, we focus on the further investigation of the pathomechanism of the synonymous C>T substitution, which is located within an exonic, methylated CpG-island and which causes significant reduction of UBE1-expression to one fifth as compared to 6 male healthy controls. Extended expression studies on RNA from white blood cells of eight carrier females of three unrelated XL-SMA families carrying the synonymous substitution demonstrated a 50% reduction of UBE1 expression compared to 8 female healthy controls. Since it has been shown that UBE1 escapes X-inactivation, these results confirm that the "silent" C>T substitution, which was shown to be absent in 7914 control X-chromosomes, leads to a significant reduction of UBE1-mRNA expression.To find out, if some exonic CpG-islands may harbour a so far unknown regulatory element, that may also be altered in a couple of diseases whose molecular basis could not be elucidated yet, we are currently performing a genome wide database screen to calculate the percentage and chromosomal distribution of genes exhibiting such exonic CpG-islands like e.g. ACHE on chromosome 7. Furthermore, we are screening SNP-databases to determine the percentage of CpG>TpG polymorphisms in these intervals and find out, if these polymorphisms may have already been associated with specific clinical phenotypes.

P183

A de novo t(1;8)(p35.2;p11.2) translocation associated with severe obesity and hypogonadotropic hypogonadism

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We report on a 15 years old female patient with severe obesity (BMI: 46 kg/m²) combined with hypogonadotropic hypogonadism resulting in amenorrhea. Cytogenetic analysis using high resolution GTG-banding revealed an apparently balanced de novo translocation described as t(1;8)(p35.2;p11.2). While the breakpoint region on chromosome 1 contains NR0B2 as a candidate gene for obesity, FGFR1 as a candidate for hyopogonadotropic hypogonadism is located within the breakpoint region on chromosome 8. Fluorescence in situ hybridization (FISH) analysis with BAC probes amongst others covering these two candidate genes showed signals on both derivative chromosomes indicating possible disruption of these genes, but exact breakpoint mapping is pending. NR0B2 (nuclear receptor subfamily 0, groub B, member 2) which is located on the predicted region on chromosome 1 is coding for an integrated factor of the cholesterol metabolism and was previously found to be mutated in patients with obesity. Mutations in FGFR1 (fibroblast growth factor receptor 1) were described as causative for autosomal dominant Kallmann syndrome of which hypogonadotropic hypogonadism is a major sign. Expression analysis of both genes in our patient is in progress.

P184

Analysis of the promoter and nuclear localization signals in MCPH1

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Biallelic mutations in the MCPH1 gene cause the autosomal recessive disorder primary microcephaly (MCPH, MIM# 606858) in combination with a cellular phenotype of premature chromosome condensation (PCC, MIM# 607117). MCPH1 is located on human chromosome 8p23.1, consists of 14 exons, and encodes the protein microcephalin containing an N-terminal and two C-terminal BRCT (BRCA1 C-terminus) domains. Subcellular fractionation assays indicated a nuclear localization of microcephalin. In silico analysis of the MCPH1 protein revealed three putative nuclear localization signals (NLS): KKKRK (NLS1), KRKRVSHGSHSPPKEKCKRKR (NLS2) and PYSGKKK (NLS3). By PCR-based in vitro mutagenesis we generated MCPH1 variants with different combinations of deleted NLS as GFP-fusion constructs. Analyzing the cellular localization of these proteins we could reveal that the presence of each of the three NLS alone is sufficient for nuclear translocation of ectopically expressed MCPH1 in COS-7 cells. These data are consistent with our observation that MCPH1 splicing variants lacking exon 8 (MCPH1Δ8) or the five 3'-exons (MCPH1Δ9–14) and therefore NLS2 or NLS3, respectively, are able to relocate to the nucleus. In a second approach we screened the MCPH1 gene for potentially regulative elements. By this, two sequences of approximately 600 bps were in silico predicted as promoters. The first element is located within the 5'-UTR and exon 1 and the second includes parts of intron 3 as well as the entire exon 4. Studies using luciferase reporter gene assays are underway to verify promoter activities for both sequences.

P185

Missense mutations in the SCA11 gene TTBK2

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The spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders characterised by poor coordination, impairment of speech, abnormal eye movements and pyramidal signs. To date 28 SCA loci have been discovered and for at least 14 the respective gene or mutation has been determined. Recently mutations in the TTBK2 gene were described to be associated with SCA11. This gene encodes tau tubulin kinase 2 which is highly expressed in the cerebellum Purkinje cells. A one-base insertion in a British family and a deletion of a dinucleotide in a family of Pakistani ancestry were identified. Both frame shift mutations created premature stop sites.

To determine the frequency and to search for further mutations concerning TTBK2, we sequenced the 18 translated exons and flanking intronic sequences of the currently longest 19-exon-transcript for 49 unrelated patients with dominant ataxia. We found seven variations at DNA level. A 157-bp-frameshift-deletion in exon 11 was detected in 8.5% of ataxia patients and in 12.5% of 95 unrelated control individuals. Remarkably, the 19-exon-transcript being the only transcript harbouring exon 11 could not be detected in lymphoblasts of patients and con-

trol individuals by RT-PCR. Furthermore four novel DNA variations were found in the TTBK2 coding region: the two missense mutations E1247G and R1515H, each occurring in one ataxia patient, as well as the two silent mutations S758S and E1269E in one and four patients respectively. Beside these novel variations two known 1-bp-substitutions in the 5'-UTR at mRNA positions −37 and −67 were detected in more than 10% of the ataxia patients. In conclusion SCA11 is not a frequent cause for dominantly inherited ataxias. The presence of the 157-bp-deletion in samples from patients and unaffected individuals argues against a disease causing potential. The missense mutations E1247G and R1515H should be further investigated to determine whether they are mutations or polymorphisms.

P186

Overexpression of mouse Pxt1 leads to cell death

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Peroxisomal testis specific 1 (Pxt1) is a male germ cell-specific expressed gene with expression starting at the spermatocyte stage of mouse spermatogenesis. The PXT1 protein sequence contains a PTS1 motif responsible for its peroxisomal targeting. The mouse Pxt1 cDNA is 1015 bp in size and has a 718 bp long 3'UTR. We hypothesized that the long 3'UTR can delay the protein translation. In order to analyze the function of 3'UTR and the consequence of overexpression and premature translation of PXT1 in the testis we have generated transgenic animals with PXT1-cmyc fusion protein expressed under the control of hPGK2 promoter. In this transgenic construct the endogenous 3'UTR was replaced by 3'UTR of human growth hormone gene, which is known to be neutral for protein translation. In the transgenic males, transcription and translation of Pxt1 occur simultaneously in primary spermatocytes and cause depending on transgene expression level various histopathological changes in the testes and epididymides up to complete arrest of spermatogenesis resulting in male infertility. Moreover, we demonstrated that impaired spermatogenesis is due to increased apoptosis in transgenic testes. These results pointed us to analyse the pro-apoptotic character of PXT1. We could demonstrate that in different cell lines the overexpression of PXT1 induces apoptosis. This is so far the first evidence that a peroxisomal protein is directly involved in apoptosis.

P187

A novel VPS13B mutation in two brothers with Cohen syndrome, cutis verticis gyrata and sensorineural deafness

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We have previously described a syndrome characterized by microcephaly, cutis verticis gyrata (CVG), retinitis pigmentosa, cataracts, hearing loss and mental retardation (MIM #605685) in two brothers from a non-consanguineous Lebanese family. In view of the rarity of the disorder and the high rate of inbreeding in the Lebanese population, we assumed an autosomal recessive trait inherited from a common ancestor. A genomewide scan (10 K Array) was performed. If the parents are considered non-consanguineous, >20 chromosomal regions show segregation with the phenotype. In contrast, assumption of parental consanguinity resulted in a single 7.24 Mb region on chromosome 8q22.1-q22.2 with homozygosity by descent in the patients. It comprised 46 genes, including the Cohen syndrome (CS) gene, VPS13B. We then sequenced VPS13B in the patients and found a novel homozygous splice site mutation that activates a cryptic acceptor site in exon 52 with a consecutive 16 bp deletion in the mRNA. CVG and deafness have never been reported in CS. This may reflect a variant of CS. Alternatively, there may be an overlap of genetic conditions: Offspring from consanguineous parents may be homozygous for mutations in unlinked genes. Deafness and CVG could be caused by mutations in different loci. However, our linkage data do not suggest another causative locus. Another mutated gene or modifier locus may segregate in cis with the CS mutation and be responsible for deafness and CVG, but no deafness locus has been mapped to our 8q22 region. In contrast, the frequent association of CVG with mental retardation suggests that it may be a rare manifestation of CS. The mapping approach conducted here can serve as a paradigm in rare recessive phenotypes: The prevalence of homozygosity for the causative mutations can also be high in families without documented consanguinity, due to a distant common ancestor, especially in small populations with a high rate of inbreeding.

P188

Mouse Arfgef2 plays an important role in early embryonic development

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The switching of ADP-ribosylation factors (ARF) from inactive form (bound with GDP) to the active form (bound to GTP) is catalyzed by ARF-GEF proteins containing a Sec7 domain. Proteins containing the Sec7 domain can be classified into two major groups according to molecular weight. Mouse Arfgef2 gene encoding BIG2 protein belongs to the class of high molecular mass (>100 kDa) proteins. This protein was isolated together with BIG1 as a macromolecular complex from bovine brain. BIG2 is believed to be associated with trans-Golgi network (TGN) and recycling endosomes. In human, mutations in the ARFGEF2 gene cause autosomal recessive periventricular heterotopia with microcephaly (ARPHM). It is still not clear whether BIG1 and BIG2 play distinct or redundant roles, literature data are contradicting. We studied a gene-trap mouse line with functional disruption of the Arfgef2 gene. Heterozygous mutants did not reveal any phenotypic abnormalities thus we conclude an autosomal recessive inheritance. Using the LacZ reporter gene from the gene-trap construct we could show that this gene is expressed very early in mouse embryonic development. Moreover, using SNP markers we could demonstrate that Arfgef2 mRNA is stored in oocytes (maternal storage) and used during the first embryonic divisions. The embryonic Arfgef2 gene is activated first at the 4-cell stage. We also detected that Arfgef2 undergoes alternative splicing. Interestingly, the splicing pattern differs among tissues of adult animal. Fertilization of Arfgef2-deficient oocytes with Arfgef2-deficient sperm resulted in syngamy, but do not develop to 4-cell stage embryos. Our results indicate that BIG2 is necessary for early embryonic development in mouse and this role can not be compensated by BIG1.

P189

Split hand/split foot malformation: Determining the frequency of genomic aberrations with molecular-genetic methods

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Split hand/split foot malformation (SHFM), also known as ectrodactyly or cleft hand/foot, is a complex congenital limb defect that is characterized by a deep median cleft with absence of central rays. A typical feature of SHFM is the intra- and interfamilial phenotypic variability, with limb defects ranging from minor syndactyly to tetragenous monodactyly. It occurs in isolated form or as part of a syndrome. So far, five different genetic loci have been identified as cause of SHFM, and recently evidence was found for two new loci. These include SHFM1 (7q21, MIM #183600), SHFM2 (Xq26, MIM #313350), SHFM3 (10q24, MIM #600095), SHFM4 (3q27, MIM #605289), and SHFM5 (2q31, MIM #606708). Initially we detected a duplication at the SHFM3 locus (10q24) in a patient with monodactyly at hands and feet. To determine the frequency of genomic aberrations of the different SHFM loci, we investigated 28 patients based solely on phenotype. Familial as well as sporadic cases were included, and patients had not been mapped to a specific locus. Previously, mutations in the TP63-gene were excluded.
Using Multiplex Ligation-dependent Probe Amplification (MLPA) we investigated the SHFM1, 2, 3 and 5 loci for deletions or duplications. We detected a duplication at chromosome 10q24 (SHFM3 locus) in 7 patients (25%). These results were confirmed by quantitative Real-Time PCR (qPCR). It is not clear if the size of the duplication, which varied from 440 kb to maximal 450 kb, correlates with the phenotype. In the literature, the frequency of TP63-mutations in SHFM patients is approximately 10%. Based on our findings, duplications at the SHFM3 locus seem to be twice as common as TP63-mutations. In clinical practice, SHFM patients should be tested for duplications at 10q24 first, before screening for TP63-mutations.

P190

Evidence for an autosomal recessive form of LADD syndrome caused by the homozygous p.R570 W mutation in FGFR2

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Lacrimo-auriculo-dento-digital (LADD) syndrome is an autosomal dominant disorder affecting mainly the lacrimal system, ear-shape and hearing, teeth development, and digit patterning. We recently described causative dominant mutations in FGFR2, FGFR3, and FGF10 in several LADD families. We now present a Turkish patient with a severe form of LADD syndrome characterized by aplasia and hypoplasia of the lacrimal ducts, facial dysmorphism, low set and cup shaped ears, bilateral thumb aplasia, and postnatal growth deficiency. The patient died at 4 months of age due to recurrent pulmonal infections. The parents were consanguineous and did not show any symptoms. Molecular analysis of FGFR2 identified the homozygous c.1708C>T substitution in exon 13. We did not find this mutation in 150 healthy control individuals. This is the first recessive mutation found in LADD syndrome and the mutation is predicted to change the highly conserved arginine at position 570 in the tyrosine kinase domain of FGFR2 to tryptophane (p.R570 W).
We compared the tyrosine kinase activity of the p.R570 W mutation with wild type FGFR2 and different other mutants and demonstrated that the intrinsic tyrosine kinase activity of FGFR2 p.R570 W is drastically reduced as compared to wild type FGFR2 or to the constitutively activated p.K641R mutant identified in a patient with Pfeiffer syndrome. In comparison with three other dominant FGFR2 mutations located in the tyrosine kinase domain, we did not see a significant difference in the level of reduction. In summary, we describe the first LADD case caused by a homozygous FGFR2 mutation and show that reduced intrinsic receptor activity is the main pathophysiological mechanism.

P191

Myofibrillar myopathies – myopathies for the elders

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Myofibrillar myopathies are a group of clinically very similar adult onset muscle diseases recently described as desmin-related myopathies or protein surplus myopathies. They are correlated with microscopic, sometimes only submicroscopic sarcoplasmatic inclusions (cyto-plasmatic bodies, spheroid bodies, granulofilamentous material), giving them the name „Alzheimer disease of the muscle". Clinically the patients present with a predominantly distal muscle weakness, sometimes mixing up the myopathy with a spinal muscular atrophy (SMA). Later on also proximal muscles are affected, often accompanied by a cardiomyopathy. CK values are moderately raised (by maximal five-time the upper normal limit). Myofibrillar myopathies are – as far as we know – all autosomal dominant diseases and quite heterogeneous. At the moment five causative genes are known: desmin (DES), myotilin (MYOT), LIM domain binding factor 3 (LDB3, ZASP), filamin C (FLNC) and alpha-B Crystallin (CRYAB), with a preference for desmin mutations. MYOT, LDB3 and FLNC show mutation hot spots.
The cellular functions of the related proteins are shown in a scheme. In more than 200 independent samples from patients suspicious for a myofibrillar myopathy (hints from the muscle biopsy) we found 7 pathogenic desmin mutations, 2 mutations in the MYOT gene, 1 in the LDB3 gene, 2 in the FLNC gene and 1 in the CRYAB gene. To illustrate the symptomes and a typical clinical course of a myofibrillar myopathy, we present the pedigree of one branched family with a p.Arg350Pro mutation in the desmin gene. The variability of the phenotype of affected family members is considerable, but penetrance is 100%.

P192

Gross deletions and insertions in the senataxin gene cause Ataxia-oculomotor apraxia 2

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The recessively inherited ataxia-oculomotor apraxia 2 (AOA2) is a neurodegenerative disorder characterised by juvenile to adolescent age at onset, gait ataxia, cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia, and elevated serum AFP level. AOA2 is caused by mutations within the senataxin gene (SETX) located on chromosome 9q34. The majority of known mutations are nonsense, missense, and splice site mutations, as well as small deletions and insertions. We screened ten patients showing a clinical phenotype consistent with AOA2 for mutations in the SETX gene. All 24 coding exons and flanking intronic sequences were sequenced. Sequence analysis revealed one nonsense mutation, eight missense mutations, one splice site mutation, and two small deletions. In six patients, mutations on both alleles were identified, whereas three patients showed heterozygosity for the detected mutation. Additionally, amplification of exon 11 to 15 by PCR failed in one patient. To confirm the molecular genetic basis for AOA2 diagnosis in these four patients, gene dosis analysis via quantative PCR assay and subsequent RNA analysis were performed. In one patient we

identified in addition to the heterozygous nonsense mutation R1606X a 1280 bp-LINE1-insertion in exon 12. In a second patient heterozygous for the nonsense mutation R1606X we found a 6107 bp-deletion between intron 11 and intron 14. Patient 3 heterozygous for a 4 bp-deletion in exon 10 showed a 20729 bp-deletion between intron 10 and 15. This deletion was seen in a homozygous state in patient 4, too. Thus, four of 10 patients showed large insertion or deletion events involving repetitive elements in the SETX gene. These findings indicate that gross mutations seem to be a frequent cause of AOA2 and reveal the importance of additional gene dosis analysis for routine diagnostic.

P193

The TSPY transgenic Insl3 deficient mouse model – an approach to understanding TSPY function in spermatogenesis

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The TSPY gene is conserved in mammals and encodes the testis-specific protein, Y encoded. Within the testis, TSPY expression is restricted to germ cells. There is circumstantial evidence that TSPY is involved in spermatogonial proliferation and gonadal tumorigenesis. Because the laboratory mouse carries the Tspy gene in a naturally silenced state, we previously restored Tspy activity in mice and generated a TSPY transgenic mouse line, in which the organization and expression of the human TSPY transgene follows the human pattern. In the present study, we generated TSPY transgenic Insl3-deficient mice (TSPY-Insl3$^{-/-}$ mice) and age-matched controls (NMRI-Insl3$^{-/-}$ mice) and analyzed the histology of their testes, the germ cell number and the relative number of apoptotic germ cells in order to contribute to understanding TSPY function in spermatogenesis. Insl3 deficient mice are cryptorchid and infertile due to a spermatogenesis arrest on the level of primary spermatocytes. We examined the testes of 33 adult TSPY-Insl3$^{-/-}$ males and 36 age-matched controls histologically and identified elongated spermatids in 3 out of 33 TSPY-Insl3$^{-/-}$ males and in 1 out of 36 nontransgenic NMRI-Insl3$^{-/-}$ mice. Testicular germ cell number and apoptosis were analyzed in nontransgenic NMRI-Insl3$^{-/-}$ and TSPY-Insl3$^{-/-}$ males (five mice from each group and postnatal stage 10, 15, 20, 30, 45 and 52 dpp, respectively). Germ cell numbers were significantly increased in TSPY transgenic crytorchid mice at postnatal stages 15 and 20 dpp in comparison to age-matched controls, indicating an anti-apoptotic or proliferative effect of TSPY at these postpartal stages. We further observed a significant association of the TSPY transgene with a reduced rate of apoptotic germ cells in cryptorchid testes of the postnatal stages 30–52 dpp. Taken together our findings show that TSPY can partially rescue spermatogenesis in cryptorchid Insl3 deficient mice during a narrow time window in postnatal development.

P194

Identification of genes with specific functions in the developing human cortex

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Development of the human neocortex depends on spatially and temporally correct gene expression. Identification of developmentally important genes may provide a better understanding of brain disorders and cognitive processes. We have constructed a cDNA chip with approximately 600 genes that are known to influence some aspect of brain development, function and cognition. When we quantified the mRNA expression levels in fetal frontal cortex (prospective area A10) from weeks 15–25 of gestation, approximately 150 genes showed at least 1.5fold expression differences between gestational weeks. Comparison of our data with published microarray expression data from adult brains identified 22 genes, which appeared to be expressed (at levels detectable by microarray analyses) only during fetal brain development. Quantitative realtime RT PCR analyses revealed that some of these genes, for example TGM1, CYP11B1, HAL, and GPR64 were indeed expressed at much higher levels (up to 50fold) in fetal cortex than in adult cortex. TGM1 encodes transglutaminase 1 which has been implicated in neurodegeneration and the initiation of Alzheimer disease. Transglutaminase can cross-link amyloid beta protein assemblies that inhibit processes involved in memory and learning. CYP11B1 encodes a member of the cytochrome P450 superfamily of enzymes, which localize to the inner mitochondrial membrane and catalyze many reactions involved in drug metabolism and lipid synthesis. The histidine ammonia-lyase HAL is a cytosolic enzyme catalyzing the first reaction in histidine catabolism. GPR64 encodes the G protein coupled receptor 64, a highly conserved heptahelical receptor of the human epididymis involved in sperm maturation. Its function and signal transduction pathways in the developing cortex are unknown. Based on our findings, it is plausible to assume that these four genes perform specific functions in the developing cortex and are candidate genes for mental retardation.

P195

First evidence for locus heterogeneity of autosomal-dominant auditory synaptopathy/neuropathy (AS/AN)

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Auditory synaptopathy or neuropathy (AS/AN) is a specific subtype of sensorineural hearing loss characterized by abnormal or absent auditory nerve and brain stem responses but normal outer hair cell functions with detectable otoacoustic emissions and cochlear microphonics. The impaired auditory function is thought to be due to disturbances either at the level of the inner hair cell, its synapse, or the auditory nerve itself. Most cases with AS/AN are caused by environmental factors like hyperbilirubinemia, however there are also genetic subforms which can help to identify molecular components necessary for auditory signal transmission. AS/AN can occur in several (neurogenetic) syndromes such as Friedreich ataxia or Hereditary and Sensory Neuropathies, whereas non-syndromic AS/AN is most often inherited in an autosomal-recessive manner. In these recessive forms, mutations in either OTOFERLIN (2p23) or PEJVAKIN (2q32) have been identified. Autosomal-dominant auditory synaptopathy/neuropathy is rather rare, and just one large multigenerational family of European descent has been described, in which the still unknown AUNA1 gene has been mapped to chromosome 13q14-q21. Here we report on the second large family with autosomal-dominant AS/AN of German descent which is unlinked to the AUNA1 locus. A whole-genome linkage analysis using the 250 k SNP array revealed shared haplotypes of all ten affected individuals on two different loci on 12q24 and 13q34. We conclude that autosomal-dominant AS/AN is a genetically heterogeneous disorder and that a second AUNA locus maps to either chromosome 12q24 or 13q34.

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Comparison of the effects of MCPH1 mutations and RNAi against MCPH1 on checkpoint control and transcription

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Autosomal recessive primary microcephaly (MCPH) is a genetically heterogeneous, neurodevelopmental disorder characterised by marked reduction in brain size and mental retardation. Mutations in the gene MCPH1, encoding the BRCT-domain containing protein microcephalin, cause primary microcephaly associated with a unique cellular phenotype of misregulated chromosome condensation. Accumulating data suggest that microcephalin is involved in a number of crucial cellular processes such as chromosome condensation, DNA damage response, transcriptional regulation, and centrosome integrity. RNAi against MCPH1/microcephalin results in changes of the transcriptional regulation of a number of genes involved in checkpoint control and apoptosis, like BRCA1, CHK1, RAD51, and caspases. The RNAi experiments not only suggest that MCPH1 is a transcriptional regulator of those genes, but also that loss of MCPH1 function results in a severe checkpoint defect.

Here, we show that the cellular effects of homozygous truncating mutations in MCPH1 patient cells differ significantly from those observed following RNAi mediated depletion. In contrast to MCPH1/microcephalin depleted cells, MCPH1 patient cells are checkpoint proficient and do not show a disturbed regulation of the above mentioned genes. Moreover, we present data demonstrating that the effects of RNAi mediated depletion of MCPH1/microcephalin on the RNA and protein levels of these genes are not depending on the depletion efficiency, but on different siRNA duplexes used for RNAi and accordingly their target sequence on the MCPH1 messenger RNA. Thus, the effects of RNAi mediated depletion of MCPH1 do not reflect the cellular effects of MCPH1 truncating mutations in patient cells.

Alternative interpretations may explain these discrepancies: the mutations are hypomorphic, alternative pathways are activated in the MCPH1 patient cells to compensate for the loss of MCPH1/microcephalin function, or off-target effects of the RNAi process.

P197

Dynamic expression of the Slit-Robo GTPase activating protein genes during development of the murine nervous system

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We investigated the expression of the three known Slit-Robo GTPase activating protein (srGAP) genes in the developing murine nervous system using in situ hybridisation. The three genes are expressed during embryonic and early postnatal development in the murine nervous system, showing a distinct pattern of expression in the olfactory system, the eye, forebrain and midbrain structures, the cerebellum, the spinal cord and dorsal root ganglia, which we discuss in relation to Slit-Robo expression patterns and signalling pathways. We also report srGAP2 expression in zones of neuronal differentiation and srGAP3 in ventricular zones of neurogenesis in many different tissues of the central nervous system (CNS). Compared to srGAP2 and srGAP3, the onset of srGAP1 expression is later in most CNS tissues. We propose that these differences in expression point to functional differences between these three genes in the development of neural tissues.

P198

Establishing neuron like cells from skin-derived precursor cells in hereditary spastic paraplegia patients

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Hereditary spastic paraplegia (HSP) denotes a genetic neurodegenerative disorder that is characterised by the presence of lower limb spasticity and weakness. The common neuropathological feature of the disease is the retrograde degeneration of long axons in corticospinal tracts and posterior columns. The aim of the current study was to develop a patient derived cell culture model with a disease-relevant, neuronal phenotype that may serve as a testing system to ascertain suggested mutational mechanisms and guide gene therapeutic approaches. For this purpose skin-derived precursor cells were isolated from skin biopsies of control persons and patients with proven SPG4 HSP as a population of non-adherent cells from the dermis that proliferate and self renew as floating spheres in response to fibroblast growth factor 2 and epidermal growth factor. The spheres were cultured for more than 8–10 weeks being passaged every 7 days. Then differentiation into cells with neuronal morphology was induced by removal of fibroblast growth factor 2 and epidermal growth factor and addition of neurotrophic factors (BDNF, beta-NGF, neurotrophin-3, neurotrophin-4). 42±5.6% of these cells were nestin-positive. After 7 days of differentiation only 5–11% of these cells co-expressed the neuronal markers neurofilament-M and beta III tubulin. After 2–4 weeks of differentiation these cells had the typical neuronal morphology with axon/dendrite like structures. The neuronal cells expressed the dendritic marker MAP2abc, growth associated protein Gap-43 and some the presynaptic marker synaptophysin. Functional assays like time lapse microscopy etc. in comparison between control and patient derived neuronal like cells are currently under way to address basic cell function.

P199

No deletion of the SRY-responsive testis enhancer of SOX9 in XY gonadal dysgenesis

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Under influence of the Y-chromosomal testis-determining gene SRY, the bipotential gonadal anlagen develop into testes. Consequently, SRY mutations cause a human sex reversal syndrome known as XY gonadal dysgenesis (XY GD; Swyer syndrome). Similarly, heterozygous mutations of the autosomal SOX9 gene also cause XY sex reversal, as one of the symptoms associated with the skeletal disorder campomelic dysplasia (CD). Murine Sox9 can function as a testis-determining factor in the complete absence of Sry, as XX mice ectopically expressing Sox9 form testes instead of ovaries. Recently, a 3.2 kb testis-specific enhancer of Sox9 (TES) has been identified to which SRY binds together with SF1 thereby upregulating Sox9 transcription in a testis-specific manner. The 1.4 kb core sequence of TES (TESCO) is conserved between mouse and human. Mutation of the several SRY- and SF1-binding sites within TESCO abolished testis-specific expression in transgenic mice (Sekido and Lovell-Badge, Nature 2008).

Only about one third of XY GD cases are the result of SRY mutations. As mutational inactivation of one SOX9 allele can lead to XY sex reversal in the context of CD, we reasoned that mutation of the human TES homolog, hTES, could cause isolated XY sex reversal without affecting skeletal development. Consequently, 69 XY GD cases for which SRY mutations had been ruled out were analysed for deletions in hTES. For

this, quantitative PCR was performed using three amplicons located within hTES. We did not detect any deletion in any of the 69 cases analysed and concluded that partial or complete deletion of hTES is not a frequent cause of XY GD. While we have not searched for point mutations, our negative results may nonetheless indicate that hTES is not the functional equivalent of the mouse TES, or that additional hTES enhancers exist so that only mutational inactivation of all hTES enhancers together would lead to XY GD.

P200

Identification of SLC8A3 as a new candidate gene for septo-optic dysplasia (de Morsier syndrome) by the characterization of a translocation t(1;14)

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Septo-optic dysplasia (MIM 182230) is a congenital malformation syndrome with high variability. It is characterized by midline malformations like hypo- or dysplasia of the optic nerve and the corpus callosum, malformations of the pituitary gland, and deficiency of pituitary hormones. In single patients mutations in HESX1 have been detected. Risk factors are a young maternal age, first pregnancy, smoking, and drug abuse.
We report on a boy in whom septo-optic dysplasia was clinically diagnosed. Mutation analysis in HESX1 gave inconspicuous results. Chromosome analysis revealed a de novo translocation t(1;14)(p33;q24.2). FISH mapping of the breakpoint raised the suspicion of an imbalance in the breakpoint regions. To detect submicroscopic imbalances GeneChip-Analysis (Affymetrix 6.0) was performed. A deletion of 10–15 kb was detected at the breakpoint in 14q24.2 containing parts of SLC8A3. We suspect that haploinsufficiency for SLC8A3 is responsible for the phenotype in the patient. Sequencing of SLC8A3 in further patients with septo-optic dysplasia in whom no mutation in HESX1 was detectable is underway.
We conclude that SLC8A3 is a new candidate gene for septo-optic dysplasia (de Morsier syndrome). The known function of SLC8A3 in the brain would be in accordance with the hypothesis that vascular factors play a role in the etiology of this disorder.

P201

A comprehensive characterisation of human CD46, CD55 and CD59 transgenic swine fibroblasts – a potential source of nuclei for somatic cloning

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Transgenic swines, especially those expressing, not a single one, but a combination of the complement system regulators are essential to help overcoming a hyperacute rejection (HAR) and necessary to estimate a potential ratio of a strategy collapse.
Single and triple transgenic swine foetal fibroblasts for human coding sequences of CD46, CD55 and CD59 using a promoter of a human elongation factor 1 alpha gene were generated by lipofection method with 80% capacity. After blasticidine selection stable lines were molecularly characterised and checked for transgene integration by PCR. Forward primers were located in the EF-1α promoter region and reverse primers in the region coding CD46, CD55 or CD59 respectively.
Lines with confirmed transgene integration were subjected for further characterisation of transgene expression by RT-PCR. The transgene expression and its impact on human complement system was assessed by human complement-mediated cytolysis assay. Human serum (HS) contains complement system components which are the main reason of HAR. Each transgene expressed in single transgenic line had a protective effect on the tested cells in HS cytotoxicity assay. Also in triple transgenic lines the expression of the transgenes had a wide positive impact on the protection of cells from human complement-mediated lysis, however it was not additive. Cytogenetic analysis was performed to evaluate the chromosomal stability in the transgenic cells. Several cytogenetic staining procedures revealed, inter alia, anomalous number of the chromosomes, structural aberration and dicentromeric chromosomes. Only fully molecularly and cytogenetically characterised cell lines can be used in the future as nuclei donors for a somatic cloning and producing healthy transgenic animals.

P202

The copy number determination of the transgene in the transgenic animals produced for xenotransplantation

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The copy number of the transgene was analyzed in the transgenic animals with introduced pCMVFUT genetic construct containing of a gene encoding human α1,2-fucosyltranserase under a control of CMV promoter. The copy number of the transgene that had integrated with the genome of the transgenic animals was analyzed by qPCR with SYBR Green dye, which enabled nonspecific double-stranded DNA detection. CMVFT-2F and CMVFT-2R primers were used to amplify 149 bp fragment of DNA. Forward primer had a sequence complementary to a promoter sequence and reversed primer to a coding sequence of α1,2-fucosyltranserase. The copy number of the transgene in the examined samples was established by plotting the obtained C_T values on a standard curve, which had been set by the usage of the C_T values for the successive standard dilutions with known copy number ($1{,}43^8$ - $1{,}43^1$ copies). As a standard we used pCMVFut genetic construct hydrolyzed with NotI restriction enzyme to a linear form. The real-time PCR results helped to establish the range of 3,2–4,05 as the number of transgene copies that had integrated to the swine genome.

P203

Marinesco Sjögren syndrome (MSS): Novel SIL1 mutations and description of a less severe phenotype

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Marinesco Sjögren syndrome (MSS) is a progressive multisystem disorder with autosomal recessive inheritance. The phenotype is characterized by cerebellar ataxia, congenital or infantile cataracts, progressive vacuolar myopathy, mental retardation, and short stature. Recently, mutations in the SIL1 gene, which encodes an endoplasmic reticulum (ER) resident co-chaperone, were identified as a major cause of MSS. Here we describe the results of SIL1 mutation analysis in an extended cohort of patients with MSS or MSS-like conditions. We report five novel mutations in the SIL1 gene, including the first multi-exon deletion described in MSS. We could corroborate our earlier observa-

tion that cataracts in MSS are not always present at birth, and we also identified two unrelated patients with SIL1 mutations without mental retardation. In agreement with earlier observations, a subgroup of MSS patients did not carry SIL1 mutations. Our data extend the mutational and phenotypic spectrum associated with the SIL1 gene and confirm genetic heterogeneity in MSS.

P204

No evidence for a role of variants in the corticotropin-releasing hormone gene in the aetiology of frontal lobe epilepsy

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Nocturnal frontal lobe epilepsy (NFLE) is a heterogeneous seizure disorder, both familial and sporadic forms are observed. The familial form, autosomal dominant form (ADNFLE), can be caused by mutations in the CHRNA4 and CHRNB2 subunit genes of the neuronal nicotinic acetylcholine receptor. However, mutations in these genes are found only in about 10% of families, suggesting the involvement of additional genes.
Recently, Combi et al. described two promoter variants in the corticotropin-releasing hormone gene (CRH). CRH acts as a neurotransmitter or neuromodulator in extrahypothalamic circuits and was also suggested as a candidate gene for familial neonatal convulsions previously.
To analyze the role of CRH in our sample of 71 partial epilepsy patients we performed a mutation screening for the promoter region and the coding region of the CRH gene.
The promoter variant g.-1470C>A was detected heterozygous in 6/71 (8.5%) patients and homozygous in 1/71 (1.4%) and also in 5/50 controls (10%). These frequencies are in concordance with the frequencies reported for the European HapMap control population. Only one of these families showed ADNFLE, the others were either sporadic NFLE patients or had familial temporal lobe epilepsy. We could not detect the promoter variant g.-1166G>C in our patient sample.
In our study we identified a new variation in the promoter region in one family. This variant was not present in the patient´s affected father but was inherited from the healthy mother. The variant could not be detected in a group of 111 healthy controls.
Furthermore a non-synonymous SNP was detected that could also be identified in healthy controls.
The observation that CRH variants were preferentially found in sporadic patients or patients that had the most severe course of the disorder in their respective families would be in accordance with the proposed role as an epilepsy susceptibility factor. However, our data argue against such a role in the aetiology of ADNFLE.

P205

Novel mutations of CASK broaden the phenotypic spectrum of associated brain malformations

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We have recently shown that mutations of the CASK gene, located in Xp11.4, are associated with a severe brain malformation phenotype with postnatal microcephaly and hypoplasia of the cerebellum and brainstem. Clinically the patients presented with severe developmental delay, mental retardation, and seizures. More variable features are optic nerve hypoplasia or optic disc pallor, scoliosis, spasticity, and breathing anomalies. The majority of affected individuals are females with heterozygous de novo mutations including microdeletions that encompass the complete gene or part of CASK and an intragenic nonsense mutation. In a single male, we found a synonymous mutation in CASK that affects splicing efficiency. Our finding of heterozygous loss-of-function mutations of CASK in girls and a partly penetrant splice mutation in a severely affected boy suggested that the CASK-associated phenotype belongs to the group of X-linked disorders with reduced male viability or even in utero lethality. Here, we describe novel mutations of CASK in two patients. In the first case, we found a de novo splice site mutation in intron 2 along with an additional base alteration within the adjacent exon: c.173–2A>C and c.174T>A (p.D58E). By analyzing CASK transcripts, we identified skipping of exon 3. Clinically, this patient had normal OFC at birth and developed microcephaly (-4 SD) at age 10 months. In the second patient, a de novo microdeletion of ~2 Mb which includes the 5' part of CASK was identified. OFC of the affected girl was normal at birth; she developed microcephaly (-4.5 SD) at age 8 months and had a delay in motor milestones. Although the brain malformations in these girls were consistent with those found in previously described individuals with CASK mutation, hypoplasia of the cerebellum and brainstem was much milder. The two new cases increase the total number of patients with CASK-associated brain malformations to nine and broaden the phenotypic spectrum towards milder forms.

P206

Cockayne syndrome: Novel mutations in ERCC6 and ERCC8

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Cockayne Syndrom (CS) is a rare autosomal recessive disorder with growth retardation, microcephaly, facial dysmorphism, joint contractures, lack of subcutaneous fat, progeria, ataxia and deafness. Progressive neurological degeneration with basal ganglia calcifications and demyelination leads to severe mental retardation. Cockayne Syndrome is genetically heterogeneous with two complementation groups, CSA and CSB. Approximately 20% of the CS patients belong to the complementation group CSA presenting with mutations in the ERCC8 gene while 80% belong to CSB with mutations in ERCC 6 gene. Both the genes, ERCC8 und ERCC6, code for proteins which play a role in transcription-coupled nucleotide excision repair (NER). Here, we describe two novel mutations in CS patients. Patient 1 is the first child of consanguineous parents. The clinical diagnosis CS was at the age of 7 years. At that time he presented with a height of −5SD, weight −4SD, a head circumference −3SD, and the typical CS associated abnormalities mentioned above. Patient 1 has a homozygous substitution in the ERCC8 gene C>T (c.316C>T) resulting in a stop codon (p.Q106X) and early truncation of the protein.
Patient 2 is also a child of consanguineous parents. At birth, height, weight and head circumference were in the normal range. At 12 years he presented with height of 87 cm, weight of 7400 g, head circumference of 40.5 cm, severe mental retardation, and calcifications of the basal ganglia. He has a homozygous mutation in ERCC6 gene (c.1765_1767delTGG) resulting in a deletion of one amino acid at position 589 (p.W589del). The deletion localizes in the ATP-binding Helicase domain (amino acid position 519–695) of the ERCC6 protein which is highly conserved from Saccharomyces to human. Even though, both the patients presented with a typical CS phenotype in early childhood, the molecular diagnosis of CS was with 7 and 12 years, respectively.

P207

Genetic rescue of the infertile murine gene trap line Lis1 $^{GT/GT}$

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Hemizygous mutations of the Lis1 (Lissencephaly 1) gene causes a severe brain abnormality, type I lissencephaly, characterized by a smooth surface of the brain due to abnormal neuronal migration during early development,. Moreover Lis1 is also strongly expressed in testis. A mouse mutant Lis1$^{GT/GT}$ generated by a gene trap integration into the second intron of the mouse Lis1 gene, leads to selective disruption of a Lis1 splicing variant in testis causing infertility in homozygous male mice. To overcome this phenotype and to gain an insight into the role and requirement of Lis1 in spermatogenesis three different transgenic mouse lines were generated and analysed. The transgenic line Lispi expresses Lis1 under control of the TP2 (Transition Protein 2) promoter in postmeiotic germ cells. Generation of the double transgenic line Lis-1^{GTGT}/Lispi failed to rescue the infertility phenotype. To rule out the requirement for Lis1 in earlier stages of spermatogenesis a Lis1-cMycTag fusion protein was overexpressed in premeiotic spermatogonial cells under control of the hEF1α (human elongation factor 1α) promoter (hEF-Lis1 line) and in a second approach in meiotic germ cells under control of the testis specific PGK2 (phosphoglycerate kinase 2) promoter (PGK2-Lis1 line). The overexpression of Lis1-cMycTag fusion protein in transgenic lines was clearly detectable by western blotting and immunohistochemistry. However, it had no obvious influence on spermatogenesis of the animals. So the transgenic lines were crossed with the gene trap line to generate the "rescued" males Lis1$^{GT/GT}$ transgene positive. So far 8 Lis1$^{GT/GT}$ /PGK2-Lis1 males were mated with females, but no sperms could be flushed out from the uterus and no pregnancies were found. The detailed analysis of spermatogenesis of these males is still in progress. Currently analysis of two different Lis1$^{GT/GT}$ /hEF-Lis1 lines is carried out.

P208

Clinical and genetic evaluation of missense mutation p.R390C in the ATR-X gene

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Mutations in the ATR-X gene (MIM *300032) cause X-linked alpha thalassemia mental retardation in males. Affected individuals present with a characteristic facial gestalt during infancy and show profound developmental delay and learning difficulties, in particular, in expressive language. Female carriers are usually physically and intellectually normal due to skewed X-inactivation. Most mutations in the ATR-X gene cluster within the exons encoding the two main functional domains of the protein. The few mutations reported outside these mutation hot spots are less severe, however, expressive speech is still very limited.

We report on a 7-year old boy who suffers from psychomotor developmental delay with dyslalia. Facial dysmorphism included coarse facial features, orbital bulging, hypertelorism and large ears. Additional abnormalities were microcephaly and paw-like hands. He was born at a weight of 1750 g as the 6th child of non-consanguine parents. Mutation analysis in the ATR-X gene detected no mutation in the hot spot regions. An extended sequence analysis, however, revealed the novel missense mutation p.R390C in the boy's DNA, that had been transmitted from his mother. Previously, missense mutation p.L409S was reported in the same region outside the mutation hot spots. It has been shown in silico that amino acid residues 390–440 fold into an α-helix, where an evolutionary highly conserved potential coiled-coil motif is embedded. Female carriers harbouring p.L409S showed skewed X-inactivation. In contrast, the boy's mother carrying p.R390C showed non-skewed X-inactivation in her blood cells. This indicates a less severe selective disadvantage for cells carrying the p.R390C allele on the active X chromosome as compared to p.L409S or classical ATR-X mutations. Apart from simple-mindedness and slight behavioural problems, the female carrier of p.R390C showed no further disease-associated features suggesting reduced expressivity of p.R390C in a heterozygous condition.

P209

Quantitative sequence analysis reveals incomplete nonsense-mediated decay (NMD) of mutant FBN1 transcripts in leukocytes

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We improved and used Sanger sequencing for quantification of SNP variants in transcripts and gDNA samples. This improved quantitative sequencing revealed highly reproducible relative allele frequencies (e.g. for a heterozygous gDNA 50.0±1.4%; P=0.05, and for a missense mutation-carrying transcript 46.9±3.7%; P=0.05) with a lower detection limit of 3–9%. Furthermore, it showed excellent accuracy (e.g. for a duplicated gDNA 66.6±2.2%; P=0.05) and linear correlation between expected and observed relative allele frequencies. This quantitative sequencing procedure, which can also be used for the quantification of CNVs, methylations, mosaicisms, and DNA pools, allowed us to investigate FBN1 transcripts in blood samples and fibroblast cell lines of patients with suspected Marfan syndrome not only qualitatively but also quantitatively. We identified a total of 19 novel and 18 known FBN1 sequence variants leading to a premature termination codon (PTC), 26 of which we analyzed by quantitative sequencing both at gDNA and cDNA levels. The relative amounts of PTC-containing FBN1 transcripts in fresh and PAXgene-stabilized blood samples were significantly higher (33.0±3.9% to 80.0±7.2%; P=0.05) than those detected in affected fibroblasts with inhibition of nonsense-mediated mRNA decay (NMD) (11.0±2.1% to 25.0±1.8%; P=0.05). In contrast, in fibroblasts without NMD inhibition no PTC-containing allele could be detected. Our results provide evidence for incomplete NMD in leukocytes and emphasize the importance of RNA-based analyses not only in FBN1 but also in other genes.

P210

Molecular and cytogenetic characterisation of human albumin transgenic goat fibroblasts as a source of nuclei in the somatic cloning

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The production of pharmaceutically important human proteins in the mammary gland of transgenic animals constitutes an important field of biotechnology.

In this study goats' fibroblasts were transfected by lipofection with transgene, which contained human gene encoding albumin under the

control of the tissue specific WAP promoter. Transfected fibroblasts were cultured with selective medium with blasticidine.
Transgene integration was examined by PCR method. Chromosomal aberrations were examined using the GTG-binding pattern. Fluorescence in situ hybridization (FISH) enabled the mapping of transgene specific DNA sequences.
Transgenic cells are going to be used as a source of nuclei in the experiments of obtaining transgenic goats by somatic cloning technique. The application of tissue specific WAP promoter allows to reduce the expression of the trangene to mammary gland. Human albumin is going to be found only in the milk of animals being accurately in the period of lactation. After the separation of albumin from milk components it would be applied in medical treatment.

P211

Posttranslational modifications of transthyretin aresSerum markers in patients with Mycosis Fungoides

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Primary cutaneous T-cell lymphoma (CTCL) comprise a heterogeneous group of non-Hodgkin´s lymphoma involving memory T-cells, predominantly of the CD4+ T-helper subpopulation, which preferentially migrate into skin. CTCL represents the most common primary cutaneous lymphoma (65%) whereas Mycosis Fungoides (MF) represents the most common disease (50% of all primary cutaneous lymphomas).
Up to now only a few biomarkers are described in CTCL espacially in MF.For serum biomarker discovery in patiens with Mycosis Fungoides 25 samples and 26 unaffected control sera were applied on IMAC30 ProteinChip arrays. The detected spectra were bioinformatically analysed using XLminer software resulting in a list of differentially expressed proteins. Some of these were identified with a specificity of 100% and a sensitivity of 82.6% as Transthyretin (TTR) or Transthyretin modifications. Further analysis of differentially expressed proteins might give more insight into this disease.

P212

Late-onset PEO and proximal myopathy in a patient with mutations in the POLG2 gene

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Objective: Polymerase gamma 1 (POLG1) mutations are a frequent cause of both autosomal dominant and recessive complex neurological phenotypes. In contrast, only a single pathogenic mutation in one patient was reported in POLG2, the gene encoding the p55 accessory subunit of pol gamma, so far.
Patient and methods: Here we describe the clinical, histological, biochemical and genetic data of a 62 year-old woman, carrying two novel heterozygous sequence variants in the POLG2 gene.
Results: First symptoms started at age 30 years with bilateral ptosis, followed by exercise intolerance and proximal weakness in the late forties. CK was occasionally elevated up to 500 U/L. Muscle histology and respiratory chain enzyme activities were normal. Southern blot and long range PCR detected multiple mtDNA deletions, but no depletion in muscle DNA. Sequencing of POLG1, Twinkle, OPA1 and ANT1 did not reveal any pathogenic mutations. Two novel heteroallelic changes were detected in POLG2, c.1191+7_1191+8insT in intron 6, and an insertion of 24 bp in exon 7 (c.1207_1208ins24). Transcript and segregation studies in the family revealed that the intronic change (c.1191+7_1191+8insT) is unlikely to be pathogenic, whereas the 24 bp insertion into exon 7 causes missplicing and loss of exon 7 on cDNA.
Conclusions: POLG2 mutations may represent a rare cause of autosomal dominant PEO. The clinical presentation of our patient is in keeping with the previously described clinical phenotype.

P213

Four patients with speech delay, seizures and corpus callosum abnormalities sharing a 0.440 Mb deletion in region 1q44 containing the HNRPU gene

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Structural genome aberrations are frequently associated with highly variable congenital phenotypes involving mental retardation and developmental delay. Although some of these aberrations may result in recognizable phenotypes, a high degree of phenotypic variability often complicates a comprehensive clinical and genetic diagnosis. We describe four patients with developmental delay, seizures, hypotonia, hypertelorism, an abnormal corpus callosum and other CNS anomalies who share deletions of chromosomal region 1q44. High resolution oligonucleotide and SNP array-based segmental aneuploidy profiling showed that these four patients share a 0.440 Mb interstitial deletion, which does not overlap with previously published consensus regions of 1q44 deletions. Two copies of AKT3 and ZNF238, two previously proposed dosage sensitive candidate genes for microcephaly and agenesis of the corpus callosum, were retained in our patients. The deletion shared by our patients contained the FAM36A, HNRPU and EFCAB2 genes. Since the HNRPU gene is involved in the regulation of embryonic brain development, this represents a novel plausible candidate gene for the combination of developmental delay, speech delay, hypotonia, hypo- or agenesis of the corpus callosum and seizures in patients with 1q44 deletions. Since only one of the two patients with deletions including the ZNF124 gene showed a Dandy Walker complex, mere hemizygosity for this gene is not sufficient to cause this anomaly. This study demonstrates that high resolution molecular cytogenetic analyses provide a valuable adjunct for dissection of clinical phenotypes and identification of candidate genes in patients with complex clinical phenotypes.

P214

A germline mutation in the hypocretin (HCRT) gene in a patient with narcolepsy

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Narcolepsy is a sleep disorder characterized by attacks of disabling daytime drowsiness and low alertness. The normal physiologic components of REM sleep, dreaming and loss of muscle tone are separated and also occur while the subject is awake, resulting in half-sleep dreams and episodes of skeletal muscle paralysis and atonia. Patients report from excessive daytime sleepiness and nocturnal sleep disruptions. An association of the disease with the HLA DRB1*1501 and DQB1*0602 genotypes has been described. Pathological findings are decreased numbers of hypocretin-secreting neurons in the hypothalamus and decreased hypocretin levels in cerebro-spinal fluid. Onset of narcolepsy usually occurs in the second decade of life, although earlier and later onset has been reported. The prevalence is estimated of approximately 1/4000 in Caucasians. Peyron and colleagues described a missense mu-

tation in the hypocretin (HCRT) gene, predicted to be pathogenic, in a patient with narcolepsy with a dominant mode of inheritance (Nat Med 2000;9:991–997). This has been the only mutation in this gene reported so far. Here we report a 47-years old male patient suffering from increased tiredness in the daytime, numbness, walking problems and decreasing efficiency. He reported to fall on sleep during driving car sometimes and the benefit of afternoon nap. Multiple sclerosis has been diagnosed four years ago. He is suffering from schizoaffective mixed psychosis for 15 years. HLA genotype suggests presence of narcolepsy. Family history reveals increased drowsiness in his father. Sequence analysis of the coding region of HCRT from genomic DNA revealed a heterozygous base substitution affecting the start codon and, therefore, is predicted to abolish translation. It presents the second HCRT germline mutation reported in a narcolepsy patient and provides further evidence for an identifiable genetic cause in some of the cases.

P215

Promoter studies of the Fanconi anemia core complex genes

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Fanconi anemia (FA) is a rare genomic instability disorder with the frequent presence of congenital malformations and bone marrow failure. Other characteristic features include predisposition to malignancies and cellular hypersensivity to DNA-interstrand crosslinking agents such as mitomycin C. Mutations in at least 13 genes are underlying as many complementation groups of the disease. Eight of the FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and other components such as the recently identified FAAP100 protein assemble in a nuclear complex, the FA „core complex". Identification and characterization of their gene promoters is essential for understanding regulation at the transcriptional level, including intergenic regulation. Promoter regions were suggested by in silico methods. All of these are located at the 5' termini of the FA genes, extend over approximately 1 kb at the 5' ends and are highly conserved among vertebrates. All of the putative promoters belong to the group of TATA-less promoters typical for housekeeping genes. They are characterized by a high CG content up to 85%. Well-defined major transcription start sites (TSS) were surrounded by arrays of alternative weaker TSSs, resulting in broad distributions with dominant peak shape (PB). Our results confirm the use of these specific cap sites, predicted in the database of transcriptional start sites. The FA gene promoters consist of three parts. Relatively repressor sequences with a maximum expansion of 665 bp, were detected in the 5' regions. Middle regions of up to 570 bp provided the highest transcriptional activity. Minimal promoters were delineated in the 3' region. All of the FA gene promoters act in a monodirectional way. They represent members of a group of strong promoters comparable to the SV40 promoter. Our results also show the accumulation of similar combinations of transcription factor binding sites among the FA core complex genes, especially around the 3' region of the promoter.

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Novel loci and candidate genes for autism spectrum disorder detected by SNP array based segmental aneuploidy screening

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Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by concomitantly impaired reciprocal social interaction, communicative deficits, and restricted and repetitive behavioral patterns. ASD occurs in syndromic forms (e.g. FRAX, del(22q11), Rett), and as non-syndromic cases frequently involving segmental aneuploidies. Recently, array-based genome-wide screens have demonstrated frequent copy number variation in non-syndromic ASD. Screening 56 patients (8 girls and 48 boys) with ASD and additional major or minor anomalies with the Infinium HumanHap300 SNP platform (Illumina, Inc., San Diego, CA) we found in 11 patients 9 regions with hemizygous losses and 4 with gains containing brain-expressed genes. But for one gain, none of these segmental aneuploidies were flanked by segmental duplications. Only one locus has previously shown significant association with ASD. In two families overlapping aneuploidies for part of the MCPH1 gene (in region 8p23.1) have been found. This suggests that changes in copy number of MCPH1 are a susceptibility factor for ASD. In two families overlapping de novo deletions of part of the DOCK4 gene (on 7q31.1), which regulates dendritic development in hippocampal neurons, were found. In two unrelated boys with Asperger syndrome we found maternally inherited aneuploidies of the proximal part of region 15q11.2. A third boy with a maternally inherited loss of 15q11.2 proved to have the Smith-Lemmli-Opitz-Syndrome. We conclude that SNP array-based screening of ASD patients uncovers an appreciable number of novel segmental aneuploidies containing brain-expressed genes that may represent clues toward etiological pathways of perturbed brain development likely to result in ASD.

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Knockout of the splicing factor Sfrs10 results in early embryonic lethality in mice and in upregulation of SmnΔ7 in vitro

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The SR-related splicing factor SFRS10 is like other members of this family non-essential for the splicing reaction. SFRS10 facilitates the inclusion of exon 7 into the SMN mRNA by binding to a GA-rich exonic splicing enhancer (ESE) localised in the central part of exon 7. The functional loss of the SMN1 gene causes autosomal recessively inherited spinal muscular atrophy (SMA). SMA is a neurodegenerative disorder caused by a progressive loss of α-motor neurons in the anterior horns of the spinal cord. The phenotypic severity correlates with the copy number of a second gene - SMN2. Due to a translationally silent mutation in exon 7, ~90% of the transcripts generated from SMN2 lack this exon while only 10% of the transcripts are correctly spliced. The correctly spliced full-length transcripts (FL) encode functional SMN protein. Overexpression of the splicing factor SFRS10 restores the splicing pattern of the SMN2 in vitro. Due to that effect SFRS10 is a promising candidate for an in vivo modulation of SMN RNA processing.

Little is known about SFRS10 and its whole spectrum of action in vivo. To address that issue we have generated a knockout of the Sfrs10 in mice via the Cre/loxP system. Even though heterozygous knockout mice exhibited reduced protein levels, these animals revealed no obvious phenotype. The homozygous ubiquitous deletion of the splicing factor resulted in early embryonic lethality around E7.5. Deletion of Sfrs10 in murine embryonic fibroblasts was used to estimate the potential of Sfrs10 on Smn splicing. A splicing isoform of the murine Smn - SmnΔ7 - was detected at relatively low levels. Sfrs10 deletion resulted in 3–4fold increase of that isoform whereas quantification of FL-Smn transcripts and Smn protein levels remained unchanged after Sfrs10 deletion.
Our results indicate an essential role of Sfrs10 during embryogenesis whereas it seems to be dispensable in certain tissues and cells such as fibroblasts also with regard to FL-Smn splicing.

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Studies on the cytoplasmic polyglutamine binding protein 1 (PQBP1) for understanding its role in mental retardation

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Mutations in the polyglutamine binding protein 1 (PQBP1) gene cause X-linked mental retardation. Identical and similar mutations resulted in high clinical variability, ranging from moderate mental retardation to much more severe forms, including microcephaly, short stature and spasticity. The characterisation of the PQBP1 complex is a logical starting point to gain more insight into its cellular functions and to unravel the pathomechanism of the disease. We have pulled-down the PQBP1 complex using a human neuronal cell line, which stably expresses tagged PQBP1, and analysed it by ESI-TOF mass spectrometry. The list contained proteins, which play an established role in mRNA metabolism. We have concentrated on these and have confirmed PQBP1's interactions with all newly found partners investigated, including polypyrimidine tract-binding protein-associated splicing factor (PSF), KH-type splicing regulatory protein (KSRP), DEAD box polypeptide 1 (DDX1) and Caprin-1 by co-immunoprecipitation. In addition, we have investigated if these interactions are dependent on RNA. Using mouse primary neurons we could show that PQBP1 and its interactors colocalise in cytoplasmic RNA granules, which play a role in the localisation and translation of specific mRNAs in dendrites and synaptic plasticity. After the induction of oxidative stress, the PQBP1 complex is recruited to stress granules, which are organelles containing transiently silenced messenger RNAs. In addition, a portion of PQBP1 fractionates with polysomes like it is known for the Fragile-X mental retardation protein (FMRP), a translational repressor. Taken together, our findings strongly suggest that PQBP1 plays a role in RNA metabolism in the brain and might influence translation regulation as well as the localisation of certain transcripts in neurons.

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INPP4B, a candidate gene for a rare form of autosomal dominant mental retardation

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Mental retardation (MR) affects 2–3% of the population. For the identification of novel MR genes both balanced and unbalanced chromosomal aberrations represent powerful tools. We report two patients with MR and chromosomal translocations with a common breakpoint region in 4q31.21. Female patient 1 carries a de novo 4;7 translocation [46,XX,t(4;7)(q31.21;q11.23)]. She presented with failure to thrive and showed a delay in speech and motor development. Mapping of the breakpoint regions by fluorescence in situ hybridization identified BAC clones overlapping the two breakpoints. The breakpoint in 4q31.21 was shown to directly disrupt the INPP4B gene. A de novo 4;14 translocation with the karyotype 46,XY,t(4;14)(q31.1;q32.2) was identified in a mentally retarded boy who had in addition a septal defect, cryptorchidism, and mild facial dysmorphic signs related to a mandible malformation. By array CGH, we detected a heterozygous deletion of ~6.1 Mb in 4q31.1q31.22, one of the translocation breakpoint regions. The deletion covers 24 genes among which the INPP4B gene was present. The inositol polyphosphate 4-phosphatases type I (INPP4A) and type II (INPP4B), two highly homologues proteins, are strongly expressed in brain and catalyze the hydrolysis of the 4-position of phosphatidylinositol 3,4-biphosphate [PtdIns(3,4)P_2]. PtdIns(3,4)P_2 directly binds and activates AKT proteins, which play a critical role in controlling various cell physiological processes. Notably, Akt3 knockout mice show a significant postnatal reduction in brain size and weight, and haploinsufficiency of AKT3 has recently been suggested to cause mental retardation and postnatal microcephaly. In line with this, Inpp4a-deficient mice have a severe neurological phenotype characterized by postnatal cell loss in the cerebellum and hippocampus. Taken together, we hypothesize that haploinsufficiency of INPP4B might be associated with MR in the two translocation patients.

P220

Homozygosity mapping for non-syndromic autosomal recessive mental retardation in two consanguineous families from Kuwait and Turkey

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Mental retardation (MR, IQ<70) affects approximately 1–3% of the general population and can be divided into syndromic and non-syndromic forms. In contrast to syndromic MR, only a few causative genes have been reported for non-syndromic MR (NSMR). It has been postulated that almost a quarter of undiagnosed NSMR cases have an autosomal recessive etiology. Studies of consanguineous families with affected children represent the best available strategy for the identification of further genes.
We recruited two consanguineous families from Kuwait and Turkey affected by NSMR. Both families have a total of five children. Two sons from the Kuwaiti family have severe mental retardation. Two sons and one daughter from the Turkish family have severe mental retardation which began following ~6 years of normal development.
We first excluded cytogenetic abnormalities, fragile (X) syndrome and small deletions and duplications (using Illumina 550 K SNP-chip). On the basis of the family history we assumed an autosomal recessive etiology and performed genome wide homozygosity mapping using the Affymetrix 10 K SNP-chip. We were able to define one homozygote region in each of the families. The affected children of the Turkish family were homozygous for ~22 Mb at 8p12-q12 (36.8 58.1 Mb). The Kuwaiti family showed a risk region at 13q13-q21 (between 36.0 and 65.3 Mb). We then verified the homozygous regions and their boundaries by genotyping STR-markers.
Our study defines circumscribed risk regions and thus represents an essential step in the identification of autosomal recessive genes for NSMR. Our goal is to facilitate the development of diagnostic strategies and to understand the complex neuronal network which underlies

normal and abnormal brain functioning. Our ongoing work aims to identify the causal mutations by sequencing all genes in the homozygous regions (around 1.000 exons per region).

P221

Bdp1 – a candidate gene causing atypical spinal muscular atrophy (SMA) with brain atrophy?

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Spinal muscular atrophy (SMA) is an autosomal recessive, neurodegenerative disorder characterized by the degeneration of α-motor neurons in the anterior horns of the spinal cord. Based on the age of onset and severity of the phenotype, SMA has been classified into type I to type IV. In rare cases SMA patients exhibit additional atypical features such as axonal neuropathies or brain atrophy. These phenotypes correlate with large deletions in the SMA region (5q13). Marker analysis showed deletions in genes distally to the SMA region including the BDP1 ("B double prime 1") gene. BDP1 is a subunit of the transcription factor IIIB complex and plays an important role in transcription initiation of genes transcribed by RNA polymerase III. BDP1 is ubiquitously expressed with notable abundance in the cerebellum. We found a "de novo" deletion of one BDP1 allele in two patients who showed a SMA phenotype plus brain atrophy (based on Southern blot analysis) and hypothesized that haploinsufficiency of BDP1 may cause brain atrophy and neuronal dysfunction.
To test this hypothesis we developed conditional knockout strategy for the murine Bdp1 via the Cre/loxP system. We have already generated heterozygous knockout mice ($Bdp1^{+/-}$). The $Bdp1^{+/-}$ mice seem to be phenotypically normal compared to wildtype littermates and first analyses are in progress. The final goal is to generate and analyse mice lacking the Bdp1 ubiquitously ($Bdp1^{-/-}$) as well as tissue specific.
To investigate the role of the BDP1 protein in mammals in more detail we started to characterize the murine Bdp1 on RNA and protein level. Via RT-PCR the expression levels in different tissues are planned to be analyzed. From the human BDP1 locus different splice variants of the transcript and protein isoforms are generated. Analysis of protein fractions of different tissues via Western Blot analyses will give us more information about possible protein isoforms.

P222

Breakpoint characterization of large deletions in FBN1 in patients with Marfan syndrome

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Marfan syndrome (OMIM 154700) is caused by heterozygous mutations in the FBN1 gene. Cardinal features involve the ocular, cardiovascular and skeletal systems. Overlapping syndromes such as the Loeys-Dietz syndrome (LDS1A, OMIM 609192; LDS1B, OMIM 610168; LDS2A, OMIM 608967; LDS2B, OMIM 610380) are caused by mutations in the TGFBR1 or TGFBR2 genes and show either a Marfan-like phenotype (LDS2A, LDS2B) or a more severe phenotype characterized by arterial tortuosity and aneurysms as well as craniofacial involvement (LDS1A, LDS1B).
In 100 patients, we applied MLPA (multiplex ligation-dependent probe amplification) to screen for large deletions in the FBN1 and TGFBR2 genes. All patients were tested negative for a mutation in FBN1 by sequencing. 50 of these patients were also tested for a mutation in the TGFBR1 and TGFBR2 genes by sequencing, and were found to be negative for such a mutation. To date, we have identified six large deletions in the FBN1 gene (exons 24–26, 50–54, 55–58, 58–63, 1–65, and 6–65).
Here we show the determination of breakpoints for two large deletions of the FBN1 gene: deletion of exon 1 to 65 and exon 6 to 65 according to MLPA. The extension of both FBN1 gene deletions in the 3' and/or 5' direction was determined by high resolution array CGH (3 probes per 1000 bp). Breakpoint spanning PCR confirmed the deletion and allowed the exact breakpoint determination by bidirectional sequencing. Deletion sizes were 256,594 bp (exon 6 to 65 of FBN1 and the DUT gene) and 676,354 bp (FBN1, DUT, SLC12A1, MYEF2 and SLC24A5 gene). We show that neither patient presented clinical manifestations other than classical MFS.
We could show that high resolution array CGH is highly suitable for the subsequent characterization of large deletions and allows for straightforward breakpoint determination by simple PCR and sequencing techniques.

P223

Elucidating the basis for molecular interaction of reticulon1 with spastic paraplegia proteins spastin and ZFYVE27

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Reticulon 1 (RTN1) belongs to reticulon family of proteins, which constitute a family of membrane proteins localized primarily to the endoplasmic reticulum (ER) membrane. Human RTN1 has three isoforms namely RTN1-A, RTN1-B and RTN1-C. Human RTN1-A and B but not RTN1-C were shown to interact with the AP-2 adaptor complex, a key player in endocytosis. Whereas RTN1-C, the small isoform of RTN1 was shown to interact with several SNARE proteins and these studies suggest that RTN1-C might play a key role in membrane fusion events such as exocytosis. Taken together these observations indicate that RTN1 plays diverse role both in endocytosis and exocytosis through different isoforms.
We have previously reported the interaction of reticulon1 (RTN1) with spastin using in vitro and in vivo experiments. Spastin encoded by SPG4 gene is most commonly mutated in autosomal dominant hereditary spastic paraplegia (AD-HSP). Interestingly, our recent Yeast Two Hybrid screening identified RTN1 as interacting protein of ZFYVE27, another HSP gene (SPG33). Furthermore, we could validate the interaction between ZFYVE27 and RTN1 through direct Yeast two Hybrid assay, co-immunoprecipitation and co-localization studies. Interestingly, over-expression of RTN1-C induced the neurite like morphology in cultured fibroblasts. This is in relevance with the fact that ZFYVE27 also promotes the neurite formation and thus indicates that ZFYVE27 and RTN1 indeed participate in the same biological pathway. RTN1 interaction with ZFYVE27 and spastin thus highlights that abnormalities in endocytosis/exocytosis could lead to the pathomechanism of neurodegenerative diseases such as HSP. Generation of loss of function mouse model for Rtn1 might help us to understand the function of Rtn1 in greater detail in conjunction with neurodegenerative diseases. Towards this end, we have generated a knock-out construct for Rtn1 using BAC mediated homologous recombination and further studies are in progress.

P224

Expression profiling of murine Scyl1 and splice variants by real-time PCR and RNA in situ hybridisation

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In mdf-mice, a 1bp-insertion in the Scyl1 gene on chromosome 19 induces nonsense-degrading of the Scyl1 RNA leading to an autosomal-recessive muscle-deficiency. The Scyl1 protein is highly conserved in

eukaryotes, mainly expressed in neurons and enriched at central nervous system synapses and neuromuscular junctions. There is yet only few information on its exact function, but it seems to be a component of the nucleocytoplasmic transport machinery. The phenotype of mdf-mice comprises tremor, degeneration of specific neurons in distinct regions of the brain and a proggressive paralysis of the hindlimbs, caused by neurogenic atrophy of the skeletal muscle. Additionally, the affected animals are slightly smaller than wild type littermates, rarely fertile and have a reduced lifespan of 8 months. These traits turn the mdf-mouse into a putative model for motor-neuronal deseases, particularly cerebellar athrophy. As part of the functional analysis of Scyl1, we profiled and localized the expression of the full-length RNA and the new transcipt-variants mSV13 and mSV15, which were discovered by our group. To profile the expression pattern over a range of different tissues, we used variant-specific TaqMan-probes in real-time PCR. The results allow the comparison of the expression levels of the analyzed tissues, as well as the splice-variants themselves and show a significant deviation for variant mSV15, namely a very low expression in the testis and a remarkably high one in the lung. As for the localization of transcripts, RNA in situ hybridisations were performed on brain and testis preparations, showing high expressions in the cortex, the hippocampus, the brain stem, and in purkinje cells of the cerebellum for all variants, as well as in sertolli cells of the testis. In this tissue, no mSV15 expression could be detected, verifing the results of the TaqMan-assay.

P225

Expression analysis of genes within the dyslexia susceptibility locus DYX2

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DYX2 on chromosome 6p21-p22 is the most replicated susceptibility locus for dyslexia (reading and spelling disability). DYX2 contains two gene clusters, KIAA0319/TTRAP/THEM2 and VMP/DCDC2/KAAG1, and each of those clusters harbors one candidate gene, namely KIAA0319 and DCDC2 (doublecortin domain containing 2). We have previously reported association between a two-marker haplotype in intron 7 of DCDC2 (rs793862 (A/G) and rs807701 (C/T)) and dyslexia (Schumacher et al. 2006 Am J Hum Genet). We hypothesized that the risk haplotype (A-C, rs79-rs80) could lead to a lower expression of DCDC2 as compared to the non-risk haplotype (G-T, rs79-rs80). Previous studies have already identified that a lower expression of DCDC2 results in an abnormal neuronal migration (Meng et al. 2005 PNAS), which is believed to be one neurological reason for the dyslexia phenotype.
In the present study, we analyzed the expression of the six genes located within the two gene clusters in 15 EBV-transformed lymphocyte cell lines carrying different rs79-rs80-haplotypes. We found that the "short" transcript of DCDC2 can be detected at a higher level in cell lines with the risk haplotype as compared to non-risk haplotype carriers. In contrast, neither the "long" transcript of DCDC2 nor any other of the investigated genes showed an effect of rs79-rs80-haplotypes on expression levels. Our data suggests that the "short" variant of the DCDC2-transcript contributes to the development of dyslexia by a yet unknown mechanism.

P226

Novel, de novo and known mutations in the SCN1A gene are associated with a wide range of clinical phenotypes

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Epilepsy is a heterogeneous group of multifactorial diseases, the vast majority determined by interactions between many genes and environmental factors; however, there are epilepsy syndromes that can be caused by a single gene mutation and are frequently inherited according to classical mendelian genetic principles. The myoclonic astatic epilepsy, the severe myoclonic epilepsy in infancy, and the severe idiopathic generalized epilepsy of infancy with generalized tonic clonic seizures belong to the myoclonic epilepsies of early childhood, a subgroup of the genetically determined idiopathic generalized epilepsies. Thus, the discovery of SCN1A mutations in severe myoclonic epilepsy of infancy and in generalized epilepsy with febrile seizures plus has led to the establishment of epilepsy as a disorder of ion channel function and, furthermore, has led to the introduction of genetic tests. In particular, Na(v)1.1 (gene symbol SCN1A) is registering with more than 330 mutations to date. The associated phenotypes range from benign febrile seizures to extremely serious conditions, such as Dravet´s syndrome (SMEI).
In our study we have analysed 104 patients most frequently diagnosed as Dravet's syndrome, but also patients with seizures after vaccination. Summarized we found 2 entire gene deletions, 5 nonsense-, 11 missense-mutations, 5 small deletions comprising also the branching site and 7 yet unclear intronic mutations. Taking into account only the clear disease causing mutations (23) they account for 22% of these patients. The spontaneous mutations account for the majority of these patients which has some impact for genetic counselling.
On a long-term basis, there is hope that progress in the pharmacological treatment will result on the understanding of the pathogenesis at the level of alterations of the ion channels caused by mutation.

P227

Detection of a 7 Mb de novo deletion in a patient with distal Athrogryposis Type 2B (DA2B)

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We report on a 5 year old male patient presenting distal Athrogryposis with club feet, ulnar deviation and slight camptodactyly as well as facial dysmorphism including high forehead, small mouth, broad nasal bridge, epicanthus, high palate, brachycephalus, relative short neck, and dysplastic ears. Although motor development was delayed, speech and mental development was considered normal. The deformation of hands and feet and the facial features correlated with Type 2B of distal Athrogryposis according to the Bamshad classification (Krakowiak et al., 1998). Using an Affymetrix 250 K Nsp Gene Chip array we found a 7 Mb deletion located on chromosome 8q21.11–8q21.13. In this region 662 SNPs and 24 refseq genes are situated. FISH analyses showed a de novo deletion of the BAC clone RP11–89J14. Up to now mutations in three genes (MYH3, TNNT3 and TNNI2) are described as causal for the phenotype of distal Athrogryposis Type 2B. These genes encode for proteins that are relevant in muscle formation and contraction. In silico analysis of the gene content of the deletion showed that none of the 24 genes is directly involved in these processes. However, some of them are possible candidate genes because of their known function. For example ZFHX4, which encodes a homeodomain- zink finger protein, is published in connection with hereditary congenital ptosis (Nakashima et al., 2008), and possible involved in neural and muscle differentiation (Hemmi et al., 2006). This case shows that large genomic deletions are not necessarily leading to mental retardation but may cause any type of genetic disorder.

P228

Identification and functional analyses of highly conserved regions in the NIPBL gene

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Cornelia de Lange Syndrome (CdLS) is a dominantly inherited multisystem developmental disorder with a characteristic face, short stature and mental retardation. Mutations in three genes (NIBPL, SMC1A, SMC3) that encode functional components of the sister chromatid cohesion complex have been demonstrated in 60% of cases. Mutations in SMC1A or SMC3 cause a milder phenotype and are present in about 5% of patients. NIPBL mutations are present in 55% and are consistent with a model of haploinsufficiency.
Because mutations any of these genes are not detectable in 40% of patients with CdLS, we screened for mutations in highly conserved regions and regulatory elements of the NIPBL gene. Utilizing computer-based promoter prediction, we identified a putative promoter element of about 5 kb, including the 5´ part of intron 1. This region was amplified and inserted into a luciferase reporter plasmid and its activity was verified in several transiently transfected cell types. Using a series of truncated promoter constructs, we identified a 1 kb region as the promoter region, whereas the core promoter element was narrowed down to 140 bps upstream of exon 1. Initial analyses of 13 patients identified three polymorphisms within the promoter region, two of which are not reported but were found in the unaffected parents.
Similarly, in silico analyses identified 11 evolutionary conserved non-coding regions of NIPBL and sequence analysis of 96 patients revealed a single de novo mutation within the first intron. To analyze the functional significance, this region was also tested in the luciferase reported assays and, consistent with a functional role, we found that it alters promoter activity. Studies are ongoing to analyze additional patients and the functional relevance of these NIPBL alterations.

P229

Molecular karyotyping reveals CDKL5 deletions as frequent cause of mental retardation of unknown origin

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In order to further elucidate the causes of mental retardation we performed a genome-wide copy number variant (CNV) survey in 160 patients with mental retardation of unknown origin using the Affymetrix 6.0 SNP platform.
Surprisingly, two of the 160 patients (1.3%) showed microdeletions involving the CDKL5 gene. Mutations in this gene were only recently identified as causative for an atypical variant of Rett-syndrome characterized by intractable early-onset seizures often accompanied by Rett-like features.
Patient 1 is a 12 months old girl, born after uncomplicated pregnancy, with secondary microcephaly, severe mental retardation, severe hypotonia and drug resistant seizures. Karyotyping, testing for Angelman syndrome and MECP2-analysis showed normal results. By array analysis a 230 kb deletion on Chromosome Xp22.13, including parts of the CXorf20 gene, the complete SCML2 gene and exon 1 of the CDKL5 gene was detected.
Patient 2, a 7 years old girl was born after an uncomplicated pregnancy and is severely mentally retarded. She had convulsions in her first year of life and shows stereotypical movements of her hands. Her speech is nasal and limited to a few words, eye contact is possible. MECP2 and TCF4 testing was normal. In this patient we detected a 157 kb deletion on the X-chromosome, including a large part of the CDKL5 gene, furthermore the RS1 gene and parts of the PPEF1 gene.
Our findings demonstrate for the first time, that CDKL5 microdeletions are a major pathomechanism in female patients with severe mental retardation and seizures and seems to constitute a relatively frequent cause of mental retardation.

P230

DNA and histone methyltransferase overexpressing mice as a model for epigenetic diseases

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Gene expression is regulated, among others, by epigenetic mechanisms. One of these mechanisms is the methylation of DNA and/or histones. Erroneous methylation can cause a vast spectrum of diseases. In this study two mouse models are created, in order to investigate if the overexpression of enzymes that are responsible for the methylation of DNA or histones is sufficient to cause erroneous methylation and disease.
One CAG promoter driven transgene each was cloned for the DNA methyltransferase Dnmt1 and for the histone methyltransferase G9a, respectively. The transgenes were tested for in-vitro functionality in cell culture using 3T3 murine fibroblasts. Because the ubiquitous overexpression of Dnmt1 has been reported to be embryonic lethal, conditional transgenes were made using the Cre-LoxP system. This technique allows the initial expression of the marker protein EGFP only. Upon cross-ins with Cre-recombinant mouse lines, the EGFP sequence is cut out and degraded. This leads to the expression of the methyltransferase under control of the CAG promoter. To easily distinguish the endogenous methyltransferases from the transgenic ones, an RGS-His and an HA-tag were added to the N-terminal end of the Dnmt1 and G9a transgene, respectively.
Pronucleus injections with the Dnmt1 transgene resulted in six founder lines verified by PCR, Southern Blot and EGFP fluorescence. These founders were crossed in with CMV-Cre and vav-Cre mouse lines to investigate the ubiquitous and the hematopoietic overexpression of the methyltransferase, respectively. The cross-ins resulted in 7 CMV-Cre and 14 vav-Cre offspring that carry a recombined version of the Dnmt1 transgene. Preliminary TaqMan analysis of expression shows an approx. 2-fold Dnmt1 overexpression in liver and kidney, but not in the spleen of recombined transgenic mice.
Upcoming analyses will test the in-vivo functionality of the transgenic protein and the phenotypic consequences of Dnmt1 overexpression.

P231

Investigations on the pathomechanisms of the P56S VAPB mutation and description of the first European ALS8 case

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The P56S mutation in the vesicle-associated membrane protein-associated protein B (VAPB/ALS8) causes an autosomal dominant mo-

tor neuron disease which has thus far only been described in Brazil [Nishimura et al., AJHG 75, 2004]. The phenotypes range from late-onset distal to proximal SMA and atypical and typical ALS. We describe a German patient who presented with onset of a lower motor neuron disorder at the age of 41 years with proximal leg muscle weakness and fasciculations, but no upper motor neuron signs. The deceased mother and maternal grandfather had suffered from a similar disease. Haplotype analysis indicated that the mutation was not derived from the same founder as the one in the Brazilian families. Thus, the P56S VAPB mutation can be found in non-Brazilian patients and is not due to a single founder.The pathomechanisms of ALS8 are largely unknown. VAPB interacts with proteins involved in the exocytosis of synaptic vesicles [Nishimura et al., BBRC 254, 1999]. VAPB also seems to play a role in the unfolded protein response [Kanekura et al., JBC. 281, 2006] and in the transport of ceramide from the ER to the Golgi apparatus [Kawano et al., JBC 281, 2006]. Mutated VAPB aggregates when expressed in cell culture models and disrupts the interaction of VAPB with several proteins [Mitne-Neto et al., Protein Expr Purif. 55, 2007]. We found that mutated VAPB forms aggregates within transfected cells including the motor neuron cell line NSC-34 whereas wild-type (wt) VAPB does not. In cells co-transfected with wt and mutated VAPB, the wt protein is recruited into aggregates by mutated VAPB, explaining the dominant mode of inheritance of ALS8. Furthermore, the mutant protein is strongly polyubiquitinylated. Accumulated polyubiquitinylated P56S VAPB might obstruct the proteasomal degradation pathway. Finally, we discovered five alternative transcripts of VAPB in cDNAs from different human tissues. The role of these transcripts is currently under investigation.

P232

A novel Fanconi Anemia candidate gene: EMSY

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Fanconi Anemia (FA) is a rare autosomal or X-chromosomal recessive disease with great genetic and phenotypic heterogenity. FA is characterized by bone marrow failure, high cancer risk and various, non-obligatory, yet typical developmental anomalies. Hypersensitivity of FA cells to DNA crosslinking agents such as mitomycin C (MMC) results in chromosomal breakage and G2-phase arrest in the cell cycle. To date, 13 FA genes FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M and -N have been reported. Their products and additional proteins interact in the FA/BRCA DNA damage response network. Because there are still FA patients who cannot be assigned to any of the reported complementation groups, it is hypothesized that further FA genes must exist. EMSY was first reported as a BRCA2-interacting protein and appeared as one of the most promising FA gene candidates. This protein could represent a link between the I/D complex, whose monoubiquitination is a key step of the FA pathway, and downstream FA proteins that initiate translesion synthesis and homologous recombination. Cells with down-regulated EMSY expression show FA-typical MMC sensitivity. Following DNA damage, EMSY is recruited to nuclear foci containing proteins like γH2AX and BRCA2, indicating activation of the FA/BRCA pathway. In this study we screened more than 20 unassigned downstream FA cell lines for EMSY by Western Blotting. While all of them revealed positive bands, EMSY was sequenced in lines with fainter bands. Extensive alternative splicing, but no mutations were identified to date. Potential interaction between EMSY and FANCD2 prompted us to screen another group of FA cell lines for EMSY mutations. These lines had been assigned to complementation group D2 by Western Blotting, but FANCD2 mutations remained elusive despite reasonable search. These EMSY screens are underway. Nevertheless, interaction studies and drug sensitivity profiles of knockdown cells recommend EMSY as a potential new FA gene.

P233

Fraser syndrome – molecular and phenotypic spectrum

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Fraser syndrome (FS; OMIM 219000) is characterized by cryptophthalmos, cutaneous syndactyly, renal, genital, laryngeal and other malformations. Mutations of FRAS1 or FREM2 have been reported in patients with FS, but in a recent study more than half of the patients had no detectable mutation in either of these genes (van Haelst et al. Am J Med Genet 2008). FRAS1, FREM1, and FREM2 form a macromolecular ternary complex located at the basement membrane, which is involved in the structural adhesion of the skin epithelium to its underlying mesenchyme in the embryo as well as in kidney development.
Patients and methods: 23 unrelated patients with clinically typical or probable FS were investigated by direct sequencing of the genes FRAS1 and FREM2.
Results: Mutations (mostly truncating ones) in either gene were identified in 20 patients (17 FRAS1, 3 FREM2). In 2 patients, a disease-causing mutation could only be identified on one FRAS1 allele. No mutation was found in 3 patients. All of them had symptoms compatible with FS, but not the complete phenotype. Most mutations were novel. Two recurrent mutations were found in patients of Western European and Slavic origin, respectively.
Conclusion: The proportion of mutation-positive cases is significantly higher in this cohort than previously published. This is likely due to patient ascertainment. FRAS1 mutations are account for the majority of FS cases. Affected patients show significant clinical variability, but their phenotype does not significantly differ from patients with mutations of FREM2.

P234

Lack of association between neprilysin (NEP) gene polymorphisms and Complex Regional Pain Syndrome (CRPS) in a German cohort

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Complex Regional Pain Syndrome (CRPS) is a chronic neurological disorder chracterized by disabling pain, swelling and impairment of motor function. The disorder usually develops after minor trauma or surgery but also a spontaneous onset has been described. Patients with CRPS reveal enhanced sensitivity to experimentally applied substance P (SP), a neurokinin responsible for neurogenic inflammation. The extended sensitivity is even present at symtome-less stages of the disease. A genetic background for the disease is assumend based on some familial cases and studies describing association of HLA antigens and CRPS. A functional candidate gene approach prompted us to determine whether neprilysin (NEP) as a SP degrading endopeptidase influences disease susceptibility in a German cohort of patients with CRPS. DNA was obtained from 325 CRPS patients and 376 controls. Initially, in a case-control study we demonstrated association of a GT- repeat polymorphism in the promoter region of NEP with CRPS ($p < 0.05$). In order to confirm this result, subsequently 21 SNPs throughout the NEP gene region were genotyped using allelic discrimination taqman assays. No significant differences in genotype frequencies were observed between controls and CRPS patients. In conclusion, our study did not reveal any association between NEP polymorphisms and CRPS. In addition, our data demonstrate the limitations of candidate gene association studies relying on a single associated polymorphism.

P235

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

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The mammalian HSP110 gene family consists of four proteins: HSPA4 l/HSPH3, HSPA4/HSPA2, HSP110/HSPA1 and GRP170/HSPH4. Expression analyses revealed that Hspa4 l is high in testis and moderate in other tissues, while Hspa4 and Hsp110 are ubiquitously expressed in various tissues. Immunohistological analysis in testis sections from different stages of postnatal development showed that Hspa4 l is highly expressed in spermatogenic cells from late pachytene spermatocytes to post-meiotic spermatids. In contrast to meiotic and postmeiotic expression of Hspa4 l, Hspa4 and Hsp110 are highly expressed in premeiotic spermatogonial cells. To study the physiological role of Hspa4 l and Hspa4 in vivo, we generated Hspa4l- and Hspa4-deficient mice. Hspa4l-deficient mice were born at expected ratios and appeared healthy. However, approximately 42% of Hspa4l-/- male mice suffer from fertility defects. Whereas the seminiferous tubules of Hspa4l-/- in testes contain all stages of germ cells, the number of mature sperm in the epididymis and sperm motility is drastically reduced. Reduction of sperm count is due to elimination of significant number of developing germ cells via apoptosis. No defects in fertility were observed in female mutants. Analysis of Hspa4-knockout on the hybrid genetic background revealed that Hspa4-deficient mice display increased incidence of male Infertility. To determine whether Hspa4 and Hspa4 l are functionally redundant, we have generated and characterized double homozygous mutations in Hspa4 and Hspa4 l.

P236

Cryptic Yp duplication including the SHOX gene in a boy with severe multicystic renal dysplasia and macrosomia at birth

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We report on a patient with a severe unilateral multicystic renal dysplasia, macrosomia and hydrocephalus at birth (birth height 55 cm, P 97^{th}, birth weight 5040 g, >P 97^{th}, SD 3,97; head circumference at birth 38 cm, >P 97^{th}, SD 2,21). A unilateral nephrectomy was performed at the age of 9 months. Clinical examination at the age of 2 years revealed a normal physical and mental development (height 92,5 cm, P 90^{th} -97^{th}; weight 15,5 kg, BMI 18,12; head circumference 51 cm, P 75^{th}-90^{th}). Since the patient's karyogram appeared to be normal, we performed Molecular Karyotyping using Array-CGH-analysis. A 1.3 MB genomic duplication was found within the PAR1 region of one of the sex chromosomes. The duplicated area includes eight genes and ranges from SHOX to ASMT. Subsequent FISH with a SHOX-specific cosmid probe revealed that a) the duplication resides on the Y-chromosome and b) was inherited from the boy's father, who also possesses a SHOX duplication on his Y chromosome.The father has a normal phenotype. Therefore, duplication of 7 genes (additional to SHOX) within the PAR1 region does not seem to be severely pathogenic. This can either be due to the nature of these particular genes or to the fact, that the duplication event does not affect the transcriptional level of these genes. Since his father has a normal kidney function, the severe clinical phenotype of the patient must be caused by another genomic event. However, besides the duplication within PAR1, no other genomic imbalance became evident by Array-CGH-analysis. We therefore conclude, that the causal mutation is either too small to be detected by Array-CGH-analysis and/or is not accompanied by gain or loss of genomic material.

P237

Mutation spectrum of autosomal recessive cutis laxa type 2 and analysis of ATP6V0A2 function in the Golgi compartment

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The main clinical features of autosomal recessive cutis laxa type 2 (ARCL2, MIM 219200), Debré type, are severe skin wrinkling, delayed closure of the anterior fontanel, downslanting palpebral fissures, a varying degree of developmental delay and general connective tissue abnomalities. Wrinkly skin syndrome (WSS, MIM 278250) is an overlapping entity with somewhat milder symptoms. Both disorders show a congenital glycosylation defect (CDG) on the level of the Golgi apparatus. We could recently demonstrate that mutations in the ATP6V0A2 gene on chromosome 12q24 are causative for both conditions. The gene encodes the V-type H+-ATPase a2 subunit, a membrane protein that is localised to the Golgi apparatus and to endosomal compartments, where it plays a role in proton translocation and membrane trafficking.

Here we present nine ARCL2 patients with proven CDG who were subjected to sequence analysis. We found 12 different novel ATP6V0A2 mutations including five frameshift, three nonsense, three missense and one in-frame deletion of exon 16. Functional analysis of patient fibroblasts revealed a significant delay in the retrograde translocation of Golgi membranes to the endoplasmic reticulum after Brefeldin A treatment. Furthermore, after RNAi-mediated knock-down of V-ATPase subunits a1, a2 and a3 in HeLa and other cell lines loss of a2 not only led to swelling and disruption of the Golgi stacks, but also evoked a glycosylation defect as detected by immunoblot analysis. In comparison, alterations of the endosomal compartment were much milder.

In summary, we were able to expand the mutational spectrum of the ATP6V0A2 gene and to confirm that the Brefeldin A effect in fibroblast from ARCL Debré type patients is highly reproducible. Moreover, we demonstrated that the a2 subunit plays a specific role in the maintenance of Golgi integrity and function.

P238

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

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

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

P239

Spectrum of PORCN mutations in focal dermal hypoplasia: How to survive lethal mutations?

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Focal dermal hypoplasia (FDH, Goltz syndrome, MIM #305600) is a multisystem birth defect characterized by widespread lesions of dermal hypoplasia or even aplasia. The resulting skin changes are arranged along the lines of Blaschko, indicating mosaicism. Another major diagnostic sign is longitudinal striation of the long bones, likewise hinting to functional mosaicism. Associated variable features include areas of hairlessness, periorificial papillomas, hypoplasia or aplasia of bones, malformations of the autopod, coloboma, and microphthalmia or unilateral anophthalmia. In addition, hypodontia or oligodontia, hearing loss, myelomeningocele, bifid ureter, horseshoe kidney, omphalocele or papillomatosis of the larynx may be found.
Recently, we had shown that the disease is caused by loss-of-function mutations of PORCN in Xp11.23 (Nature Genetics 2007, 39: 833–835). The attachment of palmitoleic acid by the O-acyltransferase PORCN prepares Wnt signaling molecules for the transport through the Golgi apparatus for secretion, signal gradient formation, and receptor binding.
We have extended the spectrum of known mutations considerably by analyzing PORCN in 24 novel patients:
i) In sporadic cases PORCN is affected preferentially by nonsense mutations whereas large deletions encompassing a variable spectrum of neighboring genes are mostly familial. Different mechanisms, non-homologous end joining (NHEJ) and non-allelic homologous recombination (NAHR) gave rise to the microdeletions.
ii) The missense mutations known so far almost exclusively exchange highly conserved amino acids in membrane spanning domains or in the lumenal loops.
iii) To override the consequences of lethal X-chromosomal PORCN mutations, male patients are somatic mosaics or show more than one X-chromosome. Females, survive either due to extreme skewing of X-chromosome inactivation, which is particularly evident in familial deletions, or they appear to be likewise somatic mosaics.

P240

Epigenetic influences on the origin of intrauterine growth defects

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Fetal growth is regulated by a variety of well balanced mechanisms. Amongst others, epigenetic marks contribute to the adequate dosages of growth factors especially insulin like growth factor 2 (IGF2). Disturbance of genomic imprinting of IGF2 result in aberrant growth observed e.g. in the conversed Silver-Russell syndrome (SRS, growth retardation) and Beckwith-Wiedemann syndrome (BWS, overgrowth). In both conditions a variety of different molecular defects have been reported that lead to a comparable phenotypic appearance within the syndromes. Only a small part of these molecular defects are gene mutations, the vast majority result from epigenetic alterations in two imprinting clusters on 11p15.5. Among these are microdeletions in the maternal H19/IGF2 imprinting-control region (ICR1) in familial BWS cases, described recently by us and others.
ICRs are allele-specifically methylated regions controlling in cis epigenetic modifications and transcription of genes in an imprinting cluster. 11p15 epimutations in BWS and SRS are converse alterations of the methylation marks. In spite of the known association of epimutations and the two syndromes, the causative transcriptional events are not well known. We therefore aimed to analyse the quantitative effects of different epigenetic defects on the transcription of the main candidate genes. As readout we quantitatively analysed the transcription of IGF2 and CDKN1C in fibroblasts of patients with BWS (maternal ICR1 microdeletions, 11p15.5 duplication) and SRS (matUPD 11p15, hypomethylation of ICR1) and compared the mRNA levels to control fibroblasts (normal epigenotype 11, ICR1 microdeletion without BWS). We observe no significant transcriptional alteration for CDKN1C, but substantial differences in the IGF2 transcription levels. Based on our observations we propose a molecular cascade that explains the origin of both syndromes in the light of the observed molecular defects and also depicts the remaining discrepancies.

P241

Mutations in the gene for autosomal recessive polycystic kidney disease (ARPKD) may modify the phenotype in patients with HNF1ß mutation

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HNF1ß is a widely expressed transcription factor that plays a critical role in embryonic development. HNF1ß mutations can cause young-onset diabetes, hyperuricemia, abnormal liver function, and urogenital developmental disorders like cystic dysplastic kidneys. Biallelic somatic inactivation may lead to renal cell carcinoma. We here present unpublished clinical and genetic data of a cohort of 203 families analysed for HNF1ß mutations by sequencing and MLPA. Clinical variability associated with HNF1ß mutations is dramatic and not understood so far. In some of our pedigrees, phenotypes range from prenatal Potter sequence to very mild manifestations in elderly family members. Even more sur-

prisingly, the renal phenotype may even vary in the same patient with cystic dysplasia in one kidney and a polycystic disease pattern in the other kidney. It is thought that certain criteria reminiscent of autosomal recessive polycystic kidney disease (ARPKD) as oligohydramnios, absence of corticomedullary differentiation and a kidney length more than + 3 SD exclude the possibility of HNF1ß anomalies. We demonstrate evidence that all these features can be present and phenotypes of patients with HNF1ß mutation may mimic polycystic kidney disease. We hypothesized that changes in the PKHD1 gene for ARPKD may modify the phenotype, because HNF1ß directly regulates PKHD1 and inhibition of its expression contributes to the formation of renal cysts in Hnf1ß mutated mice. Thus, we analysed the 66 coding PKHD1 exons in patients with HNF1ß mutation and an early-onset and/or polycystic kidney phenotype. Among changes of unknown significance, we identified a truncating PKHD1 mutation in an ARPKD patient previously shown to carry an HNF1ß mutation. Reporter assays are under way. As far as we know, this represents a new regulatory mutational mechanism with mutations in a transcription factor and its activated gene.

P242

Clinical, biochemical and molecular characterisation of 106 patients with fish-like malodour

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Fish-like body malodour may be due to trimethylaminuria, an autosomal recessive disorder caused by a deficiency of the flavin-containing monooxygenase isoform 3 (FMO_3). This enzyme is required for the oxidation and detoxification of many chemicals including endogenous amines as well as commonly used drugs; reduced FMO_3 activity results in reduced oxidation of dietary-derived, malodourous free trimethylamine (TMA) to its non-volatile, non-odourous N-oxide (TMAO). We carried out FMO_3 gene mutation analyses in 106 patients with fish-like malodour from 97 independent families. In 60 patients additional biochemical analyses were performed, showing an abnormal excretion of free trimethylamin in urine in 38 cases. In seven individuals malodour was related to carnitine medication. Homozygosity or compound heterozygosity for loss-of-function variants in the FMO_3 gene (including the previously described "variant allele" polymorphism [E158 K; E308G]) were found in 53 individuals. There were 31 known or suspected disease-causing mutations including 18 novel variants. 18 individuals were compound heterozygous for a disease-causing mutation and the variant allele, or homozygous for the variant allele. Genotypes indicating FMO_3 deficiency were found in five individuals with malodour on carnitine treatment despite sometimes normal biochemical results. Our data indicate that variants in the FMO_3 gene cause a spectrum of phenotypes ranging from severe constant malodour to intermittent malodour depending on exogenous factors such as carnitine treatment. Diagnosis is difficult particularly in attenuated forms of FMO_3 deficiency as biochemical tests may give normal or equivocal results, and no mutations may be found in some individuals with strong biochemical evidence of FMO_3 deficiency.

P243

Mapping an internal ribosome entry site (IRES) in the 5´ UTR of the human DDX3Y gene transcripts expressed in testis tissue

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The human $DDX3Y$ gene belongs to the DEAD-box RNA helicase gene family and is localised to the AZFa region in the proximal long arm of the human Y chromosome. Interestingly, although $DDX3Y$ transcripts were expressed in all analysed tissues, protein was found only in premeiotic male germ cells—spermatogonia and leptotene spermatocytes (Ditton et al., 2004). We proposed that this translational control might be associated with the use of distinct transcriptional start sites (TSS) of the gene resulting in the 5´ UTR extension of transcripts only in male germ cells. We identified that these testis transcripts underwent different splicing processes and that EGFP/3XFLAG reporter constructs with the corresponding sequences points to some translational repressor elements in the extended 5´ UTR sequence (Jaroszynski et al., in prep.). Additionally, our in silico analyses suggested that $DDX3Y$ testis transcripts with an extended 5´ UTR are also providing putative internal ribosome entry site (IRES) structures used for translation initiation when the common cap dependent translation initiation is impaired. For mapping experimentally these putative IRES function in the extended 5´ UTR of the $DDX3Y$ we designed a bicistronic vector, containing Renilla and Firefly luciferase reporter genes, transcribed under the control of the strong CMV promoter. Different fragments of the 5' UTR sequence were cloned in front of the Firefly luciferase gene in order to test their IRES potential by increase of the Firefly expression. In transfected cells the Renilla gene, serving as internal control, would be translated in the 5' cap dependent manner. Our assays indicated strong expression of the Firefly protein under a putative IRES control only for the testis specific extention of the transcript initiated with the first transcriptional start site (TSS-I; Rauschendorf et al., in prep.). This result strongly suggests IRES dependent translation of the $DDX3Y$ transcripts only in human male germ cells.

P244

DHCR7 mutations in Turkish SLOS patients and clinical description of four patients homozygous for the common p.T93 M mutation

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Smith-Lemli-Opitz syndrome [SLOS (MIM 270400)] is an autosomal recessive multiple congenital anomaly/mental retardation syndrome caused by mutations in the D7-sterol reductase (DHCR7, E.C.1.3.1.21) gene. Based on case frequency surveys the incidence of SLOS has been estimated to range from approximately 1:20.000 to 60.000 in populations of European origin. We have investigated a total of 12 SLOS patients from 11 families of Turkish origin. Apart from three patients homozygous for p.T93 M, three other patients were heterozygous for this mutation. Furthermore two Turkish SLOS patients have been detected carrying homozygous mutations p.G410S and c.964–1G>C respectively, indicating that p.T93 M is the most common mutation in the Turkish population. This mutation is also common in SLOS patients from Spain and Italy. Homozygous and compound heterozygous patients carrying this mutation show an attenuated phenotype. We report clinical details in four SLOS patients from Italy and Turkey (Kayseri and Ankara) who are homozygous for the mutation p.T93 M. All showed

a mild to moderate phenotype with severity scores between 5 and 20. 7DHC was clearly elevated in two patients in whom data were available (patient 1: 612.8 mmol/l; patient 2: 80.6 mmol/l). Considering that atypical SLOS cases caused by homozygositiy or compound-heterozygosity for p.T93 M may be more likely to be undiagnosed, we analysed the frequency of this mutation in 771 control individuals from Kayseri (n=455) and Ankara (n=316) by allele-specific PCR. Remarkably, no carrier for p.T93 M was identified, indicating that the allele frequency is lower than 1:420 (p=0.95, confidence interval 0–0.0024). Our results indicate that carrier status for SLOS in Turkey is rare and that there is no evidence to suggest that there should be a large number of non-diagnosed patients with mutation p.T93 M.

P245

Angelman syndrome due to a novel intragenic UBE3A gene deletion

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Angelman-syndrome (AS) is a rare neurogenetic developmental disorder characterized by severe mental retardation, absent speech, EEG abnormalities, seizures, ataxia, and bouts of inappropriate laughter. Known causes are maternal deletions of chromosome 15q11-q13, chromosome 15 paternal uniparental disomies, UBE3A-mutations or imprinting defects leading to a loss of function of the gene ubiquitin protein ligase E3A (UBE3A) which is subjected to genomic imprinting. In about 10% of suspected AS cases no genetic abnormalities can be detected by standard diagnostics. However, deletions encompassing the UBE3A gene which are not detectable by common methods have been described previously.
Therefore, looking for genomic rearrangements of the UBE3A gene, we tested samples of 500 AS cases, in which no causative genetic alterations could be identified so far, by semi-quantitative multiplex PCR. In an affected daughter and her healthy mother we detected a familial intragenic UBE3A deletion between introns 3 and 14. This 58 kb deletion was further characterized by microsatellite and quantitative-PCR analyses. Sequence analysis of the junction fragment confirmed the deletion and revealed an additional insertion of 87 bp.
In order to obtain clues about the mechanism underlying the rearrangement, we examined the breakpoint as well as the neighboring sequences. No repetitive elements surrounding the breakpoint could be detected, but in the flanking sequences we found short sequence homologies that might be responsible for the genomic rearrangement presented here. However, the mechanism leading to the 87 bp insertion remains elusive.
To our best knowledge, this is the first description of a genomic rearrangement confined to the UBE3A-gene in a patient with AS. Our findings indicate, that intragenic deletions of the UBE3A gene – although not very common – can cause AS.

P246

Mutational spectrum of Cohen syndrome and functional analysis of COH1

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Cohen syndrome is mainly characterised by mental retardation, postnatal microcephaly, facial dysmorphism, pigmentary retinopathy, myopia, and intermittent neutropenia. Mutations in COH1 (VPS13B) have been found in patients with Cohen syndrome from diverse ethnic origins. Here, we report 7 novel mutations, contributing to the growing spectrum of Cohen syndrome associated mutations and corroborating the finding of truncating mutations to cause Cohen syndrome. We were unable to establish a consistent genotype/phenotype correlation so far. However, our cohort of broadly heterogeneous patients from various origins have significantly confined the clinical spectrum of the entity. Interestingly, patients with the so called Jewish type of Cohen syndrome showed no mutation in COH1.
Analysing different human and mouse tissues by quantitative PCR, we found only one evolutionary conserved and ubiquitously expressed transcript, coding for 3997 amino acids. Since COH1 encodes a protein of unknown function, we studied the functional role of COH1. We found that COH1 represents a novel peripheral membrane protein of the Golgi matrix required for maintaining the Golgi ribbon integrity. Moreover, we were able to encircle a Golgi targeting domain to the last 315 residues of COH1. We propose that the Cohen syndrome phenotype results either from a truncated COH1 lacking the Golgi targeting domain or a fragmented Golgi complex due to diminished amounts of the COH1 protein.
The homology to yeast Vps13p implicates that COH1 serves as scaffold protein for the formation of protein sorting complexes. For an improved understanding of the distinct cellular mechanisms subsequently affected by the loss of COH1, we are on the way to examine endo- and exocytosis processes and to identify COH1 interacting proteins.

P247

Microdeletion of the FBXO11-gene and the MSH6-gene at 2p16.3 is likely to cause a distinct microcephaly, mental retardation, facial dysmorphism syndrome associated with an increased risk for intestinal cancer

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Genetic counceling in a collaborative Human genetic/Neuropediatric setting of a couple with their 14 month old son due to microcephaly, psychomotor retardation and distinct facial dysmorphic features was performed. He was born at term following an uneventful pregnancy as second child to healthy non-consanguineous parents. All biometric data were within the normal range except a relatively mild microcephaly (<10 centile, birth) progressive by age (<3 centile, 14 month) without seizures. Normal brain structures were shown by sonography/MRI. Rather mild facial features include hypertelorism, a short upturned bulbous nose with broad deep set nasal bridge and down turned corners of his mouth. Cytogenetic analysis revealed a normal karyotype. But comparative genome hybridization (CGH) using a 44 k oligonucleotide array followed by quantitative real time PCR analysis of DNA of the patient and his parents showed a de novo deletion of just 73 kb in the affected boy. The FBXO11-gene from the F-Box-protein-gene family is partially deleted. This gene is involved in the formation of subunits of the Ubiquitin Proteinligase-Complex. Since genes from that pathway can be related to disorders like Angleman syndrome, loss of this gene could be causative for his phenotype anomalies. A complete deletion of the mismatch repair gene MSH6 should not contribute to anomalies because functional haploinsufficiency for that gene is also seen in familial cases of Lynch syndrome. But adequate consequences have to be drawn for regular screening as a surveillance strategy for the patient. No further genes map to the flanking genomic regions of >100 kb of the deletion and so additional positional effects are unlikely. Since no deletion of the affected genomic segment have been described thus far it is justified to consider the FBXO11-gene as a candidate for an autosomal dominant form of mental retardation and patients with a similar phenotype could be tested for deletions or mutations of this gene.

P248

Molecular diagnostics of DMD gene in Polish patient with Duchenne/ Becker muscular dystrophy

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Duchenne/Becker muscular dystrophy (DMD/BMD) is lethal, recessive, X—linked disease, characterized by progressive muscular weakness and degeneration of skeletal muscle. It is caused by mutations within the dystrophin gene (DMD gene).
Approximately 60% of DMD and BMD patients carry large deletions comprising even several exons. The remaining mutations are duplications (6%) and point mutations (34%). Deletions and duplications causing frame shift result in the more severe DMD, whereas mutations maintain the reading frame cause the milder BMD. Deletions and duplications are focused in two "hot spots" whereas point mutations are scattered within whole DMD gene and difficult to identification. 30% of patients have mutations de novo.
The most efficient method of deletion and duplication screening is MLPA (multiplex ligand-dependent probe amplification), which allows identifying 100% of this kind of mutation and upgraded routine molecular diagnostics of this disease. Additional advantage of MLPA technique is possibility of carrier status determination. To find a point changes we use electrophoretic screening techniques as single stranded conformers polymorphism, heteroduplex analysis and sequencing (MegaBACE) to identifying.
In our studies we analyzed 150 patients with progressive muscular dystrophy from Great Poland and Silesia region. DNA from 72 patients was analyzed by MLPA and remaining 78 by PCR-multiplex. Summarising, we identify 69 cases of deletion, 14 duplication and 3 point mutation. We did not observed deletion and amplification of whole gene. The largest deletion covers 42 exons. Deletions and duplication included only one exon occurred in 32% of patient. In central part of a gene appeared 57% of this mutation and in proximal 43%. Furthermore we identified 2 missense mutations in exons: 6, 17 and one microdeletion in exon 45.
MLPA technique improoved significantly effeciveness of molecular diagnostics of DMD/BMD and allows to identificate of carriers.

P249

Generation and expression characterization of BAC-HD transgenic rats with full-length mutant huntingtin

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Background and objective: Our group has previously generated and characterized transgenic rats, which express a fragment of mutant huntingtin. This rat model mirrors many aspects of Huntington's disease, but it lacks the full-length mutant protein and therefore some aspects of the human condition might be imperfectly replicated. To overcome this potential disadvantage, we aimed to generate transgenic rats, which express full-length mutant human huntingtin in the same developmental and tissue- and cell-specific manner seen in patients.
Methods: Bacterial artificial chromosomes (BACs) containing human genomic DNA spanning the full-length gene with 97 CAGs including all regulatory elements, were microinjected into oocytes of Sprague-Dawley rats. Founders and F1 transgenic rats were screened for copy number of BAC insertion, number of integration sites, integrity of the BAC insertion and level of mutant huntingtin RNA by QPCR. Protein expression in brain was measured by Western Blot.
Results: Initially we established one line, in which the full-length huntingtin expression was detected in all the brain regions at low level. 24 new founders were therefore generated, which all showed germline transmission of the transgene. Many potential founders had multiple copies of the transgene integrated into their genome, in some cases more than 5-fold in comparison to the low-expressing line. Also 22 potential founders expressed the full-length mutant huntingtin gene. Expression of the full-length protein was confirmed in the brains of F1 transgenic rats, in several lines at high levels. We are currently phenotyping F2 animals in 2 lines.
Conclusion: We have established several lines of transgenic rats, which express full-length mutant huntingtin at high levels.

P250

PolyQ-expanded TBP leads to aggregation and neuronal death in primary neurons and transgenic mice

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Background and objective: SCA17 is a progressive neurodegenerative disease leading to cerebellar ataxia and dementia. Genetically, a CAG/CAA expansion in the TATA binding protein (TBP) is found in SCA17 patients. To further study the pathogenesis of this disorder, we aimed to develop a transgenic mouse model for SCA17.
Methods and results: Overexpression of polyQ-expanded TBP in primary neurons lead to aggregate formation and neuronal death. This prompted us to generate transgenic mice which express 64 CAG/CAA repeats containing human TBP gene under the control of the truncated human prion protein promoter. Transgenic protein expression throughout different brain regions was clearly demonstrable. Onset of motor dysfunction started by 7 months and progressed with age. By electron microscopy and immunohistochemical methods we were able to detect neurodegeneration and aggregation selectively in the cerebellum, but not in striatum and cortex. This could be confirmed using filter trap assay with mouse brain lysates. SDS-insoluble aggregates in the membrane were seen in lysates from transgenic mice, whereas wild-type brain lysates did not reveal significant retention of aggregated material in cellulose acetate.
Conclusion: We present detailed morphological and phenotypical data for this rodent model of SCA17, which will be a valuable tool to conduct preclinical therapeutic studies and to further study the pathogenesis of this so far incurable disease.

P251

Can proximal symphalangism due to a NOG mutation be associated with growth acceleration?

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Proximal symphalangism (SYM1, MIM 185800) and multiple synostosis syndrome (SYNS1, MIM 186500) are genetically heterogeneous and can be caused by heterozygous NOG gene mutations. Growth abnormalities have not yet been reported in human NOG-related disorders

but the suppression of noggin in transgenic mouse models was found to accelerate osteogenesis.
We report a family (father and son) affected by proximal symphalangism/multiple synostosis syndrome, where growth acceleration was the predominating clinical feature. In both patients, shortened first rays and joint synostoses of fingers and toes as well as common facial features and hyperopia were present, while conductive hearing loss was detected only in the son. The boy was referred to the pediatric department from age 3.5 years because of overgrowth along with hyperphosphatemia and elevated alkaline phosphate activity. When last examined at age 10, his height was 160 cm (0.5 cm >97th percentile) and his weight was 63 kg (1 kg >97th percentile). His father was of similar stature (185 cm) but details of his growth velocity were not available. In both patients a novel heterozygous missense mutation c.696C>G, p.Cys232Trp, of the NOG gene was detected.
Accelerated growth has not yet been outlined in NOG-related diseases, however, one Mexican family with a nonsense mutation of NOG and tall stature has been reported. Recent studies of animal models suggest that noggin reduction promotes osteogenic differentiation. We believe that accelerated growth with corresponding biochemical and radiological changes is part of SYM1/SYNS1 caused by NOG mutations.

P252

LRRK2 interacts with key mediators of cytoskeleton signalling

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Background: The Parkinson's disease (PD) key player LRRK2 (leucine-rich repeat kinase 2) accounts for 5–6% of familial and 1–2% of sporadic PD cases. There is evidence that LRRK2 might be involved in regulation of neurite outgrowth but the underlying mechanisms are completely unknown. Here we analyse the influence of LRRK2 on cytoskeleton signalling cascades.
Results: Initial expression analysis of LRRK2 siRNA transfected human dopaminergic cells suggested a role of LRRK2 in axonal guidance and actin cytoskeleton signalling pathways. CDC42 (cell division cycle 42), an actin polymerisation enforcing gene, and ARGHEF7 (rho guanine nucleotide exchange factor 7), responsible for the activation of CDC42, were most prominently upregulated in response to LRRK2 knockdown in our experimental setup. To further investigate a potential impact of LRRK2 on CDC42 and ARHGEF7 mediated cascades we performed co-immunoprecipitation and co-localisation experiments in differentiated SH-SY5Y cells and postnatal hippocampal cultures.
Taken together our studies point towards a direct interaction of LRRK2 with CDC42, actin and ARHGEF7. Over-expressed and endogenous proteins interact in HEK cells, SH-SY5Y cells and whole brain preparations. Actin, CDC42, ARHGEF7 and LRRK2 partially co-localize in differentiated SH-SY5Y cells and postnatal hippocampal cultures.
Outlook: In ongoing studies we examine the mutual impact of ARHGEF7 and LRRK2 functions as guanine nucleotide exchange factor, GTPase and kinase and the potential physiological relevance of this interaction.

P253

Characterisation of a SCA3 mutant mouse model and analysing of mitochondria-associated apoptosis in the pathogenesis of SCA3

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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. The gene trap model harboured a truncated ataxin-3 including the Josephin domain and the first ubiquitin interactive motif (UIM1), but lacking the VCP binding site and the polyQ strech. By expressing only the Josephin domain and UIM1 we got a SCA3 like phenotype with an onset of symptoms at the age of one year with a reduced lifespan, neurological symptoms e.g. gait ataxia, clasping, stereotypes and reduced body weight and cage activity. In the brain of this model we found cytoplasmic/ extracellular inclusion bodies induced most likely by the Josephin domain. This supports the hypotheses that the Josephin domain can cause similar symptoms as the polyQ strech and forms aggregates, too. We further analysed the influence of ataxin-3 in the ERAD system in the gene trap model and found that mitochondria- associated apoptosis might be involved in the pathogenesis in SCA3. In in vivo and in vitro experiments we found differentially regulated genes which are involved in the mitochondria-associated apoptosis. The data would be analysed by western blot, quantitative real time analysis, electron microscopy and biochemical analysis.

P254

mRNA expression profiling of the CNGA3 knockout mouse with focus on the dorsal-ventral gradient in retinal degeneration

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Purpose: The CNGA3$^{-/-}$ mouse is an animal model lacking the A subunit of the cone specific cyclic nucleotide gated channel. The phenotype is characterized by a loss of cone photoreceptor function and a progressive degeneration of the cones, particularly in the ventral part of the retina. To elucidate this dorsal ventral gradient in degeneration we performed whole genome expression analyses of dorsal and ventral areas of CNGA3$^{-/-}$ and wildtype retinas at the age of 4 weeks.
Methods: Expression analysis of CNGA3$^{-/-}$ and wildtype retinas was performed using Illumina MouseWG-6 v1.0 Expression BeadChips. Differential regulated transcripts with a minimum change in expression level of 1.5 fold (p-value ≤0.05) were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. To verify the data several transcripts were analyzed by qRT-PCR. The study was performed in accordance with the ARVO Statement for the use of Animals in Ophthalmic and Visual Research.
Results: Physiological differences in expression patterns of the dorsal and ventral retina were analyzed in wildtype samples, showing 264 differently regulated transcripts. Differences between the wildtype and CNGA3$^{-/-}$ retinas appeared with 579 differently regulated transcripts in the dorsal areas and 608 in the ventral retina. In contrast to the wildtype, CNGA3$^{-/-}$ mice showed a differently regulation of 227 transcripts

in the ventral retina. The results could be successfully verified by qRT PCR.
Conclusions: Expression analysis of different retinal areas showed clear differences in expression patterns of the dorsal and ventral regions. Analysis in CNGA3$^{-/-}$ retinas discovered clear differences in expression patterns. These results could help to elucidate the molecular events leading to the dorsal-ventral gradient of cone degeneration in CNGA3$^{-/-}$ mice.

P255
Screening for genomic variants in ZFP57 in Silver-Russell syndrome patients
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Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterized by intrauterine and postnatal growth restriction and morphological abnormalities including a small triangular face, relative macrocephaly, asymmetry of the head and limbs, and clinodactyly V. In >38% of SRS cases a hypomethylation of the H19/IGF2 DMR in 11p15 can be detected which may lead to an altered expression of imprinted genes in this region and hence to the typical SRS phenotype. Mackay et al. (2008) recently identified ZFP57 mutations as a cause of hypomethylation of multiple imprinted loci including the TND, GRB10, PEG1/3 and KCNQ1OT1 DMRs. To determine whether ZFP57 mutations also affect the H19/IGF2 DMR we screened 10 SRS patients with 11p15-hypomethylation for mutations within the coding region of this gene. Thereby homozygosity for a novel variant in exon 6 of ZFP57 was detected in one patient. This C to T transition results in an amino acid substitution from arginine to glutamine within a not conserved region. To further verify this variant we investigated 80 healthy German probands as controls as well as further 20 SRS patients with 11p15-hypomethylation by performing a restriction digest. Two controls and the parents of the patient showed heterozygosity for this variant while it could not be detected in the 20 additionally screened SRS patients. Except this new and probably apathogenic polymorphism and some registered SNPs no further variants were detected in the coding region of ZFP57.
In conclusion, ZFP57 mutations seem to be not causative for 11p15-hypomethylation in SRS patients and hence for the aetiology of SRS.

P256–P294 Genetic analysis, linkage and association, complex genetics/diseases

P256
Genetic variation in a putative Wnt signaling pathway gene (NEP1) is associated with colorectal cancer
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Background: By the use of RNAi screening, NEP1 (nuclear enriched protein) was identified to be a putative regulator of the Wnt signaling pathway. This pathway plays an important role in cell proliferation, differentiation and polarity during development (embryonic) and tissue renewal (in adult organisms). More importantly, dysregulation of the Wnt signaling plays a key role in colorectal carcinogenesis. We wanted to investigate whether genetic variation in NEP1 affects susceptibility to colorectal cancer.
Methods: We used a tagging single nucleotide polymorphism (SNP) approach and selected eight tagging SNPs, that represent a total of 123 SNPs and cover all the known common (allele frequency >10%) variation in NEP1 with a pairwise r2 value of linkage disequilibrium (LD) >0.8. A case-control study was carried out using a well-characterized study population containing 752 colorectal cancer case samples and 755 control samples from the Czech Republic. The TaqMan® allelic discrimination method was used for genotyping. Odds ratios and 95% confidence intervals were calculated for association between genotypes and colorectal cancer.
Results: Three of the chosen tagging SNPs were significantly associated with the risk of colorectal cancer. Two of these SNPs showed a significant correlation with an increased risk of colorectal cancer. The third SNP was associated with a decreased risk of colorectal cancer.
Conclusion: Our study showed that genetic variation in NEP1 influences the risk of colorectal cancer.

P257
First evidence for a splice site mutation associated to X-linked Emery-Dreifuss muscular dystrophy
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X-linked Emery-Dreifuss muscular dystrophy (EDMD) is characterized by progressive skeletal muscle wasting and weakness, contractures of major tendons and cardiac conduction defects. In 1994, X-linked EDMD was associated to mutations of the STA gene, which is encoding a transmembrane protein of the inner nuclear membrane called Emerin. More than 90% of the mutations represent non-sense mutations in the coding region resulting in a loss of emerin function. By mutational analysis, we found for the first time a hemizygous mutation g.IV3de −10_-27 in two unrelated EDMD patients – a 19 years old male from Poland and a 60 years old male from Belgium. Both patients showed the typical features of the disease including slowly progressive weakness, muscle wasting, cardiac rhythm disturbances and cardiomyopathy. No functional Emerin could be detected in muscular biopsies of the patients. The mutation affects six nucleotides of the acceptor splice site in intron 3 of the STA gene. To find evidence for the pathogenic effect of this splice site mutation total RNA was isolated from blood lymphocytes of the patients, and RT-PCR was performed using specific primers spanning intron 1 through 5. cDNA fragments were cloned from the RT-PCR products into a pJET vector and propagated in E.coli. 97 colonies were selected. Following colony PCR, the insert sizes were estimated by agarose gel elctrophoresis. Samples of each fragment size were directly sequenced. In the Polish patient, skipping of exon 4 (64.9%) causes a frameshift which putatively leads to a premature termination signal 10 amino acids after K87 of the emerin peptide. Interestingly, in the Belgian case, the mutation lead apparently to the use of a cryptic splice site in exon 4, which putatively results in an in-frame mutation leading to a loss of 37 amino acids (p.D88_D124del) in exon 4. Both effects found in vitro, support the hypothesis that the g.IV3de −10_-27 mutation in STA is causative for EDMD.

P258

Prevalence of hemophilia A carriers among female patients with severe bleeding complications

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Female carriers of mutations in the F8 gene often show a reduced factor VIII activity (FVIII:C) and/or a disproportional lower FVIII:C relative to von Willebrand factor (VWF) activity in plasma. Consequently these women are prone to bleeding complications after surgery or trauma. Therefore, female patients presenting with such symptoms are often suspected to be carriers of hemophilia A (HA), even when the family history for the disease is negative. The aim of this study was to determine the proportion of HA carriers among such patients, according to the residual FVIII:C.

We analysed extensively the F8 gene in 27 women with severe bleeding complications, FVIII:C/VWF:C disproportion, normal (n=14) or reduced FVIII:C between 10% and 50% (n=13) and negative family history for HA. All patients were initially tested negative for the intron 22 and intron 1 inversions as well as for large deletions/duplications using MLPA analysis. Subsequently, sequence analyses of the F8 coding region including the intron/exon boundaries and X-inactivation assay were performed.

We detected four amino-acid changes and one splicing error in the F8 gene in five of the patients. Skewed X-inactivation was present in three of the mutation positive cases. The prevalence of mutation carriers differed significantly between the groups of patients with FVIII:C <50% and those with FVIII:C 10–50%. Thirty percent of the women with reduced FVIII:C were shown to be HA carriers, whereas only 7% of those with normal FVIII:C were heterozygous for a F8 mutation.

Our study suggests that the proportion of HA carriers among female patients with severe bleeding complications is significantly higher as compared to the random population, especially when FVIII:C is reduced. The estimated risk for HA in the male offspring is thus significantly elevated in such individuals.

P259

SNP array-based genome scan for copy number variants associated with bipolar disorder

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Recent studies using SNP array data have identified rare copy number variants (CNVs) that are associated with CNS phenotypes such as mental retardation, autism, and schizophrenia. To date, only one study has systematically tested the influence of such microdeletions/-duplications on the development of another common neuropsychiatric disorder, bipolar (BP) disorder (Zhang et al. 2008). In the present study, we therefore conducted a systematic screen for common and rare CNVs in a sample of 471 patients with a DSM-IV diagnosis of BP I disorder and 855 population-based controls. All individuals were of German origin and genotyped on Illumina´s HumanHap550 arrays, comprising more than 560,000 SNPs. To identify potential CNVs, we analyzed each individual's SNP fluorescence intensity data with two programs, QuantiSNP and PennCNV, both using Hidden-Markov model algorithms. We systematically filtered for intensity changes (indicative of a potential CNV) that comprised at least 30 SNPs and were located in or near a gene (±20 kb), and compared the number of observations in patients and controls with a one-sided Fisher's exact test. The overall number of CNV events did not significantly differ between patients and controls. However, we identified two rare CNVs that were significantly over-represented in BP patients compared to controls: a 92 kb microdeletion on chromosome 7 (patients:controls=0.63%:0%, p=0.0430, OR=5.61) and a 500 kb microduplication on chromosome 15 (patients:controls=1.27%:0.23%, p=0.0252, OR=5.61). We are currently following up these CNVs together with 5 singleton CNVs (observed in a single patient and in no control), in large, independent samples of BP patients and controls using two novel methods: An allele-specific copy number PCR assay and a quantitative interspecies competitive PCR assay, both based on MALDI-TOF mass spectrometry.

P260

Interleukin-1 polymorphisms and their relation to External Apical Root Resorption (EARR)

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Recent papers have discussed a genetic predisposition for external apical root resorption (EARR), in particular IL-1A and IL-1B gene polymorphisms are discussed as genetically predisposing factors. Recently, an association of the allele 1 of the IL-1B polymorphisms (+3954) with EARR in familial cases has been reported. We therefore aimed to confirm this association in a cohort of sporadic EARR cases. Orthopantomographs (OPG) exhibiting EARR (n=90) were metrically and statistically analyzed for expression and were compared to a control group (n=162). Additionally, the percentage of affected teeth per individual was determined. A subgroup of the EARR patient samples (n=49) was investigated for an association of the genomic IL-1A (-889) and IL-1B (+3954) polymorphisms. In the case of the IL-1A variant, a significant difference of genotype distribution was found between EARR patients and the control group: the genotype 2–2 could be seen significantly more frequent in the EARR group. Furthermore, the extent of resorption grades seemed to be influenced by the genetic constitution. The genotype distribution of the IL-1B polymorphism was comparable to the distribution in the control sample. In particular, allele 1 of the IL-1B polymorphism which has been described as being associated with family histories of EARR was observed less frequently in the cohort of patients than in the control group. The available data on IL-1A polymorphism point to an association of genotype 2–2 with EARR. These results present the first step towards a future genetic testing procedure that assesses preorthodontically the risk for root resorption.

P261

Dissection of phenotype reveals association between schizophrenia and Glutamate Receptor Delta 1 (GRID1) gene promoter

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Recent linkage and association data have implicated the glutamate receptor delta 1 (GRID1) locus in the etiology of schizophrenia. In this study, we sought to test whether variants in the promotor region are associated with this disorder. The distribution of CpG islands, which are known to be relevant for transcriptional regulation, were computationally determined at the GRID1 locus, and the putative transcriptional regulatory region at the 5'-terminus was systematically tagged using HapMap data. Genotype analyses were performed with 22 haplotype-tagging SNPs in a German sample of 919 schizophrenia patients and 773 controls. The study also included two SNPs in intron 2 and one in intron 3 which have been found to be significantly associated with schizophrenia in previous studies. For the transcriptional regulatory region, association was obtained with rs3814614 (p=0.0193), rs10749535 (p=0.0245), and rs11201985 (p=0.0222). For all further analyses, the patient samples were divided into more homogeneous subgroups according to sex, age at onset, positive family history and history of major depressive episodes. The p-value of the schizophrenia association finding for the three markers decreased by approximately one order of magnitude, despite the reduction in the total sample size. Marker rs3814614 (p=0.0005) also withstood correction for multiple testing. No support was obtained for previously reported associations with the intronic markers. Our results suggest that genetic variants in the GRID1 transcriptional regulatory region play a role in the etiology of schizophrenia, and that future association studies of schizophrenia may require stratification to ensure more homogeneous patient subgroups.

P262

Alopecia areata – genetic fine mapping in Dundee Experimental Bald (DEB) rats

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Alopecia areata (AA) is a chronic inflammatory disorder of hair follicles in the actively growing anagen stage. It is characterized by one or several circular regions of hair loss on the head. Isolated hair loss on other parts of the body can occur, and hair loss can further extend, culminating in alopecia totalis or alopecia universalis.
Alopecia areata has been suggested as a tissue-specific autoimmune disease, considering the potential role of loss of immune privilege. Frequent familial occurrence of the disease demonstrates a strong genetic susceptibility with a multifactorial background.
We have been analysing the Dundee Experimental Bald (DEB) rat as a rodent model of AA. An intercross of DEB with PVG rats gave an F2 population with which we performed a whole genome-scan for linkage with microsatellite markers. This analysis resulted in suggestive loci on chromosomes 5 and 8, and one highly significant locus on chromosome 19. Further fine mapping of the locus on chromosome 19 identified a candidate interval in the region between 35 cM to 40 cM.
A detailed search through candidate genes including Cadherin 3, which plays an important role in cell adhesion, Nol3, a regulator of apoptosis, Hsd11b2, which catalyzes the conversion of corticosterone to 11-dehydrocorticosterone, and Pard6a, an adapter protein involved in asymmetrical cell division and cell polarization processes, did not give clues to the causes of hair loss but showed polymorphisms in the DEB and PVG rat genomes and further minimized the region of interest.
In future experiments we will continue saturation mapping with microsatellites and SNPs, complement the mapping approach with expression studies with a focus on genes in the minimal region and culture keratinocytes, melanocytes, dendritic cells and hair follicles from rat skin for functional analyses.

P263

A genome-wide association study identifies new susceptibility variants for male pattern baldness on chromosome 20

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Androgenetic alopecia (AGA, male pattern baldness [MIM109200]) is a progressive patterned loss of hair from the scalp. The proportion of affected males increases steadily with age. The pathogenesis of AGA is androgen dependent and a polygenic predisposition is the major requirement for the phenotype. Several studies have identified the androgen receptor gene (AR) on the X-chromosome as the first gene contributing to the development of AGA (e.g. Hillmer et al., Am J Hum Genet,2005). In order to identify novel predisposing genes, we used a genome-wide association (GWA) approach investigating more than 550,000 SNPs (Illumina HH300K- and HH550K-BeadChips). The discovery sample comprised 296 males with early-onset AGA and 347 controls, all from German descent. We then investigated the 30 best autosomal SNPs in an independent replication sample of 319 affected and 234 unaffected German individuals and found highly significant association for five SNPs. All of them were located on chromosome 20p11 and therefore displayed a new susceptibility locus for AGA. Follow-up analyses did not provide any evidence for interaction effects between the AR locus and the new locus on chromosome 20p11, suggesting that the 20p11 locus has a role in a yet-to-be-identified androgen independent pathway. Two ESTs, BQ013595 and BE789145, map to the associated region on 20p11. The closest RefSeq gene is PAX1 (paired box 1) ~100 kb telomeric of this region. Of those three candidates, only PAX1 showed considerable expression in scalp. Although PAX1 is located outside the associated LD block, the expression data suggest that PAX1 might confer the AGA-relevant effect at this locus and that a regulatory variant within the associated LD block may modulate its expression. In conclusion, our work places the genetic basis of AGA in a genome-wide context. We identified a novel genomic region conferring a strong effect on the development of early-onset AGA, with no obvious connection to the androgen pathway.

P264

A coding variant in the serotonin receptor 3C subunit is associated with diarrhea-predominant irritable bowel syndrome

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Serotonin type 3 (5-HT_3) receptors are ligand-gated ion channels. To date, five 5-HT_3 receptor subunits A, B, C, D and E are known forming pentameric receptors of diverse structure and function. 5-HT_3 receptors are involved in the regulation of gut motility, visceral sensation and secretion. 5-HT_3 receptor antagonists are beneficial in some but not all patients with diarrhea-predominant irritable bowel syndrome (IBS-D). As we recently found variants of the 5-HT_3 subunit genes HTR3A and HTR3E to be associated with IBS-D, the aims of this study were to investigate whether variants of the HTR3C subunit gene may also contribute to the IBS phenotype and to perform pharmacological analyses to provide insight into the functional consequences of respective variants.

HTR3C genotyping in a pilot study cohort of 197 IBS and 100 healthy subjects revealed the c.489C/c.489C genotype of the HTR3C c.489C>A (rs6766410, p.N163 K) coding variant be associated with female IBS-D (P= 0.0019; OR = 4.98 CI = [1.75–14.16]), whereas no association could be found in males. Calcium influx analyses of the 5-$HT_{3A/C}$ p.163 N and $HT_{3A/C}$ p.163 K receptors resembling the homozygous genotypes revealed identical potencies of 5-HT and two 5-HT_3 antagonists. However, 5-HT showed decreased efficacy at 5-$HT_{3A/C}$ p.163 N (78.1 ± 5.9 %) compared with 5-$HT_{3A/C}$ p.163 K (100%, P <0.01, n = 14) receptors and radioligand binding assays revealed a reduced B_{max} for the 5-$HT_{3A/C}$ p.163 N (86.2 ± 3.7 %) compared with the 5-$HT_{3A/C}$ p.163 K receptor (100%, P <0.05, n = 6).

The decreased 5-$HT_{3A/C}$ p.163 N 5-HT maximum response, most likely caused by reduced cell surface expression of the mature receptor in comparison to the 5-$HT_{3A/C}$ p.163 K receptor, may result in altered 5-HT_3 receptor signal transduction in the enteric and central nervous system and thereby contribute to the pathophysiology of IBS-D in females.

P265

MutationTaster – discover the disease potential of sequence alterations

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MutationTaster is a web-based application to predict the disease-causing potential of DNA sequence alterations in humans. It integrates information from various data sources as well as internal and external analysis tools. The analyses include, among others, evolutionary conservation, potential splice site changes, possible loss of protein features and changes which might affect the mRNA level (such as loss of polyadenylation signal or a premature stop codon). MutationTaster then generates a final score by assigning different weights to the results of each analysis. The output includes not only the prediction itself but additionally all single test results and further data on gene, protein and mutation, thus providing valuable information for researchers who want to apply their own biological knowledge instead to rely solely on automatic ´black box´ predictions.

MutationTaster was trained with known polymorphisms and disease-causing mutations from the literature and could correctly predict 89% of the variations in a large test set (8% false predictions, 3% not determinable). MutationTaster has a higher accuracy than most of the similar applications and is considerably faster, a typical analysis taking less than 3 seconds. The short analysis time makes it an ideal tool to automatically evaluate the huge number of sequence alterations which will inevitably be found by chromosome-wide Next Generation Sequencing approaches. Since the manual analysis of every single alteration would be very tedious and cost-intensive, MutationTaster can be used to preselect promising candidates for further (experimental) investigations. The software can be accessed at http://neurocore.charite.de/MutationTaster/index.html

P266

The p.R230C variation in ANXA11 is strongly associated with sarcoidosis

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Sarcoidosis is an inflammatory granulomatous disorder, primarily affecting the lungs and lymph nodes. Multiple organs however can be afflicted. The disease is characterized by noncaseating granulomas and an exaggerated cellular immune response caused by increased inflammatory activity. About 20% of patients have a chronic and prolonged course which needs to be treated with corticosteroids. Up to 5% of all patients have severe complications such as lung fibrosis. Very recently, a GWA study reported an association between sarcoidosis and ANXA11, a member of the annexin family of calcium-dependent phospholipid-binding proteins was described. The ANXA11 protein is involved in calcium signalling, cell division, vesicle trafficking, and apoptosis. A strong association signal for SNPs surrounding the ANXA11 gene on chromosome 10q22.3 was found, including a common nonsynonymous SNP (rs1049550, c.688T>C, p.R230C).

To replicate this association in an independent cohort, we performed a case-control association study in 325 German sarcoidosis patients and 364 healthy matched controls. Genotyping of ANXA11 rs1049550 was performed using a Taqman assay. The C allele frequency of rs1049550 was significantly increased in the sarcoidosis cohort (C=0.6504, T=0.3496 in cases; C=0.5479, T=0.4521 in controls; p<0.0005,). It was significantly associated with an increased risk of sarcoidosis for carriers of the CC genotype (OR=2.18, 95% CI 1.39–3.43; p<0.001). The increased risk was present by analysing both, dominant and recessive models (p=0.017 and p=0.0004, respectively). Gender did not affect the increased risk for sarcoidosis. There was no statistical difference between acute and chronic sarcoidosis patients in allele and genotype frequencies, but both groups had significantly increase C allele frequencies.

The results obtained in this study confirmed the strong association between variations in ANXA11 and sarcoidosis in the German population.

P267

The p.R332 W mutation in WNT10B causes split hand/foot malformation by a reduced activation of LRP6-mediated WNT signaling

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Split hand/foot malformation (SHFM) is a complex limb malformation affecting the central rays of the extremities that leads to median clefts. It is further characterized by various degrees of syndactyly and aplasia and/or hyplasia of phalanges. Recently, mapping studies in a consanguineous SHFM family identified the homozygous p.R332 W missense mutation in the WNT10B gene. In general, various WNTs are described that can activate canonical Wnt/ß-catenin upon binding to the Frizzled and co-receptor LRP5/6 co-receptors and thereby regulate developmental processes, including vertebral limb patterning. The

functional consequences of the identified WNT10b mutation have not yet been analyzed.
In the present study, we functionally characterized p.R332 W using a luciferase reporter assay. We cloned wt and mutant WNT10b cDNA into the pCMV-SC-CF mammalian expression vector. WNT10B constructs were co-expressed with the LRP6 receptor in HEK293T cells. While wt WNT1 and WNT10B activated WNT signaling response through LRP6 in the luciferase assay, mutant p.R332 W WNT10B did not show a significant activation. Western blot analysis of mutant WNT10B in HEK293T cells revealed no difference in protein expression in comparison to wt. Furthermore, crosslinking experiments with mutant WNT10B and LRP6 are currently done to determine a possible binding deficiency of WNT10B to the extracellular domain of LRP6. Our results clearly showed that the WNT10B p.R332 W mutation reduced LRP6 receptor-mediated WNT signaling and that this is the underlying pathophysiological mechanism causing SHFM in affected family members. Further investigations will be done to determine the mechanism of impaired LRP6 receptor activation upon mutant WNT10B protein binding.

P268

Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders

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As a key player in modulating both human physiological and behavioural functions including anxiety, perception and in particular appetite, serotonin is likely to be involved in the aetiology of eating disorders. Bulimia nervosa patients have been effectively treated with the selective 5-HT_3 antagonist ondansetron. This triggered our interest in investigating the putative role of variants in the 5-HT_3 receptor genes HTR3A and HTR3B in the susceptibility to bulimia nervosa (BN) and anorexia nervosa (AN) by direct sequencing. 265 patients with AN and 91 patients with BN as well as 191 healthy controls served as a pilot study group for mutational analysis. We found the coding HTR3B variant p.Y129S (rs1176744, P = 0.004, OR = 2.06) and the 5'UTR residing HTR3A variant c.-42C>T (rs1062613, P = 0.008, OR = 5.31) to be associated with the restrictive subtype of anorexia nervosa (ANR). An intronic HTR3A variant, IVS1–19G>A (rs1176722), was identified to be associated with the ANR (P = 0.002) as well as the BN purging subtype (BNP, P = 0.005). Furthermore, the association of HTR3B p.Y129S with ANR was confirmed in an independent Spanish study group of 78 patients with AN and 331 controls (P = 0.034, OR = 2.26). Hence, our study provides first evidence for an involvement of serotonin receptor type 3 variants in the aetiopathology of eating disorders in humans.

P269

Further evidence for the involvement of MYH9 in the etiology of non-syndromic cleft lip and palate

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Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common birth defects and has a multifactorial etiology which includes both genetic and environmental components. MYH9, the gene coding for the heavy chain of non-muscle myosin II, has been considered a good candidate gene in NSCL/P on the basis of its expression profile during craniofacial morphogenesis. Reports of positive association between single-nucleotide polymorphisms in the MYH9 gene and NSCL/P in an Italian sample as well as in an ethnically mixed North American sample have provided further support for the role of MYH9 in the development of NSCL/P. In the present study, we aimed to replicate these findings by conducting a family-based association study with seven single nucleotide polymorphisms in MYH9 using a sample of 248 NSCL/P patients and their parents. Single marker analysis resulted in a highly significant association for rs7078 (P = 0.00008). In haplotype analysis the most significant result was obtained for the SNP combination [rs7078; rs2071731; rs739097; rs5995288]. Our results thus confirm the potential involvement of MYH9 in the etiology of NSCL/P in our patients of Central European origin, although further studies are warranted to determine its exact pathogenetic role.
The study was supported by the DFG.

P270

Extended follow-up analysis of association signals from two independent genome-wide association studies in bipolar disorder suggests JAM3 as susceptibility gene

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The first two genome-wide association studies of bipolar (BP) disorder applied different approaches: Baum et al. (2007) analysed DNA pools from US-American and German samples on Illumina HH550 arrays and reported consistent findings between both samples; the WTCCC (2007) employed individual genotyping in a UK patient-control sam-

ple on Affymetrix 500 K arrays. Here, we aimed to identify genes that showed independent evidence for association in both studies and to follow-up promising markers in a large European sample, of which 39.5% overlap with the German replication sample of Baum et al. A gene-based comparison resulted in 9 genes with overlapping association signals. From these genes, we selected 18 Affymetrix SNPs and individually genotyped them in our combined samples of 1,805 patients and 2,271 controls. We found that rs11223704 in JAM3 (11q25) was significantly associated with BP (P=0.0252). Hence, genetic variation in JAM3 showed consistent association with BP in three studies (Baum et al., WTCCC, present study) using mainly non-overlapping samples and can therefore be regarded as a promising susceptibility gene for BP. Further experiments suggest ubiquitious expression of JAM3 during development and adulthood as well as potential tissue-specific alternative splicing. We also tested 6 independent imputed SNPs showing association with BP in the WTCCC study and found rs9834970 (3p22.2) to be significantly associated with BP in our European Sample (P=0.00428).

P271

Variation in GRIN2B contributes to weak performance in short-term memory in dyslexic children

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A multimarker haplotype within GRIN2B, a gene coding for a subunit of the ionotropic glutamate receptor, has recently been found to be associated with variation in human memory performance (de Quervain & Papassotiropoulos 2006), and the same genomic locus has been linked to a phonological memory phenotype in a recent genome scan in dyslexic families (Brkanac et al. 2008). These findings point towards an involvement of GRIN2B in memory-related aspects of human cognition.
Memory performance is one of the cognitive compounds disturbed in dyslexic patients and, together with speed of processing, has been suggested to be a shared cognitive process between dyslexia and its comorbid disorder ADHD (attention deficiency/hyperactivity disorder). We therefore investigated whether genetic variation in GRIN2B contributes to specific quantitative measures in a German dyslexia sample and genotyped 66 SNPs in its entire genomic region. The sample consisted of 397 patients and their parents.
We found supportive evidence for markers in intron 3 to be associated with short-term memory in dyslexia and could show that this effect is even stronger when maternal transmissions only are considered. These results suggest that variation within GRIN2B may contribute to the genetic background of specific cognitive processes that are correlates of the dyslexia phenotype.

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Mapping of a novel form of autosomal recessive, complicated hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a neurodegenerative condition clinically characterised by progressive weakness and spasticity of the lower limbs. The presence of additional neurological symptoms defines the so-called complex or complicated forms of the disease. With almost 20 loci described so far, complex HSP is genetically highly heterogeneous. We investigated a large consanguineous Turkish pedigree with several members suffering of a complex HSP phenotype consistent with autosomal-recessive inheritance. Clinical examination of three patients revealed a spastic gait disorder accompanied by cloni and a positive Babinski sign. An abnormal gait was first noticed during early childhood. The two older patients, currently in their thirties, started to develop cognitive problems in their teenage years. They also show evidence of progressive sensory loss. MRI analysis of the brain further revealed a thin corpus callosum. Four distantly related individuals, also from consanguineous parents, are reported to have suffered from similar symptoms with a fatal outcome in the fourth decade of life. Genetic analysis of eight family members including all three patients excluded the most common recessive forms of HSP. A subsequent homozygosity mapping revealed linkage to a single genomic region (LOD 3.2 representing the maximum score). This region does not include any of the known loci for HSP or related neurodegenerative disorders. It spans 50 Mega bases of sequence and contains approximately 500 genes. Attempts to identify the disease causing mutation are under way. Our findings suggest the presence of a novel form of complex recessive HSP closely resembling SPG11 and SPG15 with a thin corpus callosum, mental retardation and, possibly, a shortened lifespan.

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Genome-wide linkage scan in a moroccan family with autosomal-recessive exstrophy of the bladder identifies a novel gene locus on chromosome 3p25.3

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Introduction: Exstrophy of the bladder (EB) is part of the bladder exstrophy-epispadias complex (BEEC) representing a spectrum of urogenital anomalies in which part or all of the distal urinary tract fail to close and are exposed on the outer abdominal wall. Familial occurrence is rare and no causally related genetic or non-genetic factor has been identified so far. In this study, we aimed to identify a new locus contributing to EB in a family with suggestive autosomal-recessive inheritance.
Patients and methods: A genome-wide linkage scan was performed in a large consanguineous kindred of Moroccan origin. Three affected males showed the phenotype of classic EB. We were able to collect the DNA of two affected cousins and 16 unaffected individuals.
Results: Strongest evidence for linkage was obtained for a chromosomal region on chromosome 3p25.3 with a parametric LOD score of

3.4 comprising 0.9 MB. This region contains at least seven genes. However, sequence analysis of all coding exons of these genes revealed no pathogenic mutation. Interestingly, a homozygous unknown sequence variant affecting a non-coding microRNA residing in this area was observed in both patients.
Conclusion: Since the nucleotide substitution observed does not affect the mature miRNA, it seems unlikely, that it has an effect on the silencing process(es). On the other hand, one may speculate that the sequence variant promotes uncorrect folding, thereby preventing recognition and cleavage of its primary transcript by Drosha. Further studies are underway to elucidate the functional consequences of this variation.

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Mutation analysis of the ATRX- gene using DHPLC (denaturing high performance liquid chromatography)

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Background: X-linked alpha thalassaemia mental retardation (ATRX) syndrome is a rare X- linked recessive disease with its main clinical features: mental retardation, facial dysmorphisms, genital abnormalities, and alpha- thalassaemia. Over 100 mutations in the ATRX- gene are described as the responsible gene defect for the ATRX syndrome.
Objective: Direct sequencing of all 35 exons is time-consuming, laborious, and expensive. An automated, more economic but still highly sensitive method could be helpful to screen the complete gene.
Methods/ patients: We established DHPLC by cleaving all coding regions in 42 fragments and determined the necessary melting temperatures of each fragment. By means of positive controls (DNA of known ATRX patients)* we tested the detection rate of DHPLC compared to direct sequencing. Hence, we analyzed patients with strong suspect of ATRX by both methods.
Results: We established optimal detection temperatures for every single fragment. Each of our positive controls could be detected by DHPLC. In addition we were able to find in our patients one SNP and one mutation unknown hitherto.
Conclusion: DHPLC is a highly sensitive and highly specific method to screen large genes for mutations. Its time-effectiveness and its cost savings militate even more in favour of this method.
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Analysis of transforming growth factor-beta receptor type 1 (TGFBR1) in patients with non-syndromic cleft lip with or without cleft palate of Central European descent

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Transforming growth factor-beta (TGF-β) type 1 receptor (also known as activin receptor-like kinase 5, ALK5) is expressed in palatal tissue during embryogenesis. Experimental studies in transgenic mice with a genetic deletion of Alk5 showed that TGF-β type 1 receptor is required for upper lip and midline fusion of the hard and soft palate. In humans, association of TGF-ß type 1 receptor gene (TGFBR1) and the development of non-syndromic cleft lip with or without cleft palate (NSCL/P) had been observed in a multiethnic sample of Chinese, Philippine, Indian and Turkish families. In order to re-evaluate the relevance of these findings, we carried out a family-based association study among 248 NSCL/P families of Central European descent. Genomic DNA was obtained from peripheral blood of 248 complete parent-offspring triads with NSCL/P. Genotyping and transmission disequilibrium test (TDT) were performed on all 248 triads with a total of 17 tagging single-nucleotide polymorphisms (SNPs). TDT analysis revealed no significant transmission distortion, neither at the level of individual markers nor at the level of haplotypes. Our data do not support the involvement of TGFBR1 as a major risk factor in the development of NSCL/P among patients of Central European descent.
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Genomewide association scan of neurophysiological endophenotypes reveals transregulational effects on SLC2A3 in dyslexic children

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In the present study we tested for association with mismatch-negativity (MMN), an endophenotype measuring the children's reaction to a change in speech stimuli, in a whole-genome association data set of 200 German dyslexic children. We selected 7 SNPs showing significant p-values and being located in chromosomal region with previous linkage evidence for independent replication in a sample of 186 German dyslexics. We found that SNP rs4234898 on chr 4 was significantly associated with the subphenotype MMN3 in both samples. The association result in the combined sample withstood genome-wide correction for multiple testing (p=6.09e-08 carrier T model).
rs4234898 lies within a gene desert, with the nearest genes being more than 150 kb away. None of the neighboring genes shows evidence for

brain-specific expression or function or is known to be related to neurophysiological phenotypes. Analysis using publicly available GWA expression data yielded evidence for possible trans-regulation effects of rs4234898 on SLC2A3 (also called GLUT3), the predominant facilitative glucose transporter in neurons. We could confirm the trans-regulation of SCL2A3 by own experiments in EBV-transformed cell lines from 17 dyslexic children, with 10 individuals carrying at least one T-allele showing lower expression of SLC2A3 (p=0.028 one-sided Wilcoxon-test). When performing further analysis we found that rs11100040 on chr 4 was also significantly associated the differential expression of SLC2A3 in the public expression databases. Interestingly, rs4234898 and rs11100040 form a haplotype which is associated with MMN3 (p=6.71e-08 in combined sample).
In conclusion, we performed the first genome-wide association study focussing on a neurophysiological endophenotype of dyslexia. Our results suggest a genomic region on chromosome 4 as a factor contributing to variable expression of this trait, possibly conferring its functional effect through trans-regulation of SLC2A3.

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Analysis of copy number variations (CNVs) in the schizophrenia locus SCZD10 on chromosome 15q15

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Previously we identified a locus on chr.15q15 (SCZD10) for periodic catatonia (MIM605419) a subphenotype of schizophrenic psychoses with genome-wide linkage studies of 12 extended pedigrees. We refined the critical region to a 7.49 Mb interval with further extensive haplotyping studies. In our efforts to reveal the underlying disease gene, we performed mutation analysis 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 all highly conserved non-genic elements (CNGs) in our refined linkage region of SCZD10. In total, we could identify 3099 CNGs with more than 70% identity and a length of ≥100 bp, 65 of them with an identity of 100% (VISTA: Mayor et al., Bioinformatics (2000), 16:1046). We sequenced 8 affected individuals for the 65 perfectly conserved CNGs and detected 9 SNPs within these CNGs. Three of these SNPs were not contained in public databases. But none of these three SNPs segregated with the disease. Recent reports have highlighted the role of copy number variations (CNVs) in the development of complex disorders and have suggested that distinct variations are common in schizophrenia. We therefore carried out a copy number analysis in our refined linkage region with Genome-Wide Human SNP Array 6.0 from Affymetrix. We analysed 29 individuals from 3 extended pedigrees, 15 of them affected individuals. The analysed linkage region exhibits 9 CNVs known in Database of Genomic Variants (DGV). The genomic size of these CNVs vary from 6 kb (6 markers) to 195 kb (146 markers). None of these known CNVs could be found in the analysed 29 individuals. Only one healthy individual shows a de novo heterozygous duplication of 40 kb length detected by 14 markers. The duplication is absent in both parents of this individual.

P278

Putative association of polymorphisms in DNA repair genes with chronic lymphocytic leukaemia risk and chromosomal aberrations

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Associations between polymorphisms in DNA repair genes and different forms of cancer have been shown in several studies. In this study, we analysed the association of seven SNPs in five DNA repair genes with the incidence of chronic lymphocytic leukaemia (CLL). Additionally, we investigated a putative correlation of these SNPs with chromosomal aberrations that are important prognostic markers in CLL.
We analysed 461 CLL patients and an equal number of sex and age matched controls. Particular attention was paid to patients with the favourable cytogenetic aberration del(13q) as sole aberration and patients with the unfavourable cytogenetic aberrations del(17p) and del(11q).
The frequency of the SNPs rs13181 in the nucleotide excision repair gene xeroderma pigmentosum group D (XPD) and of rs25487 in the base excision repair gene X-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1) differed between controls and all patients and/or groups of patients.
The genotypes A/A and A/C of the SNP rs13181 in XPD were differently distributed between CLL patients and controls (OR = 1.39, p = 0.03), suggesting that the risk for the development of CLL could be increased for persons with the A/C genotype.
Regarding the frequencies of SNPs in cytogenetic subgroups versus controls we found that the genotype distribution of all investigated SNPs was similar in CLL patients with favourable cytogenetic aberrations and controls.
However, in the group of CLL patients with unfavourable cytogenetic aberrations we identified two SNPs conferring an increased risk for CLL: rs13181 in XPD (A/C vs. A/A: OR = 2.69, p = 0.006) and rs25487 XRCC1 (A/A vs. G/G: OR = 2.66, p = 0.024).
Our data indicate that polymorphisms in DNA repair genes may be associated with CLL. To assess the possible utilisation of inborn genetic polymorphisms for improved prediction of the outcome of CLL, we currently investigate the influence of these SNPs on overall survival of CLL patients.

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The frequency of genes alleles predisposing to inflammatory bowel disease

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Inflammatory bowel disease (IBD) is characterized by chronic relapsing inflammation in gastrointestinal tract. This autoimmune disease is dived into two subtypes Crohn disease (CD) and ulcerative colitis (UC). If it is not possible to differentiate these IBDs the patients are diagnosed as indeterminate colitis (IC). The genetic bases of predispositions have been still studied. In our 160 severe IBD patients with average age of diagnosis 26 years, the youngest patient was diagnosed when was 3 years old and the oldest one was diagnose at the age of 69. In this group we investigated frequency of alleles in NOD2/CARD15 gene and 15-PGHD gene. The 15-PGHD gene codes dehydrogenase which is a prostaglandin-degrading enzyme and acts as an antagonist to enzyme called cyclooxygenase 2. We also studied frequency of hap-

lotype in q31 region on 5th chromosome. We estimated frequency of alleles SLC22A4 1672T and SLC22A5 / T207C. We observed increased frequency of INV4+39C>T homozygotes in group of patient under 18 years old with UC (12%) in comparison to adult patients where the INV4+39C>T homozygotes were not been observed. The frequency of A at position 168 in PGDH gene were higher in patient under 18 years old (49%) than in adult patients 34%. The observed differences in frequency of both above alleles in PGDH gene are good start point to further study the role of this gene in IBDs.

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Association of the cytotoxic T lymphocyte antigen-4 (CTLA4) with alopecia areata

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AA is a common skin disease presenting with patchy hair loss which affects approximately 1–2 % of the general population. The pattern of familiality suggests that the genetic basis is multifactorial. So far, the etiopathogenesis of AA is incompletely understood. However, AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle. The mechanism of hair follicle dysfunction is immunological and is controlled by activated T-cells.
Variants of the cytotoxic T lymphocyte antigen-4 (CTLA4) gene on chromosome 2q33 which belongs to the immunoglobulin superfamily and regulates and inhibits T-cell proliferation have been described to be associated with several autoimmune diseases, e.g. type1 diabetes, Graves' disease and Hashimoto's thyreoiditis (Ueda et al. 2003, Kavvoura et al. 2005, Plenge et al. 2005). This important role of T-cells for a number of autoimmune diseases suggests further examination of their relevance for the pathogenesis of AA.
In the present study, we aimed to examine the role of CTLA4 in the development of AA by genotyping 26 variants in a large sample of 1192 unrelated AA-patients of Central-European descent and 1280 controls. Single-marker analysis showed highly significant results for 8 markers. In the overall sample, the smallest P-value of 6.63e-007 (OR 1.33) was seen for SNP rs2637777. Subdividing the sample according to severity, family history and age at onset, we detected lowest P-values for patients with the severe form of AA comprising all affecteds with complete loss of scalp hair (alopecia totalis) or of scalp and body hair (alopecia universalis) (P=6.38e-010, OR 1.55) for SNP rs1427678.
In conclusion, our results suggest CTLA4 as a general risk factor for AA with the strongest effect observed among patients with a severe type of AA and furthermore underline the autoimmune component of this hair loss disorder.

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Investigation of theTRAF1-C5 locus in alopecia areata

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Alopecia areata (AA) is a common hair loss disorder with a complex mode of inheritance. Autoimmune mechanisms are presumed to be crucial etiologically. A common genetic background might be plausible for a number of autoimmune disorders. This phenomenon has been demonstrated in previous studies by an overlap of susceptibility alleles for HLA-DRB1 and PTPN22 for AA and rheumatoid arthritis (RA). The two genes are the most established susceptibility loci for these diseases suggesting that a common pathological autoimmune reaction pathway contributes to the development of AA and RA.
Recent studies suggested that genetic variants on the TRAF1-C5 locus on chromosome 9q exert an influence on susceptibility to RA (Plenge et al. 2007, Kurreeman et al. 2007, Chang et al. 2008). These variants are in linkage disequilibrium with two genes relevant to chronic inflammation, namely TRAF1 (encoding tumor necrosis factor receptor-associated factor 1) and C5 (encoding complement component 5).
In the present study, we aimed to examine the role of the TRAF1-C5 locus in the development of AA by genotyping 29 variants in a large sample of 1192 unrelated AA-patients of Central-European descent and 1280 controls. Our results suggest that although no significant association was observed in the patient sample overall (P-values between 0.0665 and 0.849), significant findings in the subgroups of severe cases and familial cases point to an involvement of the TRAF1-C5 locus in the etiology of AA. In future, further enlargement of the collective as well as replication in independent samples will help to further understand the contribution of this gene locus to the development of alopecia areata.

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Novel RYR1 missense mutation causes malignant hyperthermia associated with a mild form of central core myopathy

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The ryanodine receptor (RYR1) encodes the major calcium-releasing channel in skeletal muscle sarcoplasmic reticulum. Mutations in this gene have been found in association with several diseases: malignant hyperthermia (MH) as well as three congenital myopathies including central core disease (CCD), multiminicore disease (MmD) and in an isolated case of a congenital myopathy characterized histologically by cores and rods. The majority of mutations reported are missense enchanges identified in cases of MH and CCD. We detected an unpublished RYR1 mutation in a patient with an episode of MH, an event of rhabdomyolysis and a tendency to muscular crampings in physical activity and in rest. We identified a mutation in exon 102 of the RYR1 gene (c.14782A>T p.Ile4928Phe) in the patient and his mother who presented only minimal symptoms of myopathy. The mother, now handicapped by a stroke, reported on previous mild muscular weakness in climbing stairs. In adolescence she was active in sports without weakness or any event of rhabdomyolysis. She also reported a tendency towards cramping. No other family member presented symptoms of a myopathy, no

further event of MH was reported. No mutation was found in the father. The novel RYR1 mutation is located in the C-terminus, a domain that is regarded to constitute the calcium channel-forming segment, in which other core rod myopathy causing mutations and the majority of dominant mutations have been identified. The phenotypic variability in this family can be explained by autosomal dominant inheritance with reduced penetrance in the mother. Galli et al (2002) also described this phenomenon previously. He suggested somatic mosaicism in the mother and discussed also anticipation in this disease.
This case underlines the importance of screening for RYR1 mutations in patients, even with mild symptoms of myopathy.

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Polymorphisms in CLU gene are associated with pseudoexfoliation syndrome and pseudoexfoliation glaucoma in German patients

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Purpose: Pseudoexfoliation (PEX) syndrome is a generalized elastic microfibrillopathy characterized by fibrillar deposits in intra- and extraocular tissues. Genetic and non-genetic factors are known to be involved in its etiopathogenesis. In this study we focus on one functional candidate gene involved in PEX material deposition and analyze its potential association with PEX syndrome and PEX glaucoma (PEXG).
Methods: 5 single-nucleotide polymorphisms (SNPs) capturing >95% of overall genetic variance observed in Europeans at clusterin (CLU) gene locus were genotyped in 333 unrelated PEX patients and 342 healthy individuals of German origin and a genetic association study was performed. To replicate our findings, we genotyped two SNPs of the CLU gene in further 328 unrelated German PEX patients as well as in 209 Italian PEX patients and 190 Italian controls.
Results: Association with PEX was only observed for the SNP rs2279590 in intron 8 of CLU gene (corrected P=0.0347, OR=1.34) in our first German cohort. Likewise, a frequent haplotype encompassing the associated risk allele showed nominally significant association. Neither of remaining SNPs nor SNP haplotypes were associated with PEX. The association found was confirmed in a second German cohort (P =0.0244) but not in the Italian cohort (P =0.7173). In addition, the association with CLU SNP rs2279590 was more significant in German patients with PEX syndrome than with PEXG.
Conclusion: Genetic variants in the gene encoding clusterin may represent a risk factor for PEX in German patients but not in Italian patients.

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Screening of candidate genes for familial exudative vitreoretinopathy (FEVR) and related vasoproliferative ocular disorders

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Familial exudative vitreoretinopathy (FEVR) is a congenital blinding disorder characterized by an incomplete peripheral vascularization of the retina. This defect in blood vessel development entails a highly variable phenotype due to secondary complications like vitreo-retinal traction, retinal folds, or hemorrhages, which can range from symptom-free conditions to severe retinal detachment. Other clinical entities with a similar ocular phenotype include Norrie disease, Coats' disease, and retinopathy of prematurity (ROP). Three genes have been associated with FEVR so far: FZD4 (EVR1), NDP (EVR2), and LRP5 (EVR4), while a fourth locus has been linked to 11p13-p12 (EVR3). Norrie disease, which in addition to the ocular abnormalities presents with defects in the brain and inner ear, and Coats' disease, which can be considered as a unilateral form of the disease, were associated only with mutations in NDP so far. Here we report on the screening of patients diagnosed with an FEVR-/ Norrie-/ Coats'-like ocular phenotype, who were negative for mutations in NDP and FZD4. We identified a novel, potentially pathogenic LRP5 mutation (p.T672 M) in a patient diagnosed with Coats' disease, and also found a possible risk allele (rs314776). Sequence analysis of the coding regions of three candidate genes (LRP5L, PLVAP, LMO2) did not reveal any pathogenic mutations. These results suggest that mutations in other genes may lead to familial and sporadic forms of EVR. Analyses of additional candidate genes are currently in progress in our lab.

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Mammalian two- and tree-hybrid studies of RAD51 paralogs

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The five RAD51 paralogs RAD51B (RAD51L1), RAD51C (RAD51L2), RAD51D (RAD51L3), XRCC2 and XRCC3 share 20–30% protein homology and interact with each other and with RAD51 recombinase, an ortholog of the Escherichia coli recombinase RecA. These proteins execute a DNA repair process designated as homologous recombination which is vital to the error-free removal of DNAdouble-strand breaks and interstrand crosslinks. Yeast two-hybrid studies, immunoprecipitations with human cell extracts and co-expressions in a baculovirus system have shown that the RAD51 paralogs likely form two distinct complexes: RAD51C/XRCC3 and RAD51B/RAD51C/RAD51D/XRCC2. In the latter, the presence of RAD51B has been reported to stabilize the interaction of RAD51D and XRCC2. We are aiming to confirm interactions of the five RAD51 paralogs through mammalian two- and three-hybrid studies. Full-length RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 cDNAs were separately cloned into the pM vector with a GAL4 DNA-binding domain and into the pVP16 vector (both Clontech) with an activation domain. Reporter gene in this assay was firefly luciferase (pGL4.31). For transfection control, we used an expression vector for renilla luciferase (pRL-null), both from Promega. As reference interaction we utilized the interaction between FANCA (pM vector) and FANCG (pVP16 vector) protein (mean 263.4-fold induction). HeLa cells were transfected with GeneJuice® reagent (Novagen). All potential interactions were assayed in both directions each of the cDNAs cloned into pM- and pVP16- vector. Interaction between RAD51C and XRCC3 was about 61.9-/8.2-fold above the negative control (basal activity). Interaction between RAD51C and RAD51B (91.1-/111.3-fold) could also be confirmed. Interaction between RAD51D and XRCC2 has not yet been quantified. Interaction between RAD51C and RAD51D has been reported as weak in yeast two-hybrid studies and may require a mammalian three-hybrid system to occur.

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Testing functional candidate genes for laminopathies including EDMD and FPLD

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Laminopathies, including Emery-Dreifuss muscular dystrophy (EDMD) and Familial Partial Lipodystrophy (FPLD) besides others, are rare genetic disorders caused by mutations in genes encoding lamins or lamin-interacting proteins. LMNA, EMD and to a lower measure the Nesprin genes SYNE1 and SYNE2 have been associated with Emery- Dreifuss muscular dystrophy (EDMD). EDMD is characterized by contractures of the Achilles tendons, progressive skeletal muscle weakness and heart rhythm disturbances leading to dilated cardiomyopathy (DCM) and sudden cardiac death. Familial partial lipodystrophy (FPLD) has been shown to be due to mutations in the LMNA gene encoding nuclear lamins A and C. FPLD is characterized by loss of subcutaneous fat from the extremities and trunk since puberty. Moreover, predisposition to insulin resistance and its complications occur.
Since ~60% of EDMD patients are not associated to the genes mentioned, we used a functional candidate-gene approach to identify additional genes involved in EDMD. Based on reported interactions of lamina associated polypeptide 2 (LAP2) with nucleoplasmic lamin A/C and the association of the LAP2alpha isoform to DCM, 111 EDMD and 87 DCM patients were investigated for DNA variations in LAP2 (encoding six LAP2 isoforms). Among ten variations found, four changes—p.P426L (c.1481C>T) in LAP2alpha, p.D271E (c.1054T>G) in LAP2beta, and p.V423L (c.1058G>C) and p.M381I (c.1387 G>A) in LAP2gamma—were unique for EDMD patients, but segregation analysis indicated only p.P426L LAP2alpha as a mutation potentially associated to EDMD.
In 27 patients with lipodystrophy no mutations in LMNA were found. Additionally, LMNB2 and NARF encoding other components of the inner nuclear membrane were considered as functional candidate genes for those non-LMNA-associated forms of lipodystrophy. By screening the coding region, promoter, 5'- and 3⊠-UTRs of LMNB2 and NARF no mutations associated to FPLD were found.

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Do epigenetic effects at MCHR1 contribute to obesity?

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Obesity is a major cause of morbidity and mortality in western community. It is associated with an increased risk of type 2 diabetes mellitus, heart disease, cancer etc.
Melanin-concentrating hormone receptor 1 (MCHR1) plays a significant role in regulation of energy balance, food intake and body weight in humans and rodents. Wermter et al. (2005) detected an association and transmission disequilibrium with obesity for two MCHR1 SNPs (rs133072, rs133073) in extremely obese German children and adolescents. However, this finding could not be confirmed in four independent study samples from Germany, France, Denmark and USA. Accordingly, results of two other groups were somewhat contradictory. Gibson et al. (2004) did not find association of SNPs rs133072 and rs133073 with obesity in a population-based cohort of British Caucasians aged 40–65 years with a mean BMI = 26. In contrast, Bell et al. (2005) reported a weak association for SNP rs133072 and obesity in a French Caucasian study group comprising morbidly obese children and adults. These findings raise the question of age-dependent mechanisms and/or epigenetic effects.
The SNPs rs133072 and rs133073 are in tight linkage and form two haplotypes in which one allele of either SNP represents a methylation site. In addition, there are several CpG dinucleotides in close vicinity of both SNPs. We hypothesize that epigenetic factors contribute to the population- and age-specific effects reported for the association of MCHR1 alleles and obesity.
We analyzed a 315 bp genomic region containing both SNPs as well as 15 CpG dinucleotides with respect to DNA methylation in 42 human individuals aged between 22–78 years. Interestingly, we found a significant haplotype-specific difference in average methylation (p=0.0004) and an age dependent hypomethylation of the obesity risk haplotype in contrast to no alterations in methylation of the other haplotype. This supports our hypothesis that epigenetic factors may act at MCHR1.

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Mapping determinants of human gene expression in hippocampus by genome-wide association

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Much of the genetic component of human phenotypic diversity has been proposed to be the result of cis-acting influences on gene expression. Several recent studies have stressed the extent to which gene expression varies within and between populations, and have shown that allele-specific expression is relatively common among non-imprinted autosomal genes. Further, there is evidence that a portion of the inherited variation in gene expression may play an important role in susceptibility to complex diseases. While genetic factors have been mapped that influence gene expression in peripheral tissues, such data is not yet available on human fresh brain tissue. This information would be highly desirable for the quest for genetic factors contributing to the development of complex CNS phenotypes. In the present study, we aim at mapping genetic determinants of gene expression in human hippocampal segments derived from epilepsy-surgery. The University of Bonn harbours a major center for neurosurgery and one of the largest hippocampal fresh frozen tissue banks world-wide.
For systematic mapping of determinants for hippocampal gene expression, we are currently isolating DNA and RNA of 150 hippocampus samples taken from the Bonn tissue bank which then undergo a genome-wide association study (GWAS) and gene expression (GEX) analysis. Each individual DNA sample is genotyped with 660,000 SNPs. Individual gene expression levels for more than 99,9% of all known human genes (approx. 25,000 annotated RefSeq and UniGene genes) are interrogated with microarrays containing more than 48,000 probes. Gene expression levels are then systematically correlated with individual genotype information. Preliminary results will be presented. An important application of our findings will be the interpretation of GWAS results for neuropsychiatric disorders that are currently generated in the course of the NGFNplus-integrated genome research network "MooDS".

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Characterization of the epidemiology and genetic basis for Dupuytren disease

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Dupuytren disease is a multifactorial fibromatosis that causes progressive and permanent contracture of the palmar fascia with subsequent flexion contracture of the fingers. It is precipitated by a proliferation of

contractile myofibroblasts within the palmar fascia. A strong genetic predisposition exists but nearly nothing is known about the genetics and the molecular etiology and pathogenesis of this disease.
We have now embarked on a comprehensive study to unravel the genetic factors involved Dupuytren disease. To date we have collected tissue and blood samples from 230 German and Swiss patients. 48 (21%) of the recruited patients were females. 94 (41%) patients had a positive family background. In cases with positive family history both hands were affected in 68% as compared to 55% with no known family history. The mean age of first surgical treatment was 57 ± 13 years of age and ranged from 22–80 years. It was 56 years in male and 60 years in female patients. 28 patients also had knuckle pads and 11 patients showed plantar fibromatosis. 11% of the patients also had diabetes, 5% had rheumatoid arthritis. We did not observe a clear risk factor, since about one third of the patients were smokers and an association with alcohol abuse was not seen.
Fibroblasts were cultured from tissue samples, and DNA and RNA samples were extracted from blood and fresh tissues. Cultured cells expressed myofibroblast markers such as α-smooth muscle actin after stimulation with transforming growth factor β. A first whole genome association study is under way, and further large scale genetic analyses shall be performed in order to gain insight into the genetics and pathogenesis of dupuytren disease. Our findings will also contribute to the understanding of age-dependent changes in myofibroblasts.

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Deletion of LCE3C and LCE3B genes at PSORS4 contributes to susceptibility to psoriasis vulgaris, but not to psoriatic arthritis in large German cohorts

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PSORS4 is one of the confirmed Susceptibility Loci for Psoriasis vulgaris (PsV), a common inflammatory, hyperproliferative disorder of the outer skin layer (epidermis). This locus is especially interesting for PsV, since it comprises the epidermal differentiation cluster, a group of genes expressed in the upper strata of the epidermis. While several genes at PSORS4 - e. g. LOR, PGLYRP and SPRR genes—have been proposed to account for psoriasis susceptibility, very recently a copy number reduction within the LCE gene cluster was shown to be enriched in 2,831 psoriasis patients and to be associated in a large family-based cohort (Cid et al, Nat Genet in press).
We established a multiplex assay of two fluorescently marked PCR products that were detected on a capillary sequencer (ABI3730) in order to analyse two large case-control cohorts of 1,114 PsV patients, 650 patients with psoriatic arthritis (PsA) and 937 control probands for this deletion. The copy number reduction of LCE3C and LCE3B genes was also more common in German PsV patients (72.2%) than in control individuals (65.5%) ($\chi^2 = 20.071$, $p = 7.46*10^{-6}$ and OR = 1.37 [1.19–1.57]). In contrast, the frequency of the LCE deletion in PsA patients (64.8%) was very similar to that in control individuals (not significant). Stratification for the main psoriasis risk factor (PSORS1 risk allele) indicated no evidence for interaction between these two factors. Our results suggest that the deletion of these two epidermally expressed genes is a contributing factor to skin manifestation, while it does not confer risk to joint affection in German patients. The suggested molecular mechanism in case of a deletion of LCE genes is an inappropriate repair response following skin barrier disruption. This deletion is the first genetic risk factor predisposing only to skin-type of psoriasis vulgaris, supporting the concept that overlapping, but different etiological factors underlie skin and joint manifestations.

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Systematic replication of candidate genes associated with atrial fibrillation supports two new susceptibility loci

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Background: Atrial fibrillation (AF) is the most common sustained arrhythmia in humans. Besides conventional risk factors, evidence for a heritable component is increasing. Associations between single nucleotide polymorphisms (SNPs) and AF have been described, but only few were reliably replicated. We systematically validated these previous findings in two large, well-defined populations of European descent.
Methods: We screened PubMed for associations between SNPs and AF. Our study consisted of 1645 AF-cases from the German Competence Network for Atrial Fibrillation and 4073 controls from the KORA S4 study. After genotyping, logistic regression was used to calculate associations between cases and controls adjusting for age, gender and hypertension. A meta-analysis combined the results of our cohorts.
Results: Twenty SNPs were identified from literature. SNPs rs1800872 in IL10 (odds ratio (OR) 0.89, 95% confidence interval (CI) 0.78–0.95, p=0.012 and rs5051 in AGT (OR 1.07, CI 1.01–1.19, p=0.042) were significantly associated with AF in our sample. AGT rs699 was associated with AF, but failed significance.
Conclusion: Our results support two new susceptibility loci for AF. Sufficient statistical power is critical to detect true associations and is the main cause for many unreplicated genetic association findings in literature. Nine of 20 SNPs studied are not respresented on common SNP arrays. We showed that also in the time of genome-wide association studies, candidate gene analyses can provide important insight into disease mechanisms.

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Determining the genetic architecture of central cornea thickness (CCT) with GWAS using DNA pooling

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Purpose: Our aim was to identify quantitative trait loci which influence central cornea thickness (CCT). Here, cornea thickness refers to the average of both eyes, since correlation of CCT in both eyes had been determined previously. CCT values from 600 individuals of German descent showed a normal distribution.
Methods: We performed a genome-wide association analysis using SNP-MaP (SNP Microarray and DNA pooling) with 500 K SNP Affymetrix arrays. We pooled 36 DNAs from either extreme of the normal distribution in 3 replicates (n=12). Array analysis of pooled DNAs was done with a modified version of GenePool (Pearson et al., AJHG 2007). For graphical visualization of GenePool data, we developed the software GPGraphics to facilitate analysis.
Results: We correlated the difference in mean RAS values of both pools (DRASmean) with GenePool ranks for each SNP. Distinct differences

were only present among the first 2,000 SNPs. Accordingly, we defined clusters with at least 3 SNPs with a rank <1,000 as associated loci. Our analysis identified 40 loci with marked DRASmean values as indication of differential allele frequencies between both extreme quantiles. We also reproduced the association by comparing the extreme groups with pools of population based controls. 34 of these SNP loci showed strong LD in HapMap data and 26 pointed to genes. We then verified pooling data at single genotype level for two selected loci using TaqMan assays in the complete cohort. htSNPs selected to contain at least 1 SNP with a rank <2,000 confirmed significant allele frequency differences (p=0.001), even after permutation correction (p=0.011).
Conclusion: Our data show that hybridization intensities of pooled DNA correlate well with individual genotyping results. Thus DNA pooling is a useful strategy for GWAS, although loci with strong LD are preferentially detected on those arrays. Furthermore, the genetic architecture of CCT is extremely complex with at least 40 loci influencing this trait.

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Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington's disease
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Background: Huntington´s disease (HD) is caused by an unstable CAG repeat expansion in the HD gene that is inversely correlated with the age-at-onset of the disease. Since the size of the CAG repeat only explains about 42–73% of the variance of the age-at-onset, other factors might modify the course of the disease.
Objective: We aimed to identify and characterize a genetic modifier in Huntingtin-associated protein-1 (HAP1), which participates in intracellular trafficking, colocalizes and interacts with huntingtin, thus representing a good candidate for a genetic modifier.
Methods: We analyzed HAP1 polymorphisms by association studies in 980 European HD patients. As one of these turned out to modify the disease, the functional analyzes of HAP1 and this polymorphism concentrated on the examination of protein-protein interactions by yeast two-hybrid assays and coimmunoprecipitation, aggregate formation, cellular localization and apoptotic properties in cell culture.
Results: We identified one HAP1 polymorphism as a genetic modifier for HD age-at-onset. Patients homozygous for this polymorphism develop the first symptoms about 8 years later than patients with other genotypes. Functional analyses showed that polymorphic HAP1 binds more mutant huntingtin and reduces its degradation. Additionally, it increases the number of large aggregates, prevents the localization of huntingtin in the nucleus and protects against huntingtin-mediated toxicity in transfected cells.
Conclusion: We identified the first genetic modifier for HD that acts protective and has a functional importance to huntingtin-mediated toxicity and thus to HD pathogenesis.

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High resolution DNA methylation analysis of chromosome 21 gene promoters
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Differential DNA methylation is an essential epigenetic signal for gene regulation, development and disease processes. We mapped DNA methylation patterns of 190 gene promoter regions on chromosome 21 using bisulfite conversion and subclone sequencing in five human cell types, including leucocytes and trisomic fibroblasts. A total of 28626 subclones were sequenced at high accuracy resulting in the measurement of the DNA methylation state of 580427 CpG sites. Our results show that average DNA methylation levels are distributed bimodally with enrichment of highly methylated and unmethylated sequences, both for amplicons and individual subclones which represent single alleles from individual cells. Within CpG-rich sequences, DNA methylation was found to be anti-correlated with CpG dinucleotide density and GC content and methylated CpGs are more likely to be flanked by AT rich sequences. We observed over-representation of CpG sites in distances of 9, 18 and 27 bps in highly methylated amplicons. However, DNA sequence alone is not sufficient to predict an amplicon's DNA methylation status, since 43% of all amplicons are differentially methylated between the cell types studied here. DNA methylation in promoter regions is strongly correlated with the absence of gene expression and low levels of activating epigenetic marks like H_3K_4 methylation and H_3K_9 and K14 acetylation. Utilizing the single base pair and single allele resolution of our data, we found that i) amplicons from different parts of a CpG island frequently differ in their DNA methylation level, ii) methylation levels of individual cells in one tissue are very similar and iii) methylation patterns follow a relaxed site specific distribution. Furthermore, iv) we identified three cases of allele-specific DNA methylation on chromosome 21.
Our data shed new light on the nature of methylation patterns in human cells, the sequence dependence of DNA methylation and its function as epigenetic signal in gene regulation. Further, we illustrate genotype-epigenotype interactions by showing novel examples of allele-specific methylation.

P295–P298 Normal variation, population genetics, genetic epidemiology, evolutionary genetics

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Evidence for founder effect of filaggrin mutations p.R501X, c.2282del4 and p.R2447X in ichthyosis vulgaris
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The molecular basis of ichthyosis vulgaris (IV), the most common hereditary disorder of cornification in humans, has recently been ascribed to loss-of-function mutations in the gene encoding the protein filaggrin (FLG), which is important for the formation of the stratum corneum during epidermal differentiation. Here we genotyped 38 IV

patients originating from Austria, Germany, the Netherlands, Belgium and Scotland, who were homozygous, compound heterozygous or heterozygous for the most common European FLG mutations p.R501X, c.2282del4 and p.R2447X, as well as 12 normal controls for eight different single nucleotide polymorphisms (SNPs). Lying within a 54 kb region, three SNPs mapped within exon 3 of FLG, while two were flanking the gene up to 16 kb in centromeric and three up to 15 kb in telomeric direction. We assessed significant differences between the genotypes of IV patients and controls with nearly complete association of the SNP alleles with the mutations carried on conserved haplotypes and therefore conclude that the high frequency of these FLG mutations is the result of a founder effect rather than mutational hot spots. Our results support the idea, that the high prevalence of FLG mutations in European populations could be the result of an advantage for heterozygous carriers, since increased transepidermal antigen transfer due to skin barrier impairment may lead to better stimulation of the immune system, however this hypothesis requires further investigation.

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Clinical and molecular studies in 110 morbid obese patients (with BMI >40)

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Morbid obesity is heritable trait and a major risk factor for numerous other medical problems including hypertension, heart disease, type 2 diabetes, and depression. Our aim was to quantify the effects of common genetic polymorphisms on BMI and weight, respectively.
In a series of 110 patients (62 females, 38 males; median age 39 years, range 17–66 years) who underwent laparoscopic gastric banding operations for morbid obesity at the Johannes Gutenberg-University, Mainz, we studied clinical parameters and the prevalence of two common single nucleotide polymorphisms (SNPs), rs9939609 in IVS1–2 of the fat mass- and obesity-associated gene (FTO) and rs75666605 located 10 kb 5'-prime of the insulin-induced gene 2 (INSIG2). Patients were predominantly Caucasians, some 6% were of Turkish origin. All patients had a body mass index of ≥40 (BMI; calculated as weight in kilograms divided by height in meters squared); the mean BMI was 49 (range 40–77). IQ was normal and there were no anomalies/dysmorphies, indicative of a syndromic disorder. All patients had normal karyotypes, 46,XX and 46,XY, respectively.
We identified the homozygous FTO obesity SNP variant in 33 patients (30%, normal population 11,7%), the homozygous INSIG2 obesity SNP variant in 12 patients (11%, normal population 9.1%), and the combination of both variants in 2 patients (1.85%, normal population 1.06%). Observed frequencies of SNPs differ significantly from those in the normal population.
Our findings promote the idea that common genetic polymorphisms significantly contribute to BMI and weight. Patients with 0 or 1 morbid alleles (n=42) had a mean BMI of 48.97, patients with 2 morbid alleles (n=44) had a mean BMI of 49.29 (approximately 0.96 kg extra weight), and patients with 3 or 4 morbid alleles (n=22) had a mean BMI of 49.55 (approximately 1.74 kg extra weight). Homozygosity for the FTO and/or INSIG2 obesity allele significantly increases the risk for obesity, as compared to the normal population.

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5'UTR extensions of DDX3Y testis transcripts points to evolution of a primate-specific control mechanism for expression of DDX3Y in male germ cells

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DDX3Y, member of the DEAD-Box family of RNA-helicases, is located in the Y-chromosomal AZFa interval. AZFa deletions are associated with male infertility and are typically specified by complete loss of male germ cells. Since DDX3Y protein was only found to be expressed in premeiotic male germ cells, it is assumed to be the major AZFa gene. Given that, DDX3Y transcripts display 5'UTR extensions only in testis with germ cells (Rauschendorf et al; in prep.), we proposed that the human DDX3Y gene has at least two different core promoter sites and that the germ cell specific transcriptional start site (TSS-II) might be involved in its translational control. We now wanted to know whether these distinct DDX3Y core promoter structures have evolved only on the human Y chromosome or were conserved already earlier during mammalian sex chromosome evolution.
For this purpose we analysed the genomic Y sequence upstream of the DDX3Y gene of chimpanzee, Macaca, Callithrix, and mouse for the presence of similar core promoter structures and compared their function by analyses of the 5´UTR extensions of the associated DDX3Y transcripts by appropriate RT-PCR assays in different tissues. We found that the first TSS region (TSS-I), proximal to the ATG start codon, was conserved on all studied Y chromosomes and was expressed in all analysed tissues. The second germ cell specific DDX3Y TSS (TSS-II) is only used in the primate lineage as expected from absence of the associated core promoter structures on the mouse Y chromosome. Like in human, TSS-II was used only in testis tissue with an exception for the macaque brain tissue. Interestingly, all primate testis DDX3Y transcripts starting from TSS-II were also spliced like the most abundant human splice variant. From our data we conclude, that only the study of appropriate primate models, and not the mouse model, can account for a further understanding of the complex DDX3Y gene expression control in human male germ cells.

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The apo(a)/Lp(a)-trait: Evolutionary genetic analysis of an elusive risk factor for atherosclerotic diseases

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The apolipoprotein(a) protein (apo(a)) is expressed in the liver and circulates in the blood covalently bound to an LDL like particle, thus forming the Lipoprotein(a) (Lp(a)) particle. Lp(a) plasma concentrations are highly variable within and between populations (with Africans having the highest mean concentrations, two- to threefold higher than Europeans), but are always highly heritable and are mainly controlled by the LPA gene (LPA, MIM 152200). While elevated Lp(a) levels have been found to be associated with atherosclerotic diseases, the physiological function of apo(a)/Lp(a) is still unknown. The distinct differences concerning Lp(a) plasma concentration frequency distribution between world populations and the fact that sequences variations at the LPA locus should be responsible for these differences, motivated us to conduct an in depth analysis of the LPA gene in various populations from different continents by direct sequencing and experimental determination of SNP haplotypes, which is possible due to a copy

number variation (CNV) inside LPA. 40 LPA alleles each were unambiguously haplotyped in Gabonese, Chinese, and Austrians for all non-repetitive exons and flanking intron regions. SNP analyses were then extended to Khoi San and Bantu from South Africa, Egyptians, and Asian Indians. Haplotype structures were indicative of positive selection acting on the LPA locus, esp. for a distinct African LPA haplotype harbouring a non-synonymous SNP directly adjacent to a functionally important lysine binding site. Additionally, two hyper variable regions directly flanking the CNV show an unusual pattern of SNPs in the African populations. Recent studies indicate that Lp(a) is an important carrier of oxidised phospholipids (oxPL) in human plasma. OxPL play a critical role in various infectious diseases, e.g. gram-negative sepsis or leprosy. We therefore speculate that the apo(a)/Lp(a) trait might show the imprints of natural selection caused by infectious diseases.

P299–P305 Genomics, technology and bioinformatics

P299
Analysis of large samples in whole-genome association studies: A technical report on the generation, use and reliability of DNA pools

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The identification of susceptibility variants for human diseases has been increasingly successful for a number of complex disorders. However, most susceptibility variants identified so far explain only a fraction of the disease cases in the population. It is thus expected that a large number of risk-conferring SNPs with small genetic effects still await identification. To detect those genetic variants with reasonable power, large samples in the range of several thousand individuals are required. Although costs for genotyping DNA samples genome-wide have substantially decreased recently, they still represent a financial burden when such large numbers of samples are required. To circumvent this problem, DNA pooling is an attractive and cost-effective alternative. The applicability of this approach has repeatedly been demonstrated (e.g. Baum et al. 2008, Kirov et al. 2008, Shifman et al. 2008). Here we present a technical report on how DNA pools can be generated and which important considerations should be taken. We present details on the generation and analysis of two different DNA pools; one consisting of 532 patients and one consisting of 912 controls, respectively. Data on the validation procedure for each of the pools using the Sequenom®-platform will be presented, as well as some exemplary results of the whole-genome study conducted on HumanHap610 k (Illumina®, San Diego). Our data indicate that DNA pooling represents a powerful tool for analyzing large number of samples at a genome-wide scale, when some important technical considerations are taken into account.

P300
Towards a physical map for the localization of the testis-determining factor in Ellobius lutescens

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The unusual karyotype of the mole vole Ellobius lutescens (2n=17,X in both sexes) has attracted interest in the genetics of sex determination in this species. In order to find the sex determining gene, we had constructed a genomic BAC library. Replicas of it were screened with a dinucleotide repeat probe in order to find BACs with polymorphic markers, which would allow performing segregation analyses. Microsatellite markers are checked for heterozygosity in founder animals of the E. lutescens pedigrees. The corresponding BACs are mapped by FISH. Thus, we obtain a panel of markers with a coarse localization on the chromosomes. The coverage and distribution of the BACs on the chromosomes prevents false-positive or false-negative results as a consequence of recombination. This will help to map the interval of the sex determining gene just by studying the segregation of marker alleles with the sex of the animals. We will present data from our segregation analyses of this comprehensive study.

P301
High-throughput sequencing of microdissected chromosomal regions

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The linkage of disease gene mapping with DNA sequencing is an essential strategy for defining the genetic basis of disease. New massively parallel sequencing procedures will greatly facilitate this process, although enrichment for the target region prior to sequencing remains necessary. For this step various DNA capture approaches have been described that rely on sequence-defined probe sets. To avoid making assumptions on the sequences present in the targeted region we accessed specific cytogenetic regions in preparation for next generation sequencing. We directly microdissected the target region in metaphase chromosomes, then amplified it by DOP-PCR and obtained sufficient material and quality for high throughput sequencing. Sequence reads could be obtained from as few as 6 chromosomal fragments. The power of cytogenetic enrichment followed by next generation sequencing is that it does not depend on prior knowledge of sequences in the region being studied. Accordingly this method is uniquely suited for situations where the sequence of a reference region of the genome is not available including population-specific or tumor rearrangements as well as previously unsequenced genomic regions such as centromeres.

P302
Whole miRNA array expression profiling reveals similarities between embryonic stem cells and multipotent adult germline stem cells

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Spermatogonial stem cells (SSCs) isolated from the adult mouse testis and cultured have been shown to respond to culture conditions and become pluripotent, so called multipotent adult germline stem cells (maGSCs). Aim of this study was to provide for the first time a comprehensive view of the whole microRNAome of maGSCs compared with that of embryonic stem cells (ESCs). To this end, whole microRNA array expression profiling of undifferentiated maGSCs and ESCs derived from a 129/Sv mouse background was performed in two biological duplicates. The array experiments revealed almost an identical miRNA expression profile between ESCs and maGSCs. ESCs and maGSCs differed only in the expression levels of miR-19b and miR-20b which belong to a group of miRNAs called oncomirs. This group includes the members of miR-17–92 cluster and its paralogues. These

results were confirmed with quantitative RT-PCR (qRT-PCR). Next we performed a whole microRNA array expression profiling comparing undifferentiated ESCs or maGSCs with ESCs and maGSCs cultured under differentiation conditions for 21 days. We show that levels of miR-17 and miR-20a, which also belong to oncomirs, do not decrease in maGSCs cultured under differentiation conditions, but they decrease in ESCs cultured under the same conditions. We then tested with qRT-PCR expression levels of representative members of oncomirs in ESCs and maGSCs that were cultured under differentiation conditions for 5, 10 and 21 days and we confirmed the results obtained by the array experiments. The array comparison between undifferentiated cells and those induced to differentiate revealed also differences between ESCs and maGSCs in miR-711, miR-720 and miR-320, which were not confirmed when qRT-PCR was performed. Our results suggest that, with the exception of oncomirs, ESCs and maGSCs are equivalent cell types from the aspect of miRNAs.

P303

Next generation sequencing applications in Nijmegen: Mutation detection in selected regions

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Recently the next generation sequencing techniques became available. Here we report on the set-up of a next generation sequencing facility in our department.
As human whole genome sequencing is still not available for higher throughput of samples, we investigated medical resequencing techniques including region specific enrichment in combination with the Roche 454 GS FLX Titanium next generation sequencer. To focus on resequencing of a given number of genomic regions, one possibility is to create a customized oligonucleotide array which targets the region, e.g. genes or exons, of interest. This enrichment of targeted regions includes hybridization of patient DNA to this array, elution of the bound DNA and processing it to massively parallel sequencing. This approach was successfully used in conjunction of Nimblegen enrichment arrays and 454 FLX sequencing (Albert et al. 2007). So far there is no data available on reliability and robustness of this technique in identification of known disease-causing mutations. Therefore we report here on a pilot study including DNA-enrichment of 5 samples with known mutations. As a first model disease we chose autosomal recessive ataxia, for which genomic sequences of all known disease genes (TDP1, SYNE1, SETX, APTX, ATM, SACSIN, FXN) are represented on an oligonucleotide array, in addition we added a novel candidate region for ataxia, 1.3 Mb in size. At the meeting we will report on the enrichment efficiency, the representation of target regions in the sequencing output data and the efficiency in detecting known mutations and SNPs. This novel technique allows tailor-made medical resequencing approaches for molecular genetic diagnosis of heterogeneous disorders.
Other applications of the next generation sequencing technique are also investigated by us, e.g. amplicon sequencing, which might allow more efficient DNA diagnostics and paired end sequencing for genome wide detection of structural variation.

P304

Performance of whole genome-amplified DNA from single cells and cell pools on tiling oligo-arrays

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Clinical DNA is often available in limited quantities requiring whole-genome amplification for subsequent genome-wide assessment of copy-number variation (CNV) by array-CGH (Comparative Genomic Hybridization). In some scenarios, such as pre-implantation diagnosis and analysis of micrometastases, even merely single cells may be available for analysis. However, procedures allowing high-resolution analyses of CNVs from small cell numbers or single cells well below resolution limits of conventional cytogenetics are lacking. Here, we applied amplification products of single cells and pools of 5 or 10 cells to a Whole Genome Tiling Array with 2.1 million oligo probes (NimbleGen HG18 WG Tiling 2.1 M CGH v2.0D). The single cell and pool amplification products were further hybridized to chromosome 22 specific oligo tiling arrays with either median probe spacing of 104 bp (Agilent custom-made oligo array) and 65 bp (NimbleGen's HG18 CHR22 FT). Our high-resolution analyses reveal that a low amount of template DNA does not result in a completely unbiased whole genome amplification, however it results in stochastic amplification artifacts, which become more obvious on array platforms with tiling path resolution due to significant increase in noise. We implemented an entirely new evaluation algorithm specifically for identification of small gains and losses in such very noisy ratio profiles. Our data suggest that when assessed with sufficiently sensitive methods high-resolution oligo-arrays allow a reliable identification of CNVs as small as 500 kb in cell pools (5 or 10 cells), and of 2.6–3.0 Mb in single cells.

P305

Genome-wide copy number alterations and mutational analyses using second generation sequencing techniques of paraffin embedded breast tumor tissues

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Modern high-throughput sequencing techniques in combination with novel protocols for the extraction and quality monitoring of nucleic acids from formalin-fixed paraffin-embedded (FFPE) tissue samples offer the possibility to obtain an overall image of the molecular changes in cancer as a prerequisite for computational modelling. Here we show that FFPE tissues can be used for copy number detections and mutational analyses using second generation sequencing techniques. In order to enable a cost efficient re-sequencing of more than 50.000 exons for mutations detection we are using a sequence-specific enrichment of DNA. In addition to the applicability of second generation sequencing techniques to snap frozen and FFPE normal tissues we present data on the detection of copy number variations and mutations in cancer tissues and cell lines.

P306–P316 Prenatal diagnosis, reproductive medicine

P306

Prenatal oligo-based array-CGH with custom made focused design: First experiences

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Array-CGH is a now worldwide well implemented diagnostic tool for the detection of submicroscopic chromosomal imbalances. Our lab has a lot of experience with oligo-based array-CGH analysis in the postnatal diagnostics of mental retardation. Array-CGH is routinely performed using whole genome 44 k, 105 k or 244 k Oligo-arrays (Agilent). Sometimes, if imbalances with uncertain clinical significance are detected, the interpretation of the results can be challenging. This is particularly crucial in a prenatal setting. To minimize such difficulties, we decided to design our own focused oligonucleotide microarray using Agilents eArray tool. Our array design is based on the 44 k format (more than 40.000 60mer oligonucleotides) and restricted on the dense coverage of about 130 known constitutional syndromes and subtelomeric regions combined with a 1 Mb whole genome spacing.
In our ongoing process of validation we found very good concordance of the array-CGH results with our focused design compared to the whole genome design. First we used DNA samples derived from blood of patients with known aberrations and normal male and female controls. But the interest in prenatal testing of fetuses with abnormal ultrasound findings is increasing and therefore we successfully tested the DNA from fetal samples like chorion villi and cultured amniocytes., If there is no time for culturing, uncultured amniocytes can be a challenging material for use in arrayCGH, since the number of cells in amniotic fluid in early pregnancies is low. Different methods for DNA isolation, whole genome amplification and fluorescence labelling were tested to optimize DNA quantity and arrayCGH quality. Some examples of the results will be discussed.
Summarized, our results are encouraging to go on with implementing this focused array design in our lab for pre- and postnatal arrayCGH diagnostics.

P307

Meiotic chromosome segregation in a woman carrying a pericentric inversion (21)(p13q21): Prenatal diagnosis and polar body analyses

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We report the case of a 36 year old woman referred to our Center for prenatal diagnosis on chorionic villi. Karyotyping of chorionic villi cells showed a free trisomy 21, including one structural aberrant chromosome 21 showing a pericentric inversion (21)(p13q21). Cytogenetic follow up studies demonstrated that the mother herself was carrying the inversion as de novo aberration. There is no phenotypic effect in the majority of pericentric inversion heterozygote carriers, when it is a balanced rearrangement. However, infertility, miscarriages and/or chromosomally unbalanced offsprings can be observed in carriers of a pericentric inversion (Gardner and Sutherland, 2004). Therefore, the patient requested invasive prenatal diagnosis in her following pregnancy. Chorionic villi sampling was performed, and cytogenetic analysis detected a free trisomy 21, including again the inversion chromosome inv(21)(p13q21). Microsatellite analyses demonstrated meiosis 1 nondisjunction in both pregnancies. A correlation between the inversion chromosome 21 and the recurrent pregnancies with trisomy 21 (intrachromosomal effect) could not be excluded. Because of this putative risk, the patient chose for preimplanation diagnosis by polar body analyses to select oocytes with aneuploidies. More than 50% of the tested polar bodies showed non-dysjunction for chromosome 21 or chromosome 21 chromatids. In summary, these data indicate strongly an intrachromosomal effect in the meiosis of the inversion carrier, resulting in an increased risk for trisomy 21. This is the first report demonstrating a maternal inversion 21 as risk factor for trisomy 21 conceptions.

P308

Results and outcome of polar body diagnosis from a public hospital in Austria: 5 year experience

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Objective: According to the restrictive legal situation in Austria, preimplantation genetic diagnosis is limited by law to polar body diagnosis (PBD). Using fluorescence in-situ hybridisation (FISH) maternally derived chromosomal abnormalities can be detected and the corresponding embryo can be excluded from transfer.
Materials/methods: Stimulation, intracytoplasmic sperm injection (ICSI) and polar body removal were carried out as previously described and corresponding to current protocols. In our department a commercial multicolour FISH probe kit (MultiVysion™ PB, Abbott Molecular Inc.) was applied to analyse five different chromosomes (13, 16, 18, 21 and 22).
Results: We present a retrospective review of results and outcome at our laboratory. The results are based on 335 treatment cycles. 34% (454) of the successfully diagnosed polar bodies (n= 1341) were chromosomally normal, and a total of 887 polar bodies (66%) were classified as aneuploid. Patients younger than/or 30 showed by far the highest rate of euploid polar bodies. The highest rate of aneuploidy (81,8%) could be found in patients older than 45, confirming the observation that the tendency to develop aneuploidy increases proportionally to maternal age.
Conclusion: The consistent finding of a high aneuploidy rate in oocytes derived from women at an advanced reproductive age provides further explanation for their low implantation and high miscarriage rate after IFV treatment. Hence testing for aneuploidy may improve the outcome of IVF cycles. In our in-house cohort (mean age 36) we could achieve a 10% increase in biochemical pregnancy rates for patients with repeated implantation failure, advanced maternal age, recurrent miscarriage and maternal translocations. Polar body diagnosis should therefore be recommended as an additional selection tool for patients with a high risk of chromosomal abnormal embryos and offered to these patients during conventional IVF treatment.

P309

Development and evaluation of a polar body based preimplantation genetic diagnosis for two patients with FAP

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Objective: We describe the development and validation of a sensitive and highly reliable polar body analysis of two patients with Familial Adenomatous Polyposis (FAP). FAP is an autosomal dominant disease characterized by the development of hundreds to thousands of polyps of the colon and the subsequent development of colon cancer in untreated cases. For carriers of a monogenic disorder, polar body analysis

is currently the only possible method to avoid prenatal diagnosis with subsequent putative induced abortion in Germany.
Methods: The protocols for polar body diagnosis were developed on fixed single blood lymphocytes from unaffected and from affected patients. The blood cells were isolated using laser micro-dissection in combination with a low-pressure horizontal transfer system for sample positioning. This system, called single particle absorbing transfer system (SPATS), allows the absorption of a laser micro-dissected single cell and the transfer to different reaction chambers. We transferred the single cells for PCR analysis on chemically structured microscope slides which provide efficient DNA amplification in minimal reaction volumes. Multiplex-PCRs for direct and indirect detection were done in 1 µl reaction volumes. The products were divided for the different specific nested PCRs, the mutated part of the APC gene and closely linked informative marker. The amplified mutated regions were sequenced and, in order to confirm the sequencing results, were also applied to SNAPShot analysis or fragment length analysis, depending on the mutation found in the patient. After optimisation of the detection reactions the methods were tested on unaffected polar bodies.
Results: With this method seven unaffected oocytes could be identified in two ICSI cycles.

P310
Perinatal data on twin and triplet birth after ART between the years 2000 and 2008 in a level I perinatal delivery center
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Introduction: According to the Census Bureau, of the 5.690.540 children born in the years 2000 to 2007, 91.529 (1,61%) were twins and 2.430 (0,043%) were triplets. Of these twins, 14.752 (16,12%) were conceived by reproductive measures such as IVF/ICSI/Kryo-Transfer, as well as were 858 (35,30%) of the triplets, according to data published by the IVF-Registry.
Between 2000 and 2008, 69 twins (34 IVF/35 ICSI) and 8 triplets (3 IVF/5 ICSI) deliveries were followed at our medical center. Literature research data reports unfavorable perinatal statistics for multiple pregnancies conceived after IVF/ICSI.
Study aim: Comparison of validity of published perinatal statistics with our own results.
Results: Twins: 65 bi/bi and 4 mono/bi twins were followed. Median maternal age was 32 years. 59,4% were delivered by Caesarean section. Mean weight at birth was 2200 g (1150–2900 g). Gestational age at delivery was between the 29th and 39th week. Fetal sex was as follows: 32% f/f, 22% m/m, and 46% m/f. Intrauterine death occurred in 3 cases of bi/bi twins. 6 children had a pH <7,19. 56 women delivered for the first time, 9 for the second, and 4 for the third time.
Triplets: n=8. Of these, 25% showed a trichoreal/triamnial, and 37,5% bichoreal/triamnial constellation. In 3 cases IVF had led to pregnancy, as well as 5 cases after ICSI. Median maternal age was 34 years, delivered between the 32nd and 24th week of gestation. 100% delivery by Cesarean section. Mean birth weight between 1310–2460 g. No child suffered a pH <7,19. In sum, 9 males and 10 females were born. Perinatal mortality was 5 children. 7 pregnancies were I.Para and one II.Para.
Twin complications: PROM, cervical incompetence, IUFD, PE, IUGR, GDM, anemia, Potter IIa, Thrombophilia, 1 Feticide.
Triplet complications: prematurity, 100% C-section rate. No fetal malformation.
Conclusion: In sum, a higher rate of fetal death. No actual higher rate of malformation in ART. No gene imprinting found.

P311
Partial trisomy 7q: Case report (prenatal diagnosis and live birth) and literature review
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Case report: This 22 year old prima-gravida was first seen in the 29th week of gestation during a routine work-up for fever and back pain. A sonogram was performed, which showed polyhydramnion, obvious but mild bilateral distension of the 2nd and 3rd cephalic ventricles, as seen in internal hydrocephalus. In prenatal imaging we don't find an anomaly of the corpus callosum. Facial dysmorphic features, low-set ears, club relation feet, and reduced femur length was also noted, as well as hypoplastic left ventricle. Because of these findings, an amniocentesis was carried out. The fetal karyotype was 46,XX,add(7)(q3?6). Molecular cytogenetic analysis using a probe in 7qtel suggests an inversion and duplication of 7q33-qter. Mother and father were both normal with regard to karyotype. Delivery in the 38+4 week of gestation, as spontaneous birth; female, 2520 g, child length 42 cm, cephalic circumference 35cm, Apgar 3–7-8, pH 7.14. Pediatric exam: hypoplasia of corpus callosum, obvious facial characteristics with the confirmation of low-set ears, left-ventricular hypertrophy, enlarged adrenal glands, deformity of the feet.
According to the literature, mental retardation and several malformations are regular features in children with duplication of 7q33-qter.
Karyogram and sonographic pictures were demonstrated.

P312
Stage-specific upregulation of epigenetic genes during human spermatogenesis
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During spermatogenesis, the human genome undergoes massive epigenetic reprogramming. In order to identify the role of DNA and histone binding and modifying proteins at specific stages of this process, we have mined microarray-based gene expression data of testicular biopsies from men with different types of spermatogenic failure (complete tubular atrophy, Sertoli-cell only syndrome, arrest before meiosis, arrest at meiosis, arrest at round spermatides, and uniform hypospermatogenesis; n=27) and with normal spermatogenesis (n=8). The expression patterns of several spermatogenesis genes (SYCP3, TNP1, PRM1 and PRM2) revealed that the data can indeed be used to determine the stage-specific upregulation of germ cell-specific genes. Little or no germ cell-specific upregulation was seen for histone deacetylases (HDACs), DNA methyltransferases (DNMTs), several methyl-cytosine binding proteins, the histone methyltransferase SUV39H1 and the CCCTC-binding factor CTCF. In contrast, CTCFL is a germ-cell specific factor that is upregulated before meiosis. The histone methyltransferases SUV39H2 and EZH2 as well as the bromodomain containing protein BRDT are upregulated during meiosis. The methyl-cytosine binding protein MBD3L1 is upregulated after meiosis. Interestingly, the methyl-cytosine binding proteins genes MBD2 and MBD1 are subject to alternative splicing and encode testis-specific isoforms, which are upregulated before and during meiosis, respectively. We have also used the data set to determine when the imprinted, paternally expressed SNURF-SNRPN locus on chromosome 15 is activated during spermatogenesis.

P313

Comparison of nuchal translucency (NT) measurements calibrated against Nicolaides' and against sonographer-specific NT reference models: A clear vote to allow for user-specific instead of study-derived NT-medians for correct risk assessment

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Objectives: Importance of NT measurements calibrated against the sonographer's personal NT reference.
Methods: NT and corresponding CRL measurements of 36 German sonographers were applied to generate user-specific NT models by the approach of polynomial regression as published by Snijders/Nicolaides. All participating sonographers were licensed for NT screening by the FMF UK or by the FMF Germany and could prove at least 100 successful NT screens. In order to compare the measurements of each sonographer NT MoM levels were calculated on the basis of NT medians derived both from Nicolaides' model and from user-specific models.
Results: Only for a quarter (9/36) of participants individual NT medians could be found inside an acceptable range of 0.95 and 1.05 MoM if the calibration was performed against Nicolaides' model. The NT medians were >1.05 MoM in 4 (11%), <0.95 MoM in 23 (64%), and <0.9 MoM in 16 (44%) of 36 participants. If calibration was performed against user-specific references the NT medians of all participating sonographers could be found inside a range of 0.97 and 1.04 MoM.
Conclusions: Risk assessment by the use of NT measurement may definitely result in wrong risk estimation if measurements of individual sonographers systematically deviate too much from the standard model used in the applied calculation program. This adversity can easily be overcome by the use of sonographer-specific NT reference models.

P314

Subtelomere MLPA analysis of 116 women with multiple miscarriages

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Approximately 0.5% of all couples who wish to have children suffer from multiple miscarriages. In the majority of cases, the underlying etiology remains unclear. To find out whether and to what extent microdeletions and duplications that are not detectable by routine cytogenetic analyses contribute to unexplained miscarriages, we have performed a systematic MLPA analysis of the subtelomeric regions with kits P036 and P070 (MRC Holland) that are widely used for postnatal diagnosis of children with symptoms of a chromosome disorder but normal standard karytype. In 5 of 116 analyzed women with unexplained recurrent (three or more) miscarriages, we identified 5 cases with subtelomeric imbalances. All five women were phenotypically normal. The affected regions were 1p36.3, 3q29, 6p23–25, 18q23 and 19p13.3. The duplications involve the IRF4, CDC34 and C18orf22 gene; the deletions BDH and TNFRSF18, respectively. As a control population, we have analyzed 50 women with proven fertility and no history of miscarriages. On the long term, our results may improve counselling, diagnosis and treatment of women with multiple miscarriges.

P315

Prenatal Molecular Karyotyping revealed an inverted duplication with deletion of 5p spanning the cri-du-chat critical region

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Although very rare, inverted duplications with terminal deletion (inv dup del) have been reported at different chromosomal ends. We report here on the first case of an inv dup del 5p detected by prenatal diagnosis.
Chorionic villi sampling was performed in the 13th week of gestation because of fetal abnormalities detected by ultrasound examination. Ultrasound findings included generalized skin oedema, hydrocephalus, tricuspid incompetence, club feet, a hyperechogenic cystic structure at the right side of the fetal neck (differential diagnosis: teratoma, encephalocele).
Conventional cytogenetic and molecular cytogenetic techniques were applied to determine the correct karyotype of the affected fetus. Molecular Karyotyping by Array-CGH analysis using a whole genome oligonucleotide chip (35 k OpArray V4 70mer Oligonucleotid Chip; Operon, Köln) and fluorescence in situ hybridisation with BACs revealed a de novo rearranged 5p chromosome with subtelomeric deletion del(5)(pter-p15.1), duplication and inversion inv dup(5)(p14.3p12).
To date, only two patients of inv dup del 5p have been reported (Sreekantaiah et al. 1999, American Journal of Medical Genetics; Wang et al. 2008, American Journal of Medical Genetics). Both cases were identified postnatally using FISH analysis or Molecular Karyotyping. The first patient, described by Sreekantaiah et al., had a catlike cry from birth but no other findings of cri-du-chat syndrome. The patient described by Wang et al. had no catlike cry or other characteristics of cri-du-chat syndrome. In both patients the cri-du-chat critical region on 5p15.2 was not deleted. To our knowledge our case is the first case with inv dup del 5p including a deletion of the cri-du-chat critical region.
The presented case shows the power of modern cytogenetic methods, allowing a more detailed diagnosis in affected individuals, and therefore, facilitating a more reliable prenatal diagnosis.

P316

Assisted reproductive technologies do not affect DNA methylation imprints in non-syndromatic children

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DNA methylation imprints at DMRs are key regulators of mono-allelic gene expression of imprinted genes. Initial low-case studies suggest an increased risk of imprinting failures in children with AS and BWS conceived after assisted reproductive technologies (ART). However, more recent studies with larger cohorts did not confirm this initial observation for BWS patients. Here we present a comprehensive study of ten DNA methylation imprints (DMRs) in 105 children conceived by ART (35 IVF and 70 ICSI) and 70 spontaneous conceptions. All children were phenotypically normal with no evidence for imprinting syndromes. DNA methylation was performed on mother and fetal leucocytes (peripheral and umbilicial cord blood) and amnion. While we found sporadic cases with altered methylation profiles (both hyper- and hypomethylation), we did not observe any overall difference between all three cohorts. Hence, IVF and ICSI procedures apparently do not promote DNA methylation changes at DMRs in ART children.

Our analysis revealed some additional interesting features on imprint stability in the analysed cells/tissues which we will discus.

P317–P327 Genetic counselling, education, genetic services, public policy

P317

How quality techniques help to produce vital data and analysis for the criminal justice system

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IfB-LGC GmbH is one of the most respected companies in the field of forensic genetics in Europe. IfB operates in a challenging commercial, legal and scientific environment and has found that quality is a major factor upon which the decision to award work is based. Quality is fundamental to the production of accurate data which can play a part in the outcome of court proceedings and criminal justice. The results of analyses are scrutinised by courts where lawyers may seek to explore and challenge any number of avenues in casework, e.g., item continuity, document retention, contamination and examination strategy. With these types of challenges and the requirements of international standards, it is vital that the qm system is robust, ensuring that both risks and challenges are considered and managed, yet flexible enough to ensure that improvements happen quickly.
IfB´s route to accreditation has enabled the company to set up a quality management system that ensures the efficacy of data and control of methods that are valid and traceable to international standards, through an unbroken chain of measurements. This is implemented through centrally controlled SOPs. The underlying policy and arrangements are supported by both management procedures such as document control and contract review and technical procedures such as records and test and calibration methods. IfB currently has over 95% of its technical activities accredited to ISO 17025. ISO 17025 provides the recognition that a laboratory is competent to undertake work, using instrumentation that is accurately calibrated and methods that are recognised and properly validated. Accreditation to ISO 17025 for opinions and interpretations also confers recognition that an analyst is expert, has extensive knowledge and has significant standing in the analytical/forensic community. There is no doubt that accredited laboratories have a better standing, are more efficient and produce accurate and defensible data.

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Non-invasive prenatal diagnosis of chromosomal anomalies – ethical and legal implications

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Informed consent and autonomous decision-making are of utmost importance in the context of prenatal genetic diagnosis (PND). This is mainly due to the fact that pathological findings in PND usually result in a medical indication for the termination of pregnancy rather than in therapeutic options for the fetus. Empirical data, however, strongly suggests that the quality of informed consent and the degree of autonomous decision-making especially in the context of non-invasive risk screening strategies must be improved.
We analyse standards of informed consent prior to non-invasive risk screening strategies and discuss their particular importance in the context of PND. Then we will transfer these considerations to the emerging techniques of direct non-invasive prenatal detection (NIPD) of fetal numerical chromosomal anomalies in maternal blood.
If NIPD will be implemented into everyday clinical practice similarly to current probabilistic non-invasive risk screening strategies, then a definite genetic diagnosis will be created within an established mass screening setting with most likely many women not sufficiently informed about the goal and the consequences of this diagnostic procedure. This would contravene efforts to enhance the reproductive autonomy of the pregnant woman.
With NIPD available in the future, it seems to be essential to maintain a two-step approach with counselling in the first stage and decision-making and specific testing where appropriate in the second stage in order to promote autonomous choice as well as the welfare of the unborn child. This view is in line with the current German legislation process, according to which NIPD using fetal nucleic acids will be considered as a prenatal gene test requiring mandatory prior genetic counselling.

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Predictive genetic testing: medicalisation of the family – re-familialisation of the individual

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The medicalisation of the family and the re-familialisation of the individual are two intertwining processes that are apt to describe the individual's reaction to a positive predictive BRCA-test result as well as her approaches to coping.
The so called „medicalisation of the family" (Finkler 2001; Lemke/Kollek 2008), describes the notion that the results of predictive genetic testing own the potential to reshape the structure of a family, whereas, re-familialisation of the individual' is understood as the need to redefine the identity and familial position of the individual member of this „molecular family" (Nelkin/Lindee 1995) who is facing a BRCA-test result and its implications.
Two case-reconstructions of BRCA-positive individuals living in a) an enmeshed and b) a disengaged family structure are going to illustrate:
- the intertwining of the two processes in a before-after comparison,
- the dependence of the process on the existing family structure, its rules and 'interactive logic' as well as on the self-definition and interpretation of the individual (life world parameters).

It can be concluded that:
- Health, not hazard is the central focus of the family image.
- The 'break lines' of the family structure can already be outlined in the past.
- Position effects can be observed concerning all members of the family.
- The crisis of the individual is rather depended on life world parameters than on information given during genetic counselling sessions but coping can be enhanced by resource-oriented counselling.

These examples and conclusions are outcomes of a qualitative case-reconstructive hermeneutic study. They are based on interviews and observations with participants of BRCA-testing and early-detection measures offered by three different centres of the German Cancer Aid Consortium on Hereditary Breast and Ovarian Cancer which I like to thank for their cooperation.

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Brave new World? Reflections on the role of visions of biological future of man in the history of human genetics

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In modern medicine ideas of perfecting human nature as well as designing the human body are well-known. The challenge is to differentiate between real and highly-pitched expectations, between therapy and enhancement and between individual and social desire.
In his article The Return of Eugenics (1988) R.J. Neuhaus stated, that Eugenics is a movement with the attitude to improve or perfect man (the human species) by engineering and that, "the horror of the Third Reich may have effected but a momentary pause in the theory and practice of eugenics".
Throughout history utopias generated by biomedical sciences are constant topic in literature. In Brave New World A. Huxley (1932) had the vision of using medical knowledge to design the world.
The ideas of prolonging life, prevention and healing diseases as well as improvement of physical and mental characteristics have been developing throughout history.
The CIBA Symposium The Man and his future (1962) and the following discussion in Germany for example illustrates hopes and fears of human genetics in public opinion. The evolution in human genetics was named as well as their ethical ijmplications.
Today there are two sides of the medal: J. Craig Venter talks about On the Verge of Creating Synthetic Life (YouTube, Feb. 2008) and has no problem with this vision. On the opposite side are the publications of J. Habermas (2005) Die Zukunft der menschlichen Natur. Auf dem Weg zu einer liberalen Eugenik? and M.J. Sandel (2007) The Case against Perfection.
The authors have their own imagination of the future using the today's knowledge of human genetics and reproductive medicine: they create different visions of the biological future of man. These utopias make contributions to the ethical debate about Eugenics. This is the similarity between today's ideas and eugenic social understandings of the early 20th century.

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"Diese Bezeichnung kann nicht als glücklich bezeichnet werden". Understanding of "Eugenics" in the last 100 years

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„Eugenics was a movement of large ambition – to improve the genetic makeup of the human race", as M.J. Sandel stated 2007 in The Case against Perfection. [p.63]
What is the meaning of "Eugenics"? Does the understanding change within the last 100 years or comparing Germany and Anglo-American countries?
At the beginning of the 20th century in German-speaking countries there were two terms well-known: Eugenics and Race hygiene. Both were used to express the idea of improving human species by exerting control over human reproduction by influencing the social and biological environment by nurture. In 1869 F. Galton has first introduced the term "Eugenics". In German-speaking countries it became widely reviewed after his article Eugenics, its definition, scope and aims had been published. In 1895 A. Ploetz named the influence of human acting on improving the intrinsic quality of human beings "Rassenhygiene" and for him it was more than Eugenics.
This was the beginning of a discussion on whether both terms had the same or a different meaning. In the following years, up to the 1930 s, various authors tried to define the terms "Rassenhygiene" and "Eugenics" and to name their differences.
In today's bioethical debate Eugenics again is topic of discussion. People were led to believe that the understanding has changed talking about "Old and New Eugenics". According to this they believe "Old Eugenics" is the same like "Vernichtung lebensunwerten Lebens" (annihilation of people unable to cope with life) practiced in the Third Reich. This is result of imprecise historical knowledge going hand in hand with a definition of Eugenics that could be adapted by biology and anthropology as well as national economics, sociology, jurisprudence and humanities.
The debate of German and Anglo-American human geneticists illustrated that Eugenics means still the same. But there are differences about the consequences of eugenically knowledge: they changed from social to individual.

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Factors affecting the decision for prophylactic salpingo-oophorectomy in carriers of BRCA germline mutations

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Women with germline BRCA1 or BRCA2 mutations have a significantly increased risk of developing breast and/or ovarian cancer. Depending on the family history for ovarian cancer, corresponding life time risk of BRCA1 and BRCA2 mutation carriers is 18–56% and 14–27%, respectively. Therefore, bilateral prophylactic salpingo-oophorectomy (PSO) is recommended to mutation carriers.
Little is known about clinical, personal and demographic factors affecting the decision of these women for or against PSO, a surgical intervention with severe and irreversible consequences. Therefore, we analyzed 88 female BRCA1 (n=62) or BRCA2 (n=26) mutation carriers who had no personal history of ovarian cancer, genotyped and counseled in 2006–2008, with regard to PSO. Of the 88 women, 29 (33%) underwent PSO within 1–6 month following communication of their carrier status (follow up time 6–36 month).
As expected, women with no personal history of breast cancer who opted for PSO (n=8) were significantly older (mean age 45 years*) and had more children (mean number of children 1.8*) than those (n=19) who did not choose to undergo this procedure immediately (mean age 30 y.*; mean no. of children 0.6*) (Mann-Whitney test: p=0.01and p=0.02, respectively). In contrast, in breast cancer patients there were no differences in age* (p=0.64) and number of children* (p=0.46) between those women who opted for PSO (n=21, mean age 47 y., mean no. of children 1.6) and those women who did not undergo PSO (n=40, mean age 49 y., mean no. of children 1.4). Remarkably, data of the four groups did not differ significantly with respect to positive family history of ovarian cancer (Fisher's exact tests: p=0.82; data not shown). In conclusion, age and number of children had a major effect on the decision for or against PSO in women with no personal history of breast cancer, whereas family history of ovarian cancer seemed to have less influence in mutation carriers.
*at time of communication of the carrier status

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Familial breast (BC) and ovarian (OC) cancer (BOC): Expect the unexpected

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Early onset, variable penetrance, gender bias, phenocopies, and associated tumors are the hallmarks of monogenic forms of familial cancer. We present prototype pedigrees in which these general expectations are not or only partially fulfilled. Pedigrees 1 and 2 are reminiscent of the phenomenon of „anticipation". The proposita of pedigree 1 was diagnosed with BC at age 31, her mother at age 39, and the maternal grandmother at age 79. In pedigree 2, the proposita carries a BRCA1 mutation and was diagnosed with OC at age 39, her sister, her maternal aunt, and her maternal grandmother were diagnosed with BC at the respective ages of 39, 42, and 70. Remarkably, her mother has remained healthy at age 81, illustrating the variable penetrance of BRCA mutations, also evident in pedigree 4. Males can be affected by BC, in particular carriers of BRCA2 mutations. Pedigree 3 illustrates that regardless of age any BC male should alert to the possibility of a common genetic basis of other types of cancers occurring in his family. Pedigrees 4 and 5 illustrate the likely coincidence of mutation-positive BOC with frequent sporadic tumors other than BOC. Different genetic etiologies may underly BOC in the maternal and paternal lineage, as illustrated in pedigree 6: The proposita carried a BRCA1 mutation and was diagnosed with bilateral BC at age 60. Commensurate with a genetic etiology, her mother had developed BC at age 32, and her maternal niece at age 39. The proposita´s second husband also came from a cancer-prone family as depicted in pedigree 6. His brother had died at age 63 from carcinoma of the pancreas, his mother and two of her sisters had been diagnosed with BC at the respective ages of 53, 49 and 79. He himself turned out to be carrier of a BRCA2 mutation not transmitted to the common daughter of the couple. The final example (pedigree 7) illustrates how the risk assessment in a given family crucially depends on the precision of pedigree information.

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Breast cancer risk assessment using PED 6 and BOADICEA

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There are a number of models available to assess both breast cancer risk and the chances of identifying a BRCA1/2 mutation. In a recent investigation of five different carrier prediction models only the BOADICEA model predicted the overall observed number of mutations detected accurately (Antoniou et al., J Med Genet. 2008, 45:425–31). The current version of BOADICEA has been implemented as a Web-based program where users can upload a pedigree file or create a pedigree online. The online pedigree building application appears quite complicated and enables to build only simple pedigrees. However, the authors of BOADICEA claim their program will process pedigrees of any size and structure.
PED 6 (www.medgen.de) is a Windows application for creating, drawing, and editing of pedigrees. All of the pedigree drawing features conform to the Pedigree Standardization Task Force recommendations. PED 6 enables the user to export family information not only as LINKAGE or CSV (comma separated values) file, but also as a BOADICEA data file. The data files created by PED can be uploaded to the BOADICEA Web Application (BWA; www.srl.cam.ac.uk/genepi/boadicea/boadicea_home.html) to estimate the risks of breast and ovarian cancer and the probability to be a carrier of any cancer-associated mutations in the BRCA1 or BRCA2 gene.
PED 6 appears to be a valuable tool to complement the BWA. In single pedigrees risks calculated by this combination of programs differ significantly from those received by the combination of Cyrillic / LINKAGE programs currently used by the Deutsches Konsortium für Brust- und Eierstockkrebs.

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Gene tests in discourse: Discussing predictive gene tests in terms of work contracts and private health insurance with young people

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The gain of knowledge about the human genome has been immense in the last years and enables a wide spectrum of new applications. Thus more and more information about a person's individual disease risk can be gained by genetic testing, opening the future possibility for individualized surveillance and drug therapy. But there are also social, ethical and legal problems arising with the new possibilities that have to be addressed, especially where non-medical applications are concerned. Therefore a discourse regarding those issues has arisen in the society.
The aim of this project was to develop a method that enables young people as future decision makers to form a differentiated opinion about ethical issues and to participate in the social discourse. The regulation of predictive genetic testing before the offer of work, or private health insurance contracts was taken as an example. A three-day workshop was developed starting with an objective and unbiased information phase about the genetic and juristic background. It was followed by a discussion stage amongst the students comprising of a variety of didactic techniques.
The workshop was held in three secondary schools in Heidelberg. At the end of the workshop the students were asked to define their position. Most of the students voted against the implementation of predictive genetic testing in terms of work contracts and health insurance and called for a general prohibition by law. All students agreed that the workshop had helped them to take up a well-defined position on the issue and to acquire tools for differentiated opinion making.
The teaching materials were revised and optimised after the workshops, based on the students´ experiences. The resources will be published online, so that teachers from other schools can adopt the curriculum for their students in a cross-disciplinary education.
The study was supported by the Bundesministerium für Bildung und Forschung (BMBF).

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Does genetic epidemiology pave the way into a brave new world

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In December 2008, the University College Hospital, London, UK, announced that the first baby to be screened by preimplantation genetic diagnosis (PGD) to avoid hereditary breast cancer is due to be born. Using PGD to ensure a baby does not carry an altered gene, which would guarantee a baby to inherit a Mendelian disease with 100% penetrance is well-established in several countries. However, screening, for a gene that may increase the lifetime risk for a complex disease, i.e. here the breast cancer susceptibility gene BRCA1, has not been reported so far. BRCA1 is the first susceptibility gene for a complex disease that has been found by Genetic Epidemiology (GE). GE is a discipline closely allied to traditional epidemiology. Although many of the greatest suc-

cesses have been with monogenic disorders, GE today is focusing on complex diseases, which are caused by several interacting genetic and environmental determinants with reduced penetrances.
The case described above raises questions about social consequences of selection procedures, such as preimplantation diagnosis, prenatal diagnosis and newborn screening for susceptibility genes rather than inherited conditions, as well as the question about designer babies. The presentation is intended to discuss how the screening for susceptibility genes for complex diseases is consistent with the aims of GE with respect to the improvement of public health and ethical issues. I will review cases of selection in human societies, the motivation, and the subjects. I will present an overview about the legal position in several countries I will discuss how genetic information can be used for prognosis for complex diseases.
In conclusion, genetic epidemiologists have to be involved in discussions about ethical considerations and the consequences of screening processes as well as in the education of medical doctors, the improvement of counseling of patients and—future—parents, and in the development of legal norms.

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Genetic model of SMA and risk calculations

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Infantile spinal muscular atrophy is a common autosomal recessive neuromuscular disorder. Three main types of mutations are described in the literature:
1. the classical SMN1 deletion of ex 7 or ex 7–8 (≈ 94%)
2. point mutations in the SMN1 gene (≈ 2%)
3. big deletions of the SMN1 gene (≈ 4%); homozygous big deletions are lethal

Therefore we will find under SMA patients:
1. about 88,4% patients homozygous for the classical SMN1 deletion
2. about 6,9% patients compound heterozygous for the classical deletion and the big deletions
3. about 4,5% patients compound heterozygous for the classical deletion and a point mutation
4. about 0,2% patients compound heterozygous for the big deletion and a point mutation
5. about 0,1% patients homozygous for point mutations

The normal allele in the SMN1 gene may have two expressions:
1. the normal the SMN1 gene (one copy) (≈ 95,1%)
2. a duplication of the total SMN1 gene (two copies) (≈ 4,9%)

Therefore under the heterozygotes of SMA we find the following situations:
1. One copy of the normal allele and the classical deletion (≈ 89,3%)
2. Two copies of the normal allele and the classical deletion (≈ 4,6%)
3. One copy of the normal allele and the big deletion (≈ 3,5%)
4. One copy of the normal allele and the point mutation (≈ 2,3%)
5. Two copies of the normal allele and the big deletion (≈ 0,2%)
6. Two copy of the normal allele and the big deletion (≈ 0,1%)

Today the standard method for testing of heterozygotes is the MLPA method.
But not all heterozygotes can be detected by MLPA. About 7,2% of the heterozygotes have two or three copies of the SMN1 gene. If the genotype of the index patient is unknown, in some cases the MLPA method cannot give a final heterozygote test result. In these cases one has to perform risk calculations using the Bayesian theorem for interpretation of the test results.

P328–P333 Therapy for genetic diseases

P328

Clinical and molecular genetic evaluation of patients with progressive familial intrahepatic cholestasis (PFIC) and current treatment strategies

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PFIC is a genetically heterogeneous group of severe autosomal recessive inherited disorders, affecting the hepatobiliary transport and presenting with clinical signs of severe cholestasis (pruritus, jaundice). Secondary manifestations include malabsorption, poor weight gain, and liver cirrhosis. Currently, 3 genes have been implicated in the pathogenesis of PFIC and a milder variant benign recurrent intrahepatic cholestasis (BRIC): ATP8B1 (PFIC1, BRIC1), ABCB11 (PFIC2, BRIC2) and ABCB4 (PFIC3). PFIC1 and PFIC2 typically present with low gamma-GT (gGT) within the first months of life; PFIC3 occurs later and can be distinguished by an elevated gGT. Early diagnosis and delineation from related disorders allows early intervention to prevent irreversible liver damage. Current treatment options include partial biliary diversion (PBD) surgery to interrupt enterohepatic circulation of bile acids, and ultimately liver transplantation (LTX).
In 2007 an interdisciplinary center for children with liver disorders was established at our University Hospital, which offers clinical evaluation as well as all available treatment options incl. PBD and LTX. Genetic workup includes genetic counseling and a phenotype based mutation analysis. We here report the clinical and genetic data of the first 2 patients with identified mutations in ATP8B1 and ABCB11. Both patients presented with unremitting neonatal cholestasis without identifiable infectious, metabolic, or anatomic causes, but the typical PFIC pattern of serum parameters incl. low gGT and cholesterol, absent lipoprotein X, and high levels of bile acids. Persistent relief from pruritus and jaundice as well as substantial improvement of all liver function assays could be achieved by PBD at the age of 17 months. Further delineation of potential genotype phenotype correlations will not only provide new insides into the pathogenesis of PFIC/BRIC, but may also support the development of individual genotype-based treatment strategies.

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Identifying truely symptomatic mutation carriers in long QT-syndrome

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Introduction: The long QT-syndrome (LQTS) is one of the most common inherited cardiac ion channelopathies and may cause syncope and sudden cardiac death. Early identification of disease carriers (MC) is relevant, as preventive measures and therapies exist. Adequate therapy ranges from lifestyle changes such as avoidance of known triggers for cardiac arrhythmias to pharmacological treatment and implantation of a cardioverter defibrillator (ICD) in high risk patients. Even though ICD therapy may be lifesaving serious complications can occur including inappropriate shocks, vascular occlusion or lead related complications, especially in children and young adults. When family screening

is implemented most of the mutation carriers are at a relatively low risk and lifestyle changes in combination with pharmacological treatment is appropriate. Syncope in patients despite optimal pharmacological treatment in LQTS is a class IIa indication for ICD implantation*,but presupposing that syncope is caused by cardiac arrhythmias.
Methods: In two young symptomatic MC (age: 8 resp. 4 ys., pt.1 with SCN5A mutation and LQTS, pt.2 with KCNQ1 mutation) with suspicion of syncope most likely not related to cardiac arrhythmias based on thorough medical history taking we decided to use an implantable event recorder prior to the decision of ICD implantation.
Results: During 6 resp. 14 months pt. 1 and 2 continued to syncopate whereas no cardiac arrhythmias were causative, therefore we decided against implantation of an ICD at present.
Conclusion: Increasing genetic diagnosis of arrhythmia syndromes should not lead to excessive therapies as typical symptoms in certain cases may be caused by non-cardiac reasons, but misinterpretation may be fatal. Therapeutic decisions should be made in a specialized interdisciplinary center in order to assess the individual risk properly.
* Guidelines from ACC, AHA and ESC, Zipes et al. 2006 .

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Genetic diagnosis in cardiac ion channelopathies leads to genotype specific therapy

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Introduction: The most common inherited cardiac arrhythmia syndromes are the long QT-syndrome (LQTS), the Brugada-syndrome (BS) and the catecholaminergic polymorphic ventricular tachycardia (CPVT). All may cause syncope and sudden cardiac death in often otherwise healthy patients. Especially in patients with LQTS knowing the specific genotype offers the possibility of a personalized genotype-based therapy. Undetected asymptomatic mutation carriers are at a special risk while taking potentially QT-prolonging drugs, as well as in febrile states or if co-morbidity occurs.
Methods: Between 1/2001 und 11/2008 we performed in 137 index patients with the clinical diagnosis LQTS, CPVT or BS, sometimes post-mortem, complete sequencing of the 5 most prevalent LQTS disease genes (KCNQ1, KCNH2, SCN5A, KCNE1 und KCNE2), of the RYR2-gene in CPVT resp. in case of BS of the SCN5A gene. After that cascade screening of relatives was performed.
Results: In 57% (78/137) a disease causing mutation could be detected. [LQTS 65% (68/105), CPVT 60% (3/5), BS 26% (7/27)]. Subsequently 244 potentially affected relatives could be identified: in n=127 (52%) relatives the mutation was absent and in n=117 (48%) the mutation could be identified as well.
Summary and conclusion: In 57% of patients with the clinical diagnosis of LQTS, CPVT or BS a disease causing mutation could be found. Especially in patients with LQTS therapy can be adjusted to the underlying individual genotype. Subsequent screening of potentially affected relatives identified on average 1.5 (0–9) additional mutation carriers and resulted in counselling with regard to lifestyle changes, choice of career, avoidance of known genotype specific triggers for arrhythmias and timely treatment and disburdened as many relatives.

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Higher efficiency of TA-clamp modified single stranded oligonucleotides in targeted nucleotide sequence correction is not correlated to a lower intracellular degradation

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Specific single stranded oligonucleotides can induce targeted nucleotide sequence correction in an eukaryotic genes in vitro and in vivo. Our model for investigating the reasons for the low correction rates achieved with this method is the correction of a point mutation in the hypoxanthine-guanine-phosphoribosyl-transferase (hprt) gene in the cell line V79–151. Using single stranded phosphorothioate modified oligonucleotides the correction rates of this hprt mutation were low but always reproducible. One reason for low exchange rates may be a very fast intracellular degradation of the oligonucleotides. Therefore we compared the exchange rates of different 3′- and 5′-end modified oligonucleotides with their degradation rates. TA-repeat (clamp) modified oligonucleotides showed higher correction rates than those with a GC-clamp and 5′-clamps induced higher correction rates than clamps at the 3′-end. Experiments on the stability of the most effective 5′-TA and 3′-TA-clamp modified oligonucleotide indicated a very rapid cleavage and the occurrence of shortened oligonucleotides in the presence of cytoplasmic and nuclear extracts. The phosphorothioate modified oligonucleotides were more stable, but their correction rates were lower. We suggest that there is no direct correlation between the biological stability of the full length oligonucleotides and the exchange rates achieved.

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Stop codon readthrough: A therapeutic concept in Fanconi anemia null cell lines?

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PTC124 (chemical name: 3-[5-(2-fluorophenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid) is a small molecule that has been shown to induce ribosomal readthrough of premature but not normal termination codons. Currently, several clinical studies explore the potential of this substance in the treatment of diseases like cystic fibrosis and Duchenne muscular dystrophy.
We examined the effects of PTC124 (custom synthesized from commercially available precursors) in cell lines derived from patients carrying biallelic nonsense mutations in various of the Fanconi anemia genes (FANCA, FANCC, FANCE, FANCJ). 4 lymphoblastoid and 2 fibroblast cell lines were analyzed. Cells were exposed to increasing PTC124 concentrations (1 µM - 300 µM), cultured for 48 h in the presence (10 ng/ml) or absence of mitomycin C, and analyzed for cell cycle distributions using bivariate ethidium bromide—Hoechst33258/BrdU flow cytometry. In a pilot series of experiments, there was no significant difference in cell cycle distributions (FA cells typically show G2 phase arrest) between PTC124-treated and untreated FA cells. It is known that readthrough efficiency mediated by PTC124 may vary depending on the specific termination triplet. In addition, the nature of the nucleotide following the nonsense codon (the +1 position) may also be influential. However, our analysis involving UGAG, UGAC, UGAT and UAGC termination contexts did not reveal detectable cell cycle effects of ribosomal readthrough regardless of the specific type of mutation. It cannot be ruled out at present that the observed lack of biological activity might be associated with the assay system used. Further investigations involving additional sequence contexts will include analytical methods focusing on possible changes in protein expression and mRNA transcript levels, and will also make use of plasmid constructs to verify the effect of termination suppression by PTC124.

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In vitro assessment and validation of 3D skin models for congential ichthyosis following standardised procedures

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Autosomal recessive congenital ichthyosis (ARCI) is clinically and genetically heterogeneous. It is characterised by severe, generalised scaling of the skin and erythema. So far treatment for ARCI is only symptomatic and often insufficient. 3D models showing features of keratinisation disorders are known already but a model for congenital ichthyosis was not developed until recently. As described earlier we have established a model for congenital ichthyosis as a model disease for genetic keratinisation disorders with severe epidermal barrier function defects using specific RNAi-mediated gene knock down for disease-related genes in normal, healthy, primary keratinocytes just prior to 3D model preparation.

Since European regulations restrict the use of animals to prove product safety and tolerance, a variety of commercially available 3D skin models have been created and are now available to the dermatological research community. Strict validation regulations following OECD and EU guidelines are used to assess and compare these models. We have now generated control models as well as models from patient cells and models mimicking congenital ichthyosis through gene knock down and evaluated these following OECD guidelines for artificial skin models. Caffein and testosterone were used as reference substances, because of comparability with previous studies and their chemical and physical characteristics. Vast and disease typical differences in barrier permeability and function have been observed, as for caffein P_{app} was 11.1 ± 1.1×10^{-6}cm/s for the control samples and for the patient samples 24.3 ± 2.3×10^{-6} cm/s. For testosterone results reflect these findings with P_{app} = 5.8 ± 0.99 10^{-6}cm/s (control) and P_{app} = 15.0 ± 0.02×10^{-6}cm/s (patient samples). Models are now used for first therapeutic approaches using nanoparticle strategies to introduce metabolites and gene products.

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