Abstracts of lectures

1. Symposia

S2 03

Statistical analysis of phenotypic variation in retinoblastoma: a model for identification of modifier effects in hereditary cancer

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By definition, monogenic traits are determined by variant forms of single major genes. Nevertheless, it is not unusual that specific mutant alleles are associated with a broad range of phenotypic expression. This uncertainty, which limits the predictive value of mutational analysis in genetic counseling and medical care, is caused by the modifying effects of genetic and non-genetic factors. In hereditary cancer predisposition syndromes, further variation is to be expected because of the stochastic nature of the events that are required for tumor formation. Genes that modify genotype-phenotype associations have been identified in some animal models of hereditary cancer but are notoriously difficult to detect in the human. In order to recognize genetic factors that determine phenotypic expression in retinoblastoma we have developed statistical tools that helps to identify modifying effects in families with incomplete penetrance.Data required for the analysis are the number of mutation carriers in a family that have had bilateral, unilateral or no retinoblastoma. According to Knudson?s two-step mutation model, these figures are distributed according to a Poisson distribution. A maximum likelihood approach is used to estimate the mean number of tumors (m) that maximizes the observed distribution of phenotypes in a family (m_max). In a second step, an exact test is employed to determine the plausibility of the assumption of a Poisson distribution given m_max.We have analyzed all 25 families with incomplete penetrance and known RB1 gene mutation that have been reported so far. In most families, the observed distribution is close or equal to the expected distribution of phenotypes given m_max. However, values of m_max in families with identical mutations can be distinct thus indicating the effect of genetic modifiers located in cis to the RB1 gene. A distinct kind of modifying effect is observed in two other families. In both families, the observed distribution of phenotypes does not conform to a Poisson distribution. However, separate analysis of phenotypes associated with maternally and paternally transmitted mutant alleles resulted in distinct m_max values each with good plausibility. This shows that phenotypic expression in these two families is subject to a parent-of-origin effect.

S2 04

Most patients with Muir-Torre syndrome have germline mutations in MSH2 - a clear-cut genotype-phenotype correlation in HNPCC

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Sebaceous gland tumors are the characteristic cutaneous manifestation of the Muir-Torre syndrome (MTS); the clinical diagnosis of MTS can be made when a patient is diagnosed with a sebaceous gland neoplasia and an internal neoplasia. MTS is known to be a phenotypical variant of hereditary non-polyposis colorectal cancer (HNPCC); in both MTS and HNPCC germline mutations in DNA mismatch repair (MMR) genes have been identified. The diagnosis of HNPCC is important for the patient and his family as HNPCC patients and their relatives are recommended frequent cancer surveillance examinations. The identification of a germline mutation in an HNPCC family not only proofs the diagnosis but also enables predictive genetic testing of persons at risk.Aim of the study was to analyze the mutation spectrum in a large MTS patient cohort. We investigated 41 unrelated index patients diagnosed with MTS or suspicious for MTS due to the occurrence of a sebaceous tumor. 37 of the patients had been pre-selected for mutation analysis by microsatellite analysis or immunohistochemical staining for DNA MMR protein expression. We detected germline mutations in 27 patients (66%). Each mutation occurred only once. 25 (93%) of the detected mutations are located in the DNA MMR gene MSH2, only 2 (7%) are located in MLH1. This is a significant difference to the well-balanced proportion of 51% MSH2 and 49% MLH1 mutations identified by us in a sample of 105 HNPCC patients without the MTS phenotype. Our results demonstrate a clear-cut correlation between the MTS phenotype and mutations in the MSH2 gene.According to the International Collaborative Group on HNPCC the suspicion of HNPCC should be raised when a patient meets certain clinical criteria. These criteria, known as the Bethesda criteria, list various specific HNPCC malignancies; however, sebaceous neoplasias are not among these tumors. We therefore raised the critical question whether the mutation carriers from our MTS collective would have been detected by applying the Bethesda criteria as a first pre-selection step prior to tumor tissue analysis. Interestingly, 6 (22%) of the mutation carriers do not meet the Bethesda criteria, nor do their families. From this observation we conclude that sebaceous tumors are important indicators for inherited DNA MMR deficiency. The diagnosis of a sebaceous neoplasm, even in patients not meeting the Bethesda criteria, should always lead to further diagnostic work up for possible HNPCC. Supported by the Deutsche Krebshilfe and the DFG

S5 03

Characterization of deletions of the ZFHX1B region and genotype-phenotype analysis in Mowat-Wilson syndrome

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Mowat-Wilson syndrome is a recently delineated distinct multiple congenital anomalies-mental retardation syndrome characterized by recognizable facial anomalies, severe mental retardation, growth retardation and malformations such as Hirschsprung disease and congenital heart defects. The underlying genetic defects are mutations or large scale deletions of the ZFHX1B gene, but detailed genotype-phenotype analysis was not reported so far. Of 14 patients with the distinct facial gestalt of Mowat-Wilson syndrome analyzed by us, 10 had truncating mutations, and 4 had large scale deletions, thus giving a ZFHX1B defect in 100% of patients and a deletion rate of 29%. We investigated deletion sizes in patients by FISH analysis with 26 BAC clones and additional polymorphic markers and reviewed genotype-phenotype correlation.Our results show that deletion sizes and breakpoints in Mowat-Wilson syndrome patients vary widely from 300 kb to about 11 Mb, thus ruling out a true microdeletion syndrome. In general, patients with deletions are very similar to those with truncating mutations. There was no correlation between the phenotype and mutation site or size of deletion up to 5 Mb. However, one patient with larger deletion (~11 Mb) had early seizures with lethal course and hypoplasia of great toes as additional features. Regardless of the underlying mutation, seizures occurred in 82%, Hirschsprung disease in 67.6%, congenital heart defects in 47%, and agenesis of corpus callosum in 35%.

S5 04

Computer-based classification results of an extensive set of dysmorphic faces

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An extensive set of digital images (n=147) of faces from patients with certain syndromes was collected. These syndromes include Mucopolysaccharidosis type III (n=10), Cornelia de Lange (n=12), Fragile X (n=12), Prader-Willi (n=12), Williams-Beuren (n=13), 5p- (n=16), 22q- (n=23), Noonan (n=18), Sotos (n=18) and Smith-Lemli-Opitz (n=13) syndrome. The analysis is performed in two steps, first obtaining a numeric decomposition of a facial image using Gabor-Wavelet transformations and second applying statistical classification methods. We show results for kth-nearest neighbour (kNN), support vector machines (SVM) and linear discriminant analysis (LDA). Prior to performing SVM and LDA

we employed a principle components analysis to reduce the complexity of the data set. This step resulted in 30 components out of 3840 covariates in the original data set. The error rates as measured by cross validation are 37%, 49% and 31% for kNN, SVM and LDA, respectively. These results indicate that the methods presented can be helpful to guide the diagnostic process when comparing them to the 90% error rate of a random choice. Reconstructions of average or typical images of faces for a given syndrome allow to comprehend the decision process of a classification algorithm.

S6 02

A novel mouse model for Spinocerebellar Ataxia Type 3 containing 148 polyglutamine repeats

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Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene encoding a polyglutamine repeat in the respective ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases. In order to study the course of the disease we generated a transgenic mouse model for this disorder. Promoter studies of the ataxin-3-promotor itself are currently still lacking. We therefore used the well characterized promoter of the Huntingtin gene responsible for Huntington´s disease, another polyglutamine disease. In our mouse model, the Huntingtin-promoter controls the expression of the full length ataxin-3 gene containing 148 CAG repeats. We performed 7 injection cycles of the transgene and identified positive founders in our 25 offspring. We raised up to now three transgenic founders and established stable mouse lines. Western blot analyses confirmed the expression of the transgenic ataxin-3 protein with an expanded polyglutamine repeat almost exclusively in the brain. In heterozygous mice, the expression of the transgene is weaker than that of the endogenous ataxin-3 protein. Therefore a falsifying influence of overwhelming expression of a transgenic protein can be excluded for our mouse model. It rather reflects the actual conditions occurring in SCA3 patients. Studies are currently ongoing to further characterize these mice at different ages (up to 24 months) containing immunohistochemical analyses as well as motor-functional examinations. On account of the dramatic length of this polyglutamine stretch we are hopeful that these analyses will prove the suitability of our transgenic mice as a model of SCA3.

S6 04

Myotonic dystrophies: Clinical and molecular genetic similarities and differences of myotonic dystrophy type 1 and 2 (DM1/DM2)

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Similar to myotonic dystrophy type 1 (DM1), myotonic dystrophy type 2, also termed proximal myotonic myopathy (DM2/PROMM; OMIM 602668) is a multisystemic disease characterised by myotonia, muscle weakness, wasting and cataracts as the main symptoms. In contrast to DM1, predominant proximal leg muscle weakness, mild if any facial involvement, preservation of bulbar function and manual skills, hyperhidrosis, absence of significant hypersomnia and changes of mood and motivation is characteristic for DM2. Pain is quite frequent in DM2/PROMM but not in DM1. Clinical anticipation of disease onset has been observed not only in DM1, but - to a minor degree - also in DM2. However, a congenital phenotype of the disease and mental retardation in children as seen in DM1 have not been observed in DM2 so far. Both, DM1 and DM2 are associated with a repeat expansion in a non-coding gene region. DM1 is associated with a CTG repeat expansion in the non translated region in the 3' end of the dystrophia myotonica protein kinase (DMPK) gene on chromosome 19q. Recently, DM2/PROMM was shown to be caused by a CCTG repeat expansion in intron 1 of the ZNF 9 gene on chromosome 3q. The DM2 mutation is huge (up to 50 KB), highly instable and increases with age. DNA analysis in DM1 usually shows large fragments allowing determination of the length of the repeat expansion. In DM2, expanded fragments are visible as DNA smears or multiple fragments on Southernblot. In contrast to DM1 where a correlation of the length of the repeat expansion and disease severity and age of onset could be shown, a genotype-phenotype correlation in DM2 patients in whom determination of repeat lengths has been possible has not convincingly been demonstrated so far. In both diseases accumulation of mutated RNA strangs within the nuclei of muscle fibres has been demonstrated by in situ hybridisation leading to the term "nuclear RNA disease". It is hypothesized that the mutated RNA itself and/ or nuclear RNA accumulation lead to dysregulation of cellular protein synthesis.

S7 03

Valproic acid increases the SMN2 protein level: A well-known drug as a potential therapy for spinal muscular atrophy

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Proximal spinal muscular atrophy (SMA) is a common neuromuscular disorder causing infant death in half of all patients. Homozygous absence of the survival motor neuron gene (SMN1) is the primary cause of SMA, while SMA severity is mainly determined by the number of SMN2 copies. SMN2 produces only about 10 percent of full-length protein identical to SMN1, whereas the majority of SMN2 transcripts is aberrantly spliced due to a silent mutation within an exonic splicing enhancer in exon 7. However, correct splicing can be restored by over-expression of the SR-like splicing factor Htra2-beta1. We show that in fibroblast cultures derived from SMA patients treated with therapeutic doses (0.5-500 micromol/l) of valproic acid (VPA) the level of full-length SMN2 mRNA / protein increased 2-4-fold. Importantly, this up-regulation of SMN could be most likely attributed to increased levels of Htra2-beta1 which facilitates the correct splicing of SMN2 RNA as well as to an SMN gene transcription activation.Especially at low VPA concentrations, the restored SMN level depended on the number of SMN2 copies. Moreover, VPA was able to increase SMN protein levels through transcription activation in organotypic hippocampal brain slices from rats. Finally, VPA also increased the expression of further SR-proteins, which may have important implications for other disorders affected by alternative splicing. Since VPA is a drug highly successfully used in long-time epilepsy therapy, our findings open the exciting perspective for a first causal therapy of an inherited disease by elevating the SMN2 expression level and restoring its correct splicing.

S7 04

Regulation of the MID1 protein function is fine-tuned by a complex pattern of alternative splicing

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Clinical features of Opitz BBB/G syndrome are confined to defects of the developing ventral midline whereas the causative gene, MID1, is ubiquitously expressed. Therefore, a non-redundant physiological function of the MID1 product appears to be developmentally restricted. Here, we report the identification of several alternative MID1 exons in human, mouse and Fugu. We show that comparable splicing patterns of the MID1 gene occur in the three organisms, suggesting an important role in the regulation of MID1 protein function. Accordingly, we observed differential MID1 transcript patterns in a tissue-specific manner by Northern blot and RT-PCR. The identified splice variants cause loss-of-function effects via several mechanisms. Some introduce STOP codons followed by a novel polyA+ tail, leading to the formation of C-terminally truncated proteins. Dominant negative effects through altered binding to the MID1-interacting protein alpha4 in vitro could be demonstrated in a couple of cases. Others carry premature termination codons without polyA+ tails. These are degraded by nonsense mediated mRNA decay (NMD). Our data reveal a mechanism conserved in human, mouse and Fugu that regulates developmentally restricted MID1 activity and suggest NMD to be critical in the translational regulation of a ubiquitously transcribed mRNA.

2. Selected presentations

Sel 001

Mutations in BMPR1B cause brachydactyly type A2

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Brachydactyly type A2 (BDA2) is an autosomal dominant hand malformation characterized by shortening and lateral deviation of the index fingers and, to variable degree, shortening and deviation of the 1st and 2nd toes. We performed linkage analysis in two unrelated German families and mapped a locus for BDA2 to 4q21-q25. This interval includes the gene (BMPR1B) for bone morphogenetic protein receptor 1B, a type I transmembrane serine-threonine kinase known to have an important function in chondrocyte differentiation. In one family, we identified a 1456C>T mutation leading to an arginine to tryptophan amino acid change (R486W) in a highly conserved region C-terminal of the BMPR1B kinase domain. In the other family, we identified a 599T>A mutation changing an isoleucine into a lysine residue (I200K) within the GS domain of BMPR1B, a region involved in phosphorylation of the receptor. Functional analyses using a chick limb micromass culture system revealed a strong inhibition of chondrogenesis by both mutant receptors. Overexpression of mutant chBmpR1b in vivo in chick embryos using a retroviral system (RCAS) resulted either in a brachydactyly phenotype with shortening and/or missing phalanges similar to the human phenotype or in severe hypoplasia of the entire limb. These findings suggest that both mutations in chBmpR1b affect cartilage formation in a dominant negative manner.

Sel 002

Alu mediated large deletions of the MECP2 gene detected by gene dosage analysis in patients with Rett syndrome

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MECP2 loss-of-function mutations are responsible for Rett syndrome (RTT), a progressive encephalopathy withonset in infancy. Approximately a quarter of all cases of classic RTT, however, do not have an identifiable mutation within the coding region of the MECP2 gene. The reasons proposed for this significant false negative rate include mosaic mutations, rearrangements of the MECP2 gene that extend beyond its coding regions, mutations in highly conserved regulatory regions of the gene, as well as mutations in other genes. The MECP2 mutational spectrum includes recurrent small deletions in a deletion prone region (DPR) at the 3' end of exon 4. We hypothesised that larger deletions arising from the DPR occur commonly and are not being routinely screened for by PCR mediated strategies. We developed a quantitative PCR strategy (qPCR) to examine the hypothesis and applied it to samples referred for diagnostic assessment from 140 patients among whom RTT was strongly suspected but in whom MECP2 mutation screening had yielded a wildtype result. We also evaluated 31 samples from a second selected group of girls with classical RTT among whom a mutation had not been identified in the MECP2 coding region. Among those in the first group we identified 10 large deletions (7.1%). Five deletions were characterised within the second group (16.1%). Sequencing of the breakpoints in 11 cases revealed that 8 cases had one breakpoint within the DPR. Among 7 cases the breakpoint distant to the DPR involved one of several Alu repeats, six of which were members of the AluSx class. Three cases involved the same AluSx repeat and 2 cases involved another. Two inserted sequences were derived from more distant AluSx repeats, one positioned 600kb away from the site of insertion. Sequence analysis of the junction sequences revealed that 8 cases had complex rearrangements, including double and triple deletions and indels. Examination of the MECP2 genomic sequence reveals that it is highly enriched for repeat elements, with the content of Alu repeats rising to 27.8% in intron 2, in which there was an abundance of breakpoints among our patients. Furthermore, a perfect chi-sequence (gctggtgg), known to be recombinogenic in E. coli is located in the DPR. We suggest that the chi-sequence in concert with neighbouring Alu repeats, as well as the multiple simple repeat elements within the DPR, are potent factors contributing to the high rate of genomic rearrangement in this region of MECP2. At a practical level, routine mutation screening in MECP2, at least in classical RTT cases, should include quantitative analysis of the genomic sequences flanking the DPR.

Sel 003

The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes

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Mutations in the homeobox gene SHOX cause idiopathic growth retardation, the short stature and the skeletal abnormalities associated with Léri-Weill, Langer and Turner syndromes. Little is known about the mechanism underlying these SHOX-related inherited disorders of bone formation. Here we demonstrate that SHOX expression in stable cell lines and in primary cultures leads to cell cycle arrest and apoptosis. These events are associated with alterations in the expression of several cellular genes, including pRB, p53 and the cyclin kinase inhibitors p21Cip1 and p27Kip1. A SHOX mutant, such as seen in Léri-Weill syndrome patients, does not display these activities of the wild type protein. We also show that endogenous SHOX is mainly expressed in hypertrophic/apoptotic chondrocytes of the growth plate, strongly suggesting that the protein plays a direct role in regulating the differentiation of these cells. This study provides the first insight into the biological function of SHOX as regulator of cellular proliferation and viability and relates these cellular events to the phenotypic consequences of SHOX deficiency.

Sel 004

Positional cloning of COH1, a novel membrane transport protein mutated in patients with Cohen syndrome

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Cohen syndrome is a rare autosomal recessive disorder with a variable clinical picture mainly characterised by mental retardation, microcephaly, typical facial dysmorphism, progressive pigmentary retinopathy, severe myopia, and intermittent neutropenia. Less specific symptoms such as truncal obesity and joint hyperextensibility increase the probability of the diagnosis of Cohen syndrome. The Cohen syndrome locus, COH1, was mapped to chromosome 8q21-q22 in Finnish patients and in a consanguineous family of Lebanese origin. In order to identify the underlying gene defect, we refined the localisation of COH1 by analysis of further consanguineous families from Germany, Turkey, Poland, and Oman. Homozygosity mapping placed the gene in a 4.4 cM interval between D8S521 and D8S1749. This region, which is 2.1 Mb in length, contains at least 15 genes. In a large transcript spanning more than 150 kb of genomic sequence, we have now identified several muta-

tions in Cohen syndrome patients. Affected members of the Lebanese family mapped to the region showed a homozygous G>T mutation in the splice acceptor site of intron 13. Three unrelated patients from German and Polish families showed heterozygous frameshift deletions in exons 5 and 9, respectively, and a nonsense mutation in exon 4. The impact of a number of missense mutations has not been assessed so far. At present, we have characterised 24 exons of COH1. According to EST data, however, a splice variation exists in exon 2, which could involve further ESTs mapped 5' to the gene. We expect COH1 to extend towards 5', and further exons to contain the missing mutations in compound heterozygous patients. Alignment of the deduced peptide sequence of 1687 residues showed similarity with VPS13, a protein-sorting protein of yeast involved in intracellular transport. Domain analysis predicted metallopeptidase and inner membrane component motifs. Identification of mutations in COH1 as the cause of Cohen syndrome will contribute to a better understanding of the disease mechanism and shed light on its intriguing clinical variability.

3. Workshops

W1 01

Cardiofaciocutaneous (CFC) Syndrome

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The Cardiofaciocutaneous (CFC)Syndrome was first described in 1986 by Reynolds et al. It is a rare condition: until now only about 60 patients are published in the literature. All cases are sporadic. Main features of the CFC syndrome are a typical facies with sparse, curly hair, prominent forehead, bitemporal narrowing, shallow orbital ridges, down-slanting palpebral fissures, ptosis, hypertelorism, short upturned nose, depressed nasal bridge, prominent philtrum, small upper lip, deep set and posteriorly rotated ears. Other findings are hyperkeratosis, severe atopic dermatitis and short stature. More than 80% of patients show a mild to moderate mental retardation; more than 30% present cardiac anomalies, e.g. pulmonic stenosis. Failure to thrive and feeding problems are characteristic in the neonatal and infantile period. We report on 8 patients with CFC-syndrome from age 2-10 years. The spectrum of CFC syndrome is illustrated by our patients and a review of the literature. We highlight particular complications in the course of the disease with examples. CFC syndrome has to be differentiated from Noonan and Costello syndrome. Until now, no chromosomal aberrations have been found in the CFC-syndrome. Screening analysis for PTPN11 mutations in the literature and in 4 of our patients revealed no abnormalities in the coding region of the gene. Therefore, the hypothesis that the Noonan syndrome and the CFC-syndrome are two distinct entities appears to be confirmed by the molecular results.

W1 02

SALL4 mutations result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, Acro-Reno-Ocular syndrome and patients previously reported to represent Thalidomide Embryopathy

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We have recently shown that Okihiro syndrome results from mutation in the putative zinc finger transcription factor gene SALL4 on chromosome 20q13.13-13.2. Since there is considerable overlap of Okihiro syndrome clinical features with other conditions, most notably Holt-Oram syndrome, a condition in part resulting from mutation of the TBX5 locus, as well as acro-renal ocular syndrome, we analyzed further families/ patients with the clinical diagnosis of Holt-Oram syndrome and acro-renal-ocular syndrome for SALL4 mutations. We identified a novel SALL4 mutation in one family where the father was originally thought to present with thalidomide embryopathy and had a daughter with a similar phenotype. We also found two novel mutations in two German families originally diagnosed as Holt-Oram syndrome and two further mutations in two out of three families carrying the diagnosis acro-renal-ocular syndrome. Our results show that some cases of "thalidomide embryopathy" might be due to SALL4 mutations, resulting in an increased risk for likewise affected offspring. Furthermore we confirm the overlap of acro-renal-ocular syndrome with Okihiro syndrome also on the molecular level and expand the phenotype of SALL4 mutations.

W1 03

Long-term observations of spinal muscular atrophy (SMA) with respiratory distress (SMARD1)

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Diaphragmatic spinal muscular atrophy or spinal muscular atrophy with respiratory distress type 1 (SMARD1) is caused by mutations in the recently discovered IGHMBP2 gene on the long arm of chromosome 11. We herewith describe three SMARD1 patients with prolonged survival upon assisted ventilation (4, 5 and 11 years), in whom the diagnosis of Werdnig-Hoffmann disease was made. Eventually, the diagnosis of SMARD1 was established by mutations in the IGHMBP2 gene in these patients.While it has been stated that the morphological pattern in the muscle differs between classic SMA and SMARD1, our patients display typical neurogenic atrophy in the muscle with signs of reinnervation and normal peripheral nerves which makes a distinction from infantile SMA nearly impossible on the basis of histological findings. In SMA patients, where no SMN1 gene deletion or mutation can be detected, it might be useful to consider IGHMBP2 gene analysis after clinical re-evaluation. The clinical picture of SMARD is characterised by initial respiratory distress due to diaphragmatic palsy and distally pronounced weakness. Following a period of unspecific muscular hypotonia and failure to thrive, a more specific pattern of hand muscle paresis with fatty replacement of finger muscles develops. The disease course may stabilise after initial progression, however, all reported patients to date remained entirely immobile with practically nil motor function.

W1 04

Mutations in NSD1 are responsible for Sotos syndrome, but are not a frequent finding in other overgrowth phenotypes

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Recently, deletions encompassing the nuclear receptor binding SET-Domain 1 (NSD1) gene have been described as the major cause of Japanese patients with Sotos syndrome, whereas point mutations have been identified in the majority of European Sotos syndrome patients. In order to investigate a possible phenotype-genotype correlation and to further define the predictive value of NSD1 mutations we performed mutational analysis of the NSD1 gene in 20 patients and one familial case with Sotos syndrome, 5 patients with Weaver syndrome, 6 patients with unclassified overgrowth/mental retardation, and 6 patients with macrocephaly /mental retardation. We were able to identify mutations within the NSD1 gene in 18 patients and the familial case with Sotos syndrome (90 %). The mutations (6 nonsense, 8 frame shifts, 3 splice-site, 1 missense, 1 in frame deletion) are expected to result in an impairment of NSD1 function. The best correlation between clinical assessment and molecular results was obtained for the Sotos facial gestalt in conjunction with overgrowth, macrocephaly and developmental delay. In contrast to the high mutation detection rate in Sotos syndrome, none of the patients with Weaver syndrome, unclassified overgrowth/mental retardation and macrocephaly/ mental retardation harbored NSD1 mutations. We tested for large deletions by FISH analysis but were not able to identify any deletion cases. The results indicate that the great majority of patients with Sotos syndrome are caused by mutations in NSD1. Deletions covering the NSD1

locus were not found in the patients analyzed here.

W1 05

Novel mutations in ENG and ALK1 reveal exceeding clinical relevance in German HHT patients

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Hereditary hemorrhagic telangiectasia (HHT) or Osler-Rendu-Weber syndrome is an autosomal dominant disorder of the fibrovascular tissue with prevalence rates of 1:5.000 to 1:10.000. The manifestation of the disease is variable including epistaxis, arteriovenous malformations of the lungs, liver and brain, as well as gastrointestinal bleeding. Mutations in either of two genes, ENG and ALK1, can cause HHT. Both gene products are expressed on the surface of endothelial cells and are involved as receptors in the TGF-ß-signalling pathway. A third gene is discussed.We screened 18 unrelated German patients with HHT and 6 families with 45 family members, including patients with diagnosed HHT, relatives of unknown status and unaffected. The exons of both genes were amplified and consecutively sequenced. More than 10 mutations in ENG were detected including insertions, deletions, missense and intronic mutations, only one of these mutations has been previously observed. Moreover, mutations in ALK1 were identified involving only one mutation already described before. Interestingly, the majority of the missense mutations noted were localized in exon 7. Each patient examined displayed a different mutation in one of the two genes.Our investigations show that mutations in both genes can generally be detected all over the coding region. Remarkable is the high incidence of deletions and insertions in ENG rather than missense and nonsense mutations.

W1 06

MECP2 mutations in female patients with Angelman syndrome phenotype

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Angelman syndrome (AS) is a disorder of psychomotor development caused by the loss of function of the maternal UBE3A gene on chromosome 15q11-13. Identifiable genetic abnormalities are found in the majority of patients with AS. Rett syndrome (RTT) shares clinical similarities with AS, such as severe mental retardation and acquired microcephaly, but it is associated with developmental regression and stereotypic hand movements and occurs almost exclusively in females. MECP2 mutations on chromosome Xq28 have been identified in about 80 % of female patients with RTT. Two previous studies of patients with apparent AS have resulted in the detection of mutations in the MECP2 gene in 8 and 11 %. In the present study we analyzed for MECP2 mutations a panel of 140 female patients referred with a clinical diagnosis of AS but showing a normal methylation pattern at the SNURF-SNRPN locus and no mutation in the UBE3A gene. All DNA samples were screened by means of PCR - denaturing high performance liquid chromatography (dHPLC) and those showing shifts were directly sequenced. We were able to identify mutations in the MECP2 gene in 16/140 patients (11%), which was consistent with the data reported so far. All mutations found here are known to be associated with classical RTT. Reevaluation of the clinical data of those patients with MECP2 mutations showed development of symptoms consistent with RTT in some of them. Our findings draw once more attention to a phenotypic overlap of AS and RTT. Analysis of the MECP2 gene should be considered in patients with AS phenotype but without evident genetic abnormality of 15q11-13.

W2 01

Multicolor-banding (MCB) for all human chromosomes in one hybridization step with a resolution of 450 bands or more

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A reliable and comprehensive analysis of complex rearranged karyotypes cannot be achieved by GTG-banding alone. Molecular cytogenetic methods like M-FISH or SKY, followed by application of FISH banding methods like multicolor-banding (MCB) are necessary for a definition of all involved breakpoints [see e.g. Kuechler et al., Genes Chromosomes Cancer 2003, 36:207-210]. The up to present used MCB approach was applicable for 1 up to 3 chromosomes, simultaneously. For cases with only one or a few rearranged chromosomes this limitation of MCB is rather of advantage, as only the chromosomes of interest can be specifically studied. For cases with complex karyotypic changes, however, this restriction makes it necessary to do up to 24 different experiments for a comprehensive breakpoint analysis. For the rapid analysis of such cases the multicolor-banding probe sets of all human chromosomes were used as one probe set. I.e. this approach it is based on the 138 microdissection derived MCB-probes as published [Liehr et al., Int J Mol Med 2002, 9:335-339], which have been pooled and labeled with 5 fluorochromes (FITC, Spectrum-Orange, Texas-Red, Cy5 and DEAC). The feasibility of the one-step MCB probe set, which allows for a resolution of 450 bands per haploid karyotype or more, has been proven in this study for 3 complex rearranged human cell lines (LnCAP, NS2T2, WCF-10A), 2 complex aberrant leukemia cases and 4 ape-species (Hylobates lar, Gorilla gorilla, Pan troglodytes and Pongo pygmaeus). Thus, we present the first high resolution FISH-banding technique for the entire human genome.

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W2 02

SKY-COFISH to investigate Telomere – Double Strand Break fusions and their role in tumorigenesis

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Telomeres are highly specialized nucleoprotein structures that stabilize and protect the ends of linear chromosomes. The effective end-capping of mammalian telomeres requires proteins like Ku70, Ku80, and DNA-PKcs (the catalytic subunit of DNA-dependent protein kinase). These proteins are also associated with DNA double-strand break (DSB) repair through non-homologous end-joining (NHEJ). Mutations in any of these genes cause spontaneous chromosomal end-to-end fusions that maintain large blocks of telomeric sequence at the points of fusion and are not a consequence of telomere shortening [Bailey et al., PNAS 96 (1999), 14899]. Bailey et al. discovered that telomeres produced via leading-strand DNA synthesis are especially susceptible to these end-to-end fusions, suggesting a crucial difference in postreplicative processing of telomeres that is linked to their mode of replication. [Bailey et al., Science 293 (2001), 2462]. They also observed in irradiated DNA-PK deficient mouse cell lines (p53-/- scid, BALB/c, etc.) that not only DSB-DSB or Tel-Tel fusions but also by Tel-DSB fusions were created in a dose-dependent manner. With strand specific fluorescence in situ hybridization, CO-FISH, it was possible to distinguish between these different kinds of aberrations. BALB/c is radiosensitive and susceptible to radiogenic mammary cancer. The BALB/c phenotype has been attributed to a variant allele of the DNA-PKcs gene, Prkd-cBALB, which has two naturally occurring coding sequence polymorphisms that result in reduced DNA-PKcs abundance and activity, most markedly in mammary gland tissue [Yu et al., Can Res 61 (2001), 1820]. Bailey et al. could already show in DNA-PK deficient mammary cells that telomeres fuse to DSB-ends. For exact analysis of the aberrations we developed SKY-CO-FISH, a combination of spectral karyotyping and CO-FISH. With this method the origin of each chromosome segment can be identified and at the same time it is possible to tell which kind of rearrangement took place at the fusion point of the chromosomes. SKY-CO-FISH is used to screen different repair deficient mouse cell lines (i.e. BALB/c, p53 -/- scid) for recurrent chromosomal aberrations and simultaneously for Tel-DSB fusion. This analysis should help to enlighten the role of dysfunctional telomeres in DNA repair deficient background in tumorigenesis.

W2 03

Rapid detection of subtelomeric deletion/duplication by novel real-time quantitative PCR using SYBR-green dye

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Telomeric chromosome rearrangements may cause mental retardation, congenital anomalies, miscarriages and hematological malignancies. Automated detection of subtle deletions and duplications involving telomeres is essential for high-throughput screening procedures, but impossible when conventional cytogenetic methods are used. Novel real-time PCR quantitative genotyping of subtelomeric amplicons using SYBR-green dye allows rapid high-resolution screening of single copy number gains and losses by their relative quantification against a normal diploid genome. To assess the applicability of the technique in the screening and diagnosis of subtelomeric imbalances we present a blinded study, in which DNA from negative controls and patients with known unbalanced cytogenetic abnormalities involving at least one or more telomeres were analyzed by using a novel human subtelomere-specific primer set, flanking altogether 86 amplicons, in the SYBR-green I -based real-time quantitative PCR screening approach. Screening of the DNA samples from 20 unrelated controls for copy number polymorphism do not detect any polymorphism in the set of amplicons, but single-copy-number gains and losses were accurately detected by quantitative PCR in all 20 patients, except the copy number alterations of the subtelomeric p-arms of the acrocentric chromosomes in two cases. Furthermore, detailed mapping of the deletion/translocation breakpoints was demonstrated in two cases by novel real-time PCR "primer-jumping".Because of the simplicity and flexibility of the SYBR-green I-based real-time detection, the primer-set can in future easily be extended; either to perform further detailed molecular characterization of breakpoints or to include amplicons for the detection and/or analysis of other syndromes which are associated with genomic copy number alterations.

W2 04

Giemsa-dark bands split into dark and light subbands as demonstrated by multicolor banding (MCB)

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In the present study the phenomenon of region specific chromosome-swelling (Lemke et al., 2002, Am J Hum Genet 71:1051-1059; Claussen et al., 2002, Cytogenet Genome Res 98:136-146) was addressed by a molecular cytogenetic approach – the multicolor banding (MCB) technique. Chromosomes of different length were studied. Metaphases with 850 and 550 bands per haploid karyotype were obtained by BrDU and metothrexate incorporation, respectively. Chromosomes at 400 and 300 band stages were prepared from normal peripheral blood cell suspension; the latter were obtained by drying of the suspension on the slides at 60°C. A MCB probeset for chromosome 5 (as described in Liehr et al., 2002, Int J Mol Med 9:335-339) was applied. In MCB chromosome-region specific fluorescence profiles are translated in computer-based pseudo-colors using the ISIS software (MetaSystems, Altlussheim, Germany). 37 pseudo-colors along chromosomes 5 were assigned at a GTG-banding stage of 850 bands. At a 550, 400 and 300 band stage only 33, 21 and 17 pseudo-color bands, respectively, were detectable. An assignment of the disappearing pseudo-color bands with the corresponding GTG bands was done: pseudo-colors disappeared in the bands 5p15.3-p15.2, 5p14.1-13.3, 5q11.2-12.1, 5q15-21.1, 5q22, 5q23.2-q31.1, 5q34 and 5q35.3. The results obtained here by MCB are in concordance with the concept of region specific chromosome-swelling and previously published results obtained by chromosome stretching (Hliscs et al., 1997, Cytogenet Cell Genet 79:162-166, Kuechler et al., 2001, Cytogenet Cell Genet 95:12-16). Those and the MCB-results presented here indicate for an exclusive splitting of Giemsa-dark bands into dark and light subbands during preparation induced chromosome elongation. Supported by the DFG (436 RUS 17/40/00 and 17/49/02) and the Wilhelm Sander-Stiftung (99.105.1-2).

W2 05

Characterization of an evolutionary breakpoint that distinguishes human chromosome 3p25.1 and orangutan chromosome 2

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One shared and two independent inversions derive Bornean orangutan 2 and human 3 from an ancestral simian chromosome, implying that neither Bornean orangutans nor humans have conserved the ancestral chromosome form. An additional pericentic inversion derives the Sumatran orangutan 2. Three evolutionary breakpoints in human 3p25.1, 3p12.3, and 3q22.1 distinguish human chromosome 3 and Bornean orangutan chromosome 2. Here we concentrate on the 3p25.1 breakpoint region. FISH with the breakpoint-spanning BAC RP11-616m11 revealed two distinct signals on the long arm of Bornean orangutan 2 and two signals on the short and long arms of Sumatran orangutan 2. Hybridization of the overlapping BAC clones RP11-421b21 (proximal to the breakpoint) and RP11-60m1 (distal to the breapoint) mapped the breakpoint to a 60 kb segment. This region was covered with a contig of 7 overlapping long-range PCR-products, which were then used as FISH probes. This allowed us to further narrow down the breakpoint to a 17 kb sequence, which contains the Rabenosyn 5 gene, a Rab5 effector. Rab5 regulates endocytic membrane traffic by specifically recruiting cytosolic effector proteins to their site of action on early endosomal membranes. Molecular analyses are underway to show whether the pericentric inversion in the orangutan lineage affects the Rab5 expression level.

W2 06

Analysis of pericentric inversion breakpoints supports the chromosomal speciation theory for separation between early hominins and chimpanzees

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Early hominins and chimpanzees cohabited the same African areas without obvious geographic isolation and the causes of initial reproductive barriers between them are unknown. The chromosomal speciation theory implies that large-scale chromosomal rearrangements influenced speciation by allowing differences in genes to accumulate because of reduced recombination frequencies between chromosomes heterozygous for the rearrangement, rather than creation of new gene combinations or gene disruption at the breakpoints themselves. In contrast to the normal recombination between colinear chromosomes, the reduced recombination rate of chromosomes with heterozygous rearrangements disturbs gene flow and thus creates a semi-permeable reproductive barrier. Insofar, genetic differences accumulate more rapidly on rearranged chromosomes and cause incompatibilities in hybrids of the emerging subspecies. Comparing the genome of modern humans with that of chimpanzees, pericentric inversions are among the most prevalent karyotypic changes. The model of chromosomal speciation as worked out above is more likely, if reproductive fitness is not reduced dramatically by the alteration of genes at the breakpoints of the rearrangements. The most obvious approach to investigate the effects of pericentric inversions is to analyse at the molecular level, whether genes are directly affected at the breakpoints. We compared seven breakpoints of pericentric inversions on human chromosomes 4, 9, 12, and 17 with the orthologous regions on chimpanzee chromosomes and observed that they are located in intergenic regions rich of high-copy repetitive elements, like L1- and Alu-elements. Although we cannot rule out long-range position effects of these rearrangements on coding sequences in flanking areas, our findings suggest that these pericentric inversions are primarily neutral rearrangements and that their fixation is not triggered by adaptive selection imposed by the rearrangement of genes at the breakpoints. Rather, indirect effects, like the reduced recombination rates and the resulting genetic divergence governed reproductive isolation in hominids.

W3 01

The Netherton Syndrome: Novel mutations and absence of LEKTI in the epidermis

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Netherton syndrome (NTS) is a rare autosomal recessive congenital ichthyosis featuring a chronic inflammation of the skin, failure to thrive

and hair shaft defects. Because of hypersensitivity reactions to food allergens and elevated IgE it is so far regarded as a model for atopic dermatitis. We studied 7 NTS patients belonging to five different families and performed a mutation screening using direct sequencing of the SPINK5 gene. We found 3 already known mutations and identified two novel ones affecting exons 5 and 16, respectively, leading to defective serine protease inhibitor LEKTI. One of these mutations affected exon 5 and concerned the two bp deletion 398delTG resulting in a frame shift while the other mutation occured in intron 15 at 1432-13 G to A creating a strong additional acceptor splice site. We stained skin biopsies with a newly developped monoclonal antibody against LEKTI and found absence of LEKTI in the epidermis in all our NTS patients contrasting with a strong LEKTI signal in the stratum granulosum in normal controls. We assessed further disturbances in keratinization accompanying LEKTI deficiency in NTS studying either activity or immunohistochemical expression of transglutaminases being key enzymes responsible for crosslinking of the cornified envelope. All NTS patients showed both epidermal transglutaminase-1 protein and activity in typical pericellular fashion but in a grossly broadened zone which encompassed almost all suprabasal layers. In contrast, NTS patients showed no or only faint immunostaining for transglutaminase 3. In addition, and firstly reported for NTS, the epidermis of NTS patients expressed elafin and ß-defensin 2, proteins that are usually not detected in normal skin. We conclude that the mutations identified in the SPINK 5 gene including the novel splice site mutation in intron 15 have functional relevance at the posttranslational level and result in the absence of LEKTI protein in the epidermis. This is a new and constant finding. Moreover the epidermal phenotype of NTS shows similarities to that of psoriasis vulgaris as far as transglutaminase 1, elafin and human ß-defensin 2 is concerned. Anmerkungen: Unsere Arbeit zeigt erstmalig, dass beim Netherton Syndrome die ursächlichen SPINK5-Mutationen konstant zu einem Fehlen des LEKTI-Proteins in der Epidermis führen. Wir glauben, dass der Abstract sich für den Workshop Molecular Genetics oder eventl. auch für das Symposium Genomic Disorders eignet.

W3 02

Hypotrichosis simplex of the scalp is associated with nonsense mutations in the corneodesmosin gene

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Hypotrichosis simplex of the scalp (HSS; MIM 146520) is an autosomal dominant form of isolated alopecia. Usually, affected persons present with normal hair at birth. They experience a progressive, gradual loss of scalp hair, beginning at the middle of the first decade and leading to almost complete alopecia by the third decade. HSS has been previously mapped to chromosome 6p21.3. Using a positional candidate gene approach, we identified nonsense mutations in the corneodesmosin gene (CDSN) in three HSS families of various ethnic origin. CDSN is a late differentiation epidermal glycoprotein thought to function as a keratinocyte adhesion molecule. It is also expressed in the inner root sheath (IRS) of the hair follicles. Histological, immunohistological and western-blotting analyses performed on patient scalp biopsies revealed an accumulation of a truncated form of CDSN, probably as protein aggregates, in the superficial dermis and at the periphery of the hair follicles in the lower dermis. Our findings identify mutations in CDSN underlying HSS. These results underline the major role of CDSN in hair growth of the scalp, and are an important step towards understanding the biological mechanisms for scalp-specific hair loss.

W3 03

The KIAA1882 gene is disrupted in the 8q24.3 breakpoint region of a 3;8 translocation associated with Zimmermann-Laband syndrome

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Zimmermann-Laband syndrome (ZLS) is a rare disorder characterized by coarse facial appearance including bulbous soft nose, thickened lips, thick and floppy ears, gingival hypertrophy, aplasia or dysplasia of hand- and toenails, various skeletal anomalies including hypoplastic changes in the terminal phalanges, hyperextensibility of joints, and, in some cases, hepatosplenomegaly, hypertrichosis, and mental retardation. Autosomal dominant inheritance has been suggested. However, the genetic basis of ZLS is unknown. We previously reported an apparently balanced chromosomal translocation, 46,XX, t (3;8)(p21.1;q24.3), in an affected mother and daughter. By FISH analysis, we delineated both breakpoint regions and identified breakpoint spanning BAC clones: BAC RP11-889D3 overlaps the 3p21.1 breakpoint and clones RP11-192I2 and RP11-1038G5 overlap the 8q24.3 breakpoint. By database analysis, no gene was identified that was interrupted by the breakpoint in 3p21.1. In contrast, the KIAA1882 gene, that spans a region ~590 kb in 8q24.3 and consists of 23 exons, was found to be disrupted by the translocation breakpoint. Based on the intensity of the split signals of the two BAC clones and the localization of exons of the KIAA1882 gene on the BAC inserts, we mapped the breakpoint between exons 11-14. Currently, we are characterizing the breakpoint in the KIAA1882 gene by 3` RACE-PCR. Northern blot analysis and 5` RACE-PCR revealed the existence of four KIAA1882 transcripts of ~ 4.400 bp that differ in the first exon. Expression analysis of the four alternative first exons by RT-PCR revealed that one exon (1A) is ubiquitously expressed whereas the other three exons (1B, 1C, and 1D) are differentially expressed in various tissues. These data suggest that expression of the KIAA1882 mRNA is regulated by the use of alternative tissue-specific promoters. The protein encoded by the KIAA1882 gene showed no homology to known proteins in the database. Mutation screening of the 21 coding exons of KIAA1882 in seven ZLS patients with normal karyotype did not reveal so far any pathogenic mutation.

W3 04

A homozygous nonsense mutation in SOX9 in the dominant disorder campomelic dysplasia: a case of mitotic gene conversion

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Campomelic dysplasia (CD; MIM 114290), an autosomal dominant skeletal malformation syndrome with XY sex reversal, is caused by heterozygous de novo mutations in and around the SOX9 gene on 17q. We report here a patient who was diagnosed at birth with typical signs of CD, including sex reversal. This patient was, surprisingly, homozygous for the nonsense mutation Y440X (C1692A) in exon 3. Since neither parent carries the Y440X mutation, possible mechanisms explaining this de novo homozygous mutation include uniparental isodisomy, somatic crossing-over and gene conversion. As the patient was heterozygous for 6 microsatellite markers flanking SOX9, uniparental isodisomy or somatic crossing-over were excluded, as was non-paternity. Analysis of haplotypes by use of intragenic polymorphisms showed that the father was homozygous G/G, the mother heterozygous G/A, and the patient homozygous A/A for a SNP at position 1485 in exon 3, 207 bp upstream of the homozygous mutation. Two additional informative SNPs, one in intron 2 and the other at position 1925 in exon 3, 233 bp downstream of the homozygous mutation, revealed biparental inheritance in the patient. These results suggest that a de novo germline mutation Y440X occurred on one maternal chromosome, followed by a nonreciprocal transfer of the mutant sequence from the maternal to the paternal chromosome by a mitotic gene conversion event. From the SNP data, the converted segment is at least 207 bp and at most 1305 bp in size. The Y440X mutation, one of the few recurrent mutations in SOX9, is associated with a milder phenotype and longer survival, due to retention of some transactivation activity of the mutant protein. The fact that the patient survived for three months may thus be explained by homozygozity for a hypomorphic rather than a complete loss-of-function allele. While the results of the blood DNA sample analyzed gave no indication for mosaicism, this cannot be ruled out for other tissues.

W3 05

Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz Syndrome

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The Smith-Lemli-Opitz Syndrome (SLOS [MIM 270400]) is an autosomal recessive malformation/mental retardation (MR) syndrome which ranges in clinical severity from mild dysmorphism and moderate MR to severe congenital anomalies and intrauterine death (Kelley and Hennekam 2000). SLOS is caused by mutations in the delta7 sterol-reductase gene (DHCR7; E.C. 1.3.1.21) (Fitzky et al. 1998; Waterham et al. 1998; Wassif t al. 1998) which impair endogeneous cholesterol biosynthesis making the growing embryo dependent from exogeneous (maternal) sources of cholesterol. We here have investigated whether apolipoprotein E which is a major component of the cholesterol transport system in humans (Utermann 1987; Mahley 1988) is a modifier of the SLOS. Common apo E, DHCR7, and LDLR genotypes were determined in 137 biochemically characterized SLOS patients and in 59 of their parents. There was a significant difference between the SLOS patients clinical severity scores and the maternal apo E genotypes (p = 0.028) but not between severity scores and patients or paternal apo E genotypes. In line with their effects on cholesterol levels the maternal apo genotypes with the e2 allele were associated with a severe SLOS phenotype and genotypes without the e2 allele were associated with a mild phenotype. The correlation of maternal apo E genotype with disease severity persisted after stratification for DHCR7 genotype. There was no association of SLOS severity with LDLR gene variation. The data suggest that the efficiency of cholesterol transport from the mother to the embryo is affected by the maternal apo E genotype and expand the role of apo E and it´s disease associations to embryonic development and malformation.

W3 06

Quantitative TaqMan analysis of mosaic DNA methylation in Angelman syndrome

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In a small group of patients with Angelman syndrome (AS) the disease is due to an imprinting defect. Among 87 such patients, nine patients (10%) were found to have an imprinting center (IC) deletion, whereas in 78 patients (90%) no mutation could be detected. The presence of a faint maternal band in a methylation specific PCR test in 27% of non IC deletion patients suggests that these patients are mosaic for an imprinting defect that occurred after fertilization. Methylation mosaicism makes it difficult to predict the phenotype. In fact, the clinical spectrum of these patients is very broad and ranges from typical AS over mild AS to atypical AS.We have established a quantitative methylation test to study the degree of the methylation mosaicism in more detail. To quantify the percentage of methylated DNA we performed real time PCR (TaqMan) on bisulfite treated DNA with two common primers and two minor grove binding probes specific for either the unmethylated paternal or the methylated maternal allele of SNRPN. A standard curve was constructed by mixing varying amounts of DNA from a patient with Prader Willi syndrome with uniparental disomy (two methylated gene copies) and an patient with AS with a typical deletion (one unmethylated gene copy). In 21 patients tested, the percentage of normally methylated cells ranged from <2-30%. Preliminary data suggest that patients with a higher percentage of normally methylated cells have milder clinical symptoms than patients with a lower methylation level.

W4 01

First evidence for a modifying pathway in SMA discordant families SMN regulates its interacting partners and its own splicing factor Htra2-beta1

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Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder, caused by homozygous mutations of the SMN1 gene. SMN1 interacts with multiple proteins with functions in snRNP biogenesis, pre-mRNA splicing and presumably neural transport. SMN2, a nearly identical copy of SMN1, predominantly produces exon 7-skipped transcripts, whereas SMN1 mainly produces full-length transcripts. The SR-like splicing factor Htra2-beta1 facilitates correct splicing of SMN2 exon 7 through direct interaction with an exonic splicing enhancer within exon 7. In rare cases siblings with identical 5q13-homologs and homozygous absence of SMN1 show variable phenotypes, which suggest that SMA is modified by other factors. By analyzing nine SMA discordant families, we demonstrate that in all families the unaffected siblings produce a significantly higher amount of SMN, Gemin2, Gemin3, ZPR1 and hnRNP-Q protein in lymphoblastoid cell lines but not in primary fibroblasts as compared with their affected siblings. p53, an additional SMN-interacting protein, which is not a component of gems, is not subject to an SMN-dependent regulation. Surprisingly, Htra2-beta1 is also regulated by this tissue-specific mechanism. A similar regulation was found in all type I-III SMA patients, although at a different protein level than in the discordant families. For the first time we provide evidence that an intrinsic SMA modifying factor acts directly on the expression of SMN and its interacting partners, thus influencing the SMA phenotype. Additionally, we show that SMN regulates its own splicing factor, Htra2-beta1, due to a feedback mechanism. Further insights into the molecular pathway and the identification of SMA modifying gene (s) may help to find additional targets for a therapy approach.

W4 02

Association of Alzheimer disease with genotype of PLAU, the gene encoding urokinase-type plasminogen activator located at chromosome 10q22.2

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In the brain, tissue plaminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are synthesized in neurons. tPA and uPA convert plasminogen to plasmin. uPA receptor and plasmin have been implicated in potentially pathogenic mechanisms involved in Alzheimer disease (AD). PLAU, the gene encoding uPA maps tochromosome 10q22.2, and is in an interval of approximately 30 megabases that is flanked by two regions showing linkage to late-onset AD (LOAD) (Science 290:2302-2305, 2000). Therefore, PLAU may be considered both a functional and positional candidate gene for LOAD. PLAU contains a C/T polymorphism of the secondbase of codon 141 (NCBI SNP rs2227564) introducing a nonconservative amino acid exchange (P141L) in the kringle domain of uPA. We genotyped this SNP in 356 patients with LOAD and 281 cognitively normal control subjects (ctrl). All probands were Caucasians recruited in German (D, 221 LOAD, 196 ctrl), Swiss (CH, 43 LOAD, 55 ctrl), and Italian (It, 92 LOAD, 30 ctrl) memory clinics or hospitals. As expected, in all three samples there was a strong association of LOAD with APOE E4-positive genotypes (p £ 0.002, respectively). In patients of all three samples both the frequency of C allele (fC) and that of the homozygous C/C genotype (fC/C) was higher and frequencies of the C/T and T/T (T+) genotypes were lower in comparison to the respective ctrl samples. There was a significant association of PLAU genotype with LOAD in the German (p = 0.031, DF 2) and Swiss (p = 0.02, DF 2) but not the Italian (p = 0.414) sample, the latter one possibly due to the low number of ctrl. The similar distribution of PLAU allele and genotype frequencies both in patients and ctrl of the three samples together with the association of LOAD with APOE E4 in all of them and HWE-conform data suggested a homogeneity that allowed us to pool the samples. Analysis of the pooled sample revealed a strong association of PLAU genotype with LOAD (p = 0.000255, DF 2) with an odds ratio for LOAD having genotypeC/C (vs. T+) of 1.92 (95% CI 1.39 - 2.64). The genotype association of PLAU with LOAD found in this study appears to be robust as it was also shown in all subsamples stratified by gender or APOE genotype. Furthermore, stratification of samples based on combined geno-

types of APOE (E2+/E4-; E3/E3; E4+/-; E4/E4) and PLAU (C/C; T+) suggested an additive effect of both genotypes. We conclude that either allele C (P141) of PLAU may be a frequent recessive risk allele for LOAD or that allele T (L141) may confer protection. Therefore, PLAU appears to be a promising candidate gene. Both replication studies and functional studies on P141L are required to confirm the association. [Supported by DFG, grants FI 704/1-3 and FOR 267; correspondence: finckh@uke.uni-hamburg.de]

W4 03

Loss of functional ZNF41 in a female patient with a balanced translocation t (X;7)(p11.3;q11.21) and severe mental retardation: evidence for a role for ZNF41 in cognitive development

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Severe non-syndromic X-linked mental retardation (MRX) is a heterogeneous condition defined by an IQ <50 and the absence of additional clinical features. The 12 MRX genes identified to date account for less than one third of all MRX, suggesting that numerous other gene defects cause the disorder in other families. In a female patient with severe non-specific mental retardation and a de novo balanced translocation t (X;7)(p11.3;q11.21), we have cloned the DNA fragment containing the breakpoint.In silico analysis provided no indication for a causative role for the chromosome 7 breakpoint in MR, whereas on the X-chromosome, a zinc finger gene, ZNF41, was found to be disrupted. Expression studies indicated that ZNF41 transcripts are absent in a cell line of the patient, suggesting that the mental disorder in this patient results from loss of functional ZNF41. This protein contains a highly conserved transcriptional repressor domain that is linked to mechanisms of chromatin remodelling, a process which is defective in various other forms of mental retardation; ZNF41 is therefore an excellent candidate MRX gene. Screening of a panel of MRX patients led to the identification of a potentially disease-causing ZNF41 proline to leucine amino acid exchange present in affected members of one MRX family. Taken together, our results suggest that ZNF41 is critical for cognitive development. Further studies are required to elucidate its specific function.

W4 04

Molecular basis of human neuronal migration disorders leading to different forms of lissencephalies

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Lissencephalies are a heterogeneous group of brain malformations with reduced or absent gyration of the cerebral cortex. The underlying basic defect is mostly the malfunction of migratory processes during brain development. Our understanding of this complex process with its different, spatial and temporal restricted modes is increasing rapidly. The fast development of imaging technologies (MR, fMR) and the progress in molecular genetics now allows the elucidation of the underlying different pathways and systems involved. Up to date, eight different genes (LIS1, 14-3-3 varepsilon, DCX, RELN, ARX, POMT1, POMGnT1, FKTN) have been associated with different forms of lissencephaly.We have developed an interdisciplinary approach involving neurologists, pediatricians, neuroradiologists, and human geneticists to establish a well defined clinical and genetic diagnostic procedure. Here, we present the preliminary data on our cohort of approx. 100 unrelated patients. Our clinical workup includes the assessment of the family history, neurological examination, and evaluation of MRIs to classify the distinct forms of migration disorders. The mutation screening of genes follows along a flow chart based on MRI findings and clinical phenotypes. Patients without mutations in the known genes are recruited for additional analysis of further candidate genes. Furthermore, we have established in vitro and in vivo assays to improve our understanding of the function of migration associated genes in the adult CNS and during brain development.

W4 05

Somatic mosaicism for maternal uniparental disomy 15 in a girl with Prader-Willi syndrome: expression profiles of cloned cells suggest a defect in dopamine-modulated food reward circuits

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Although uniparental disomy often results from the postzygotic rescue of a meiotic non-disjunction event, mosaicism is usually confined to the placenta. We describe a girl with PWS who is mosaic for normal cells and cells with maternal uniparental disomy 15 (upd (15)mat) in blood and skin. The karyotype was normal (46,XX), but molecular studies revealed one paternal and two maternal alleles at several 15q microsatellite loci. By methylation-specific PCR analysis of the SNRPN CpG island we noted a strong methylated band and a faint unmethylated band. X inactivation was random in blood and somewhat skewed in fibroblasts. Somatic mosaicism was confirmed by cloning and genotyping of skin fibroblasts. To study the effect of upd (15)mat on the gene expression pattern, RNA samples from three normal clones and three upd clones were hybridised to Affymetrix (TM) chips containing 45,000 probe sets that represent 33,000 human genes. Proof of principle was obtained by detecting several chromosome 15 genes known to be imprinted. We did not see any evidence for novel 15q genes showing imprinted expression in fibroblasts. Differentially expressed genes on other chromosomes are candidates for downstream genes regulated by an imprinted 15q gene and may play a role in the pathogenesis of PWS. The finding of strongly reduced mRNA levels in upd (15)mat cells of the gene encoding secretogranin II (SCG2), which is a precursor of the dopamine releasing factor secretoneurin, suggests that the compulsory eating behaviour in patients with PWS may in part be due to a defect in dopamine-modulated food reward circuits.

W4 06

Molecular characterisation of the novel human and mouse FBX25 genes suggests an important role for them in neuronal development and hippocampal function.

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In an effort to characterise the translocation breakpoints in a balanced t (X;8)(p11.2;p22.3) rearrangement, we investigated a novel gene, hFBX25, disrupted by the chromosome 8 breakpoint. hFBX25 and its mouse counterpart belong to the F-box protein family that is defined by a 40-50 amino acid (aa) motif, the F-box. Members of this family have been found in organisms ranging from viruses to humans. The F-box domain confers substrate specificity to the SCF-type (Skp1/Cul1/F-box protein) of ubiquitin protein ligase complexes. Ubiquitin-dependent proteolysis mediated by SCF complexes plays a key role in several cellular processes such as cell cycle regulation, signal transduction, circadian rhythms and transcriptional regulation and silencing. Aberrant ubiquitin-mediated protein degradation has been implicated in a range of human diseases, including the neurological disorders Alzheimer's, Parkinson's and Huntington's.hFBX25 comprises 12 exons. Three alternatively spliced variants were recovered with open reading frames (ORFs) of 367, 358 and 291 aa. Overexpressed hFBX25 localises predominantly to the nucleus, but not to the nucleoli. Subsequently, we identified and characterised the mouse orthologue, mFbx25, which contains 11 exons, yielding ORFs of 366 and 357 aa. Northern blot analysis of both hFBX25 and mFbx25 indicates ubiquitous transcription but in situ hybridisation on mouse E14.5 day embryos shows exclusive neuronal tissue specific expression. Expression of mFbx25 in mouse adult brain is confined to the hippocampus and the cerebral cortex. These expression patterns suggest a major role for mFbx25, and most likely also for hFBX25, in neuronal development and hippocampal function during adulthood.

W5 01

Identification of a second 11q amplicon in AML/MDS patients with amplification of the MLL gene

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Structural rearrangements involving the mixed lineage leukaemia (MLL) gene (11q23) are among the most common recurring abnormalities in de novo and therapy-related haematological disorders, including myelodysplastic syndromes (MDS) and acute myeloid leukaemias (AML). In addition to a diversity of MLL chromosomal translocations and partial tandem duplications with subsequent self-fusion, recent reports have implicated MLL amplification as another potential mechanism of leukaemogenesis. We identified 20 AML/MDS cases with MLL gene amplification in form of ring chromosomes, hsr and dmins by classical and molecular cytogenetic studies. In an attempt to define the entire amplified region, we performed restriction landmark genomic scanning (RLGS) analysis. Eight amplified fragments were uncovered in two analysed AML samples. Virtual genome scan (VGS), a novel informatic tool for sequence prediction of RLGS fragments, rendered sequence information for four of these fragments. One of these sequences was derived from chromosome region 11q13.4. The remaining three sequences represented amplified regions between the MLL locus and the telomere of 11q. By semiquantitative PCR we tested the amplification status of the chromosomal regions covered by these fragments in six AML/MDS samples. Our results indicated the presence of two amplified regions in at least 3/6 of the patients. Subsequent CGH analysis of 4 of these patients on an array of BAC clones representing the entire chromosome 11 with a 1Mb coverage confirmed and extended this finding. The presence of an independent centromeric amplicon represented by 2,4 Mb sequence in 11q13.4-q14.1 was shown in all four tested patients. Moreover, FISH analysis revealed that the core BAC clone of the latter centromeric region is coamplified with the MLL gene in 8/13 patients. We also show that the core region of a continuous amplicon including the MLL gene contains maximally 950 kb. However, further amplified regions telomeric to the MLL were found in 9/11 analysed AML/MDS samples. Thus, our array-CGH results revealed highly complex 11q amplification. Furthermore, our study suggest that apart from the MLL gene other genes within 11q with possible leukaemogenic potential may trigger 11q amplification in AML/MDS.

W5 02

Characterization of CATS, a protein which interacts with CALM and is expressed predominantly in thymus and spleen

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The recurring translocation t (10;11)(p13;14) is found in the leukemic cells of patients with acute myeloid leukemia, acute lymphoblastic leukemia and malignant lymphoma. This rearrangement results in the fusion of AF10 on 10p13 and CALM (Clathrin Assembly Lymphoid Myeloid leukemia gene) located on 11q14. CALM plays an important role in the regulation of clathrin-coated pit formation and in receptor mediated endocytosis. The t (10;11)(p13;q14) translocation is the sole structural chromosomal abnormality in several leukemic samples studied implying that the resulting CALM/AF10 fusion protein plays a pivotal role in leukemogenesis.To further elucidate CALM function we used the N-terminal half of CALM (aa 1-335) as a bait in a yeast two hybrid interaction screen to identify novel protein interaction partners. One of these clones was derived from the C-terminal region of an unknown gene encoding 60 amino acids. Multiple tissue Northern blot analysis using this clone revealed a 1.6 kb transcript which is predominantly expressed in spleen and thymus and to a lesser extent in small and large intestines. The gene was named CATS (CAlm interacting protein expressed in Thymus and Spleen). Sequence analysis of a full length CATS cDNA revealed an open reading frame of 238 or 248 amino acids. The amino acid sequence of CATS shows no significant homologies to other proteins in the database. CATS is located on chromosome 17, has at least 5 exons and spans approximately 6 kb. Several processed pseudogenes of CATS could be identified in the human genome. The interaction between CALM and CATS was verified using GST-pulldown experiments. Further analysis showed that the CATS interacting region of CALM is between amino acids 294 and 335. The murine Cats is about 70% homologous to its human counterpart. Whole mount in situ hybridization on mouse embryos showed expression of Cats in the neural tube and in the pharyngeal arches at early embryonic stages (E9.5-10.5) and a distinct expression pattern in the genital bud and in the developing limbs at later stages (E11.5-14.5).A transiently expressed CFP-CATS fusion protein localized to the nucleus of NIH3T3 cells. Coexpression of CFP-CATS with YFP-CALM or YFP-CALM/AF10 was able to change the subcellular localization of CALM and CALM/AF10 from predominantly cytoplasmic to more nuclear. These results indicate that the subcellular localization of CALM could depend in part on the presence of CATS with a greater portion of CALM being present in the nucleus in cells with high CATS expression (e.g. lymphoid cells). The CALM-CATS interaction might thus play an important role in CALM/AF10 mediated malignant transformation.

W5 03

Chromosome 11 aberrations in newly diagnosed patients with multiple myeloma detected by interphase cytogenetics

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Multiple Myeloma (MM) is a malignancy of clonal plasma cells accumulating in the bone marrow (BM). The low proliferation index hampers conventional cytogenetic analysis on metaphase spreads. This difficulty can be overcome by interphase cytogenetic analysis. Aberrations of the long arm of chromosome 11, especially the region 11q13-q23 harboring several oncogenes and tumor suppressor genes, are among the most common abnormalities in lymphoproliferative disorders. In this study, we have analyzed numerical and structural changes of chromosome 11 by interphase fluorescence in situ hybridization (FISH). Bone marrow plasma cells from 30 newly diagnosed MM patients were enriched by magnetic-activated cell sorting for the CD138 antigen using an AutoMACS-device (median purity 95%). We hybridized a comprehensive panel of probes including 11cen, 11q13 (CCND1), 11q22.3-q23 (ATM), 11q23 (MLL), 11q23.1 (PLZF), and subtelomeric probes for 11p and 11q. Gains of 11q23-qter were found in 20 of 30 patients. Results indicated a complete trisomy 11 in 13 patients. In 7 patients, the size of the gained region differed. The high incidence of gains of 11q23-qter detected in patients at diagnosis indicates an important role also in the pathogenesis of MM.

W5 04

Adult acute lymphoblastic leukemia: Spectrum of chromosomal aberrations in 647 patients

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In adults (> 15 y), acute lymphoblastic leukemia (ALL) is a rare disease. Only a few larger cytogenetic studies have been reported as yet. We present data of the so far largest consecutive series of 647 patients which were investigated at diagnosis using chromosome banding from 01/93 until 10/99 within the scope of the German Multicenter Therapy Study Group for Adult ALL. All ALL-subtypes were included. Our aim was to explore the spectrum and frequency of recurrent structural and numerical aberrations as well as the distribution of ploidy groups. Minimal regions of gains and losses as well as recurrent involvements of chromosome breakpoints were inves-

tigated by using the previously described simplified computer readable cytogenetic notation (SCCN) and specific analysis software (1). A Complex Karyotype Aberration Score (CKAS) was calculated to evaluate the degree of the karyotype alterations. 270 patients (41.7%) showed a normal diploid and 310 patients (47.9%) an aberrant karyotype. A fraction of 67 patients (10.4%) had inadequate results. The t (9;22) was present in 35.5% and the t (4;11) in 6.8% of the aberrant cases. Aberrations of 9p, 11q23, 12p, 14q32 and del (6q) were found in 11%, 4.2%, 2.3%, 4.5% and 4.5% respectively. The frequency of t (1;19) was 1.3% and t (10;14) 1%. Gains of the whole chromosomes 8 and 21 as well as losses of chromosome 7 where found in 7.7%, 4.8% and 7.1% respectively. Recurrently involved breakpoints (>2 aberration events) and genomic imbalances were attributed to divers structural aberrations. The breakpoint analysis with the SCCN software showed a peak of three events on band 13q14 caused by two interstitial deletions and a t (12;13)(p12;q14). A frequent loss of 20q (8x) was detected by the SCCN imbalance analysis. This was caused by 5 cases with dic (9;20) and one case each del (20q11), add (20q11) and dic (20;22)(q11;p11). 13% of the aberrant karyotypes were hypodiploid, 51% were pseudodiploid, 21% were hyperdiploid, 12% were high hyperdiploid and 2% were near triploid or near tetraploid. A CKAS ranging from 1 to 18 was observed. A CKAS <3 was predominantly caused by balanced rearrangements, whereas a CKAS >6 was attributable to numerical gains or losses. Unbalanced rearrangements were associated with hyperdiploid karyotypes with a CKAS between 1 and 6. 22.6% of the patients had a complex aberrant karyotype (CKAS >/= 3 with at least 1 structural aberration), 9.4% of these had a Philadelphia translocation, and none of these cases had a t (4;11). In summary, additional to previously described ALL specific aberrations a recurrent involvement of band 13q14 and loss of 20q11 was detected by the SCCN analysis. We could show that a notable rate of patients with ALL has a complex aberrant karyotype.(1) Bradtke et al; BMC Bioinformatics 2003 4:4

W5 05

Characterization of a novel CAT gene in thyroid adenomas with 19q13 aberration

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Structural rearrangements involving chromosomal region 19q13 are a frequent finding in follicular adenomas of the thyroid and might represent the most frequent specific structural chromosomal abnormality in epithelial tumors at all. By FISH analysis recently we have been able to map the breakpoint of two cell lines derived from thyroid tumors between two cosmids in a region of approximately 25 kb. Close to that region we found a gene that shares some homology with one of the carrier families i.e. cationic amino acid transporters (CAT). We have tentatively referred to that gene as hCAT-12. By now four human CAT-genes have been identified including two isoforms (hCAT-1, hCAT-2A, hCAT-2B, hCAT-3 and hCAT-4). Like the other members of the CAT family, hCAT-12 contains transmembrane domains. We were able to identify parts of the genomic structure of that gene including its complete open reading frame. To further complete the genomic structure of CAT we have performed 3´ and 5´ RACE experiments and sequencing. As a result we were able to identify two splice variants. So far the genomic sequence of hCAT-12 spans about 8 kb and consists 6 exons. In the hCAT-12B alternative splicing results in a truncated exon 5. The total size of the hCAT-12 cDNA is about 1.3 kb. The location close to the breakpointcluster and its function as a transmembrane transporter protein makes hCAT-12 a candidate target gene for specific 19q13 alteration.

W5 06

Downregulation of BRCA1 mRNA and Protein Expression in Chronic Pancreatitis and Sporadic Pancreatic Adenocarcinoma

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BRCA1 & BRCA2 are cancer susceptibility genes that have been associated with breast and ovarian cancer, but also with pancreatic cancer. In sporadic breast and ovarian cancer, BRCA inactivation is mainly due to altered gene expression or functional inactivation, not to BRCA mutations. In the present study, we analyzed BRCA1 & BRCA2 expression in sporadic pancreatic adenocarcinoma and in chronic pancreatitis that is associated with an increased risk of malignant transformation. Gene expression was analyzed by quantitative real-time PCR and immunohistochemical analyses in normal pancreatic tissues, chronic pancreatitis and pancreatic cancer specimens. For BRCA1, our data indicate that expression is downregulated in pancreatic cancer both on the RNA and protein level. Quantitative analysis of BRCA1 protein expression demonstrated positive or negative staining in 50% of tumor specimens tested (n=50), respectively. Patients with BRCA1-positive tumors showed a significantly better 1-year-overall survival than patients with BRCA1-negative tumors (BRCA1-positive: 76%, BRCA1-negative: 40%; $p<0.05$). Interestingly, BRCA1 expression was also downregulated in chronic alcoholic pancreatitis, in particular on the RNA level (healthy control: 100%, chronic pancreatitis: 46%, pancreatic cancer: 40%). In contrast, no significant change in BRCA2 protein expression was found in chronic pancreatitis and pancreatic cancer samples. In summary, BRCA1 suppression is present in chronic alcoholic pancreatitis and sporadic pancreatic adenocarcinoma, while BRCA2 expression was not clearly changed in these diseases. Therefore, loss of BRCA1 function may be important for pancreatic carcinogenesis and may be an early event in already present premalignant lesions. It may also influence the clinical outcome of patients with pancreatic adenocarcinoma.

W6 01

Germline mosaicism for a Prader-Willi syndrome imprinting center deletion

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In a small group of patients with Prader-Willi syndrome (1%) and Angelman syndrome (2-4%), the disease is due to aberrant imprinting and gene silencing. In some of these patients the imprinting defect is the result of a microdeletion of the imprinting center (IC), which overlaps the SNURF-SNRPN exon 1 region. Whereas non-IC deletion patients with an imprinting defect appear to have a low recurrence risk, inherited IC deletions are associated with a recurrence risk of 50%. Among 55 patients with PWS and an imprinting defect, nine patients were found to have an IC deletion. The same is true for nine of 91 patients with AS and an imprinting defect. In most cases the IC deletion was found to be a familial mutation, but in three patients with PWS and two patients with AS the IC deletion was de novo or a consequence of a germline mosaicism in the father or the mother, respectively. In these families the recurrence risk ranges from 0-50%. Here we report on a family whose first child has PWS and an IC deletion. By quantitative Southern blot and microsatellite analysis the IC deletion was found to span 60 - 160 kb. In a second pregnancy, methylation analysis of the SNURF-SNRPN exon 1 region in CVS-DNA performed by Southern blot and methylation-specific PCR revealed a typical PWS pattern, i.e. the absence of a paternal band. By quantitative Southern blot analysis of SNURF-SNRPN exon 1 we found a reduced dosage in the fetus. Furthermore, microsatellite analysis with marker PAR-SN, which maps 5 kb distal to exon 10 of SNURF-SNRPN inside the IC deletion, showed that the fetus had no paternal allele at this locus. In summary, these data demonstrate that the fetus inherited the same IC deletion as the index patient, indicating that the father has a germinal mosaic for the IC deletion. The family chose to abort the pregnancy.

W6 02

Mocaicism for maternal isodisomy 22 and trisomy 22 in a fetus with malformations

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We report on a fetus with malformations and mocaisism for maternal isodisomy 22 and trisomy 22. Karyotyping of chorionic villi was performed in the 11 + 4 week of gestation due to nuchal translucency of 2.4 mm (adjusted risk for trisomy 21 – 1:5 and for Trisomy 13/18 - 1:8) and maternal age (45 years). Chromosome analysis from placental tissue revealed non mosaic tri-

somy 22 while subsequent amniocentesis at 14 + 1 week of gestation showed a normal 46,XX female karyotype. Microsatellite analysis using DNA from cultured amniocytes and the parents lymphocytes showed maternal isodisomy for chromosome 22. Detailed ultrasound examination in the 19 + 1 week of gestation revealed a distinct subcutaneous oedema (5 mm) at the back of the head in terms of a hygroma colli which was visible from the 11 + 1 gestational week on. Fetal measurements were accordant to gestational week. After detailed genetic counselling the parents elected for termination of pregnancy, which was performed at 19 + 5 weeks of gestation. Pathological examination of the fetus showed a distinct hygroma colli and deep set back rotated ears. Microsatellite analysis from cultured skin fibroblasts from the fetus confirmed maternal isodisomy 22 suggesting the existence of an additional trisomic cell line. Subsequent FISH analysis revealed trisomy 22 in about 32% of metaphases. Uniparental disomy 22 is a rare condition. Normal phenotypes in previous reports have suggested that maternal UPD 22 has no impact on the phenotype. Auricular malformations are described in patients with trisomy 22. As our case presents an obviously never described condition, mechanisms of development of maternal isodisomy 22 / trisomy 22 mocaicism and its possible impact on the phenotype are discussed.

W6 03

Five patients with severe prenatal onset of Caffey-Silverman syndrome (infantile cortical hyperostosis)

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Infantile cortical hyperostosis is characterized by swelling of bones starting in early infancy. However, several cases have been reported with a severe prenatal onset. We describe a sporadic patient and a family with 4 affected fetuses with intrauterine onset of this disease.Patient 1 was born after 29 weeks of gestation because of premature rupture of the fetal membranes caused by polyhydramnios. Broad ribs and osteolytic lesions of ribs, scapulae, and facial bones were diagnosed by X-rays after birth. Meta- and diaphyseal osteolysis was documented followed by severe hyperostosis. The 5-year-old boy showed slow remission with intermittent disease episodes. In the familial cases the first pregnancy was complicated by polyhydramnios beginning in the 26th week of gestation. Fetal ultrasound showed short ribs and bowed and short extremities. X-rays in the 28th week of gestation demonstrated hyperostosis of the corticalis of the long bones, ribs, scapulae, and ilia. The child died after cesarean section at the 31st week of gestation. Postnatal X-rays confirmed cortical hyperostosis. In a second triplet-pregnancy polyhydramnios was diagnosed at 22nd week of gestation in two of the three fetuses. Pre- and postnatal X-rays revealed marked cortical hyperostosis in all three patients. The pregnancy was terminated after premature rupture of fetal membranes at 23rd week of gestation.Our 5 patients with cortical hyperostosis demonstrate the variability even of the severe prenatal form of the disease. The familial cases support the suggested autosomal recessive mode of inheritance. Autosomal dominant inheritance with reduced penetrance in a parent cannot be excluded.

W6 04

Characterisation of a supernumerary marker chromosome derived from chromosome 2 by Multicolor-FISH and Comparative Genomic Hybridisation

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Chromosome 2 is not often involved in the formation of marker chromosomes. Only six cases with a small supernumerary marker chromosome (SMC) derived from chromosome 2 have been verified to date by FISH. Four of these cases showed phenotypical abnormalities, while the other two are phenotypically normal. The differences in the clinical symptoms of patient with chromosome 2 SMCs might be due to variable genetic content of the marker, the degree of mosaicism, and the possible presence of uniparental disomy. We described a new case of SMC derived from chromosome 2 detected in about 50% of metaphases of cultured amnioncells. Conventional G-banding had failed to identify the chromosomal origin of the marker. The origin of chromosome 2 was determined using Multicolor-FISH (M-FISH). More detailed characterisation was archived by Comparative Genomic Hybridisation (CGH). The CGH profile showed gain in the region of 2p13.1->2q13. Based on the cytogenetic and molecularcytogenetic data the karyotype of the fetus was thus defined as 46,XX/47,XX,r (2)(p13.1q13). Parental chromosomes had been normal indicating a de novo origin of the additional material in the fetus. Furthermore, with polymorphic microsatellites we determined paternal origin of SMC with no evidence for UPD 2. After termination of pregnancy post mortem examination showed a dystrophic female fetus with no internal malformations, but some facial dysmorphic features like pronounced hypertelorism, broad nose and nasal bridge, microretrognathy and low-set ears.This report illustrates the importance of the precise definition of the origin of SMCs to establish genotype-phenotype correlation, which are particularly important when SMCs are identified in prenatal diagnosis.

W6 05

RISCALW a windows program for risk calculation in Duchenne muscular dystrophy

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A prerequisite for risk calculation in families is an appropriate genetic model for the disease of interest. Because of empirical evidence that the formerly used simple genetic model for Duchenne muscular dystrophy (DMD) is not sufficient, a general model to include germline mosaicism and heterogeneous new mutation rates depending on sex and mutation type has been formulated. Based on this model RISCALW was develloped. RISCALW is a user-friendly Windows program for risk calculation in families with DMD. It runs on any standard personal computer under the Windows operating system.A range of different genetic models including germline mosaicism, sex and mutation specific mutation rates may be used. It is possible to include marker genotypes, creatininkinase values and FISH-deletiontest results.Pedigree data input is required in a modified LINKAGE format in the pedigree window; the user can make selections for the risk calculation in the parameter window; the results are displayed in the log window. The main editing features are accessible for pedigrees and results. Pedigrees can be included via the Windows clipboard from any wordprocessor. Pedigrees and results can be added to any wordprocessor in the same manner. Detailed online help is available. RISCALW has been extensively tested for more than 100 families. The user is able to perform risk calculation in large families in an easy way and thus explore the dependence of results on different model assumptions for example different mutation rates in male and female. RISCALW is freely available from the authors for scientific use.

W6 06

Preliminary results of the prospective German ITA study

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In early 2002 the Institute of Human Genetics in Goettingen started a prospective study on ITA, a new risk marker for fetal aneuploidies. ITA (invasive trophoblast antigen) is a special hyperglycosylated isoform of human chorionic gonadotropin (hCG). Its concentration in maternal serum and urine is said to be highly increased in case of fetal trisomy 21 and extremely decreased in case of fetal trisomy 18. Presently we have measured ITA levels in more than 2000 urine and more than 3000 serum samples of volunteer probands between 10th and 18th week of gestation. ITA levels show a an extremely steep decrease between 10th and 14th week and afterwards slowly shift over into basal levels that keep constantly low after 16th week of gestation. From the ongoing follow-up we are reported on aneuploid fetal outcome in 16 cases so far: seven trisomies 21, three trisomies 18, three Turner- and three Klinefelter cases. In six out of seven cases with trisomy 21 the ITA levels are above the 95th percentile.The ITA levels of three cases with trisomy 18 are below the 5th percentile, one is below the 1st percentile. For the six cases with Turner and Klinefelter syndrome no atypical ITA levels could be observed in general. Although more ITA values of affected pregnancies are needed for solid conclusions we assume that 1. ITA is a risk marker mainly for fetal trisomy 21 2. ITA is more discriminative in the second than in the first trimester 3. Preanalytical cofactors (gestational dating, maternal weight, sample handling) play an important role for the interpretation of ITA results.

W7 01

Functional characterization of TRPM5 , a transient calcium-activated cation channel

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Transient receptor potential (TRP) proteins are biological important signalling molecules, that are grouped into three subfamilies, named after their founding members TRPC (canonical TRPs), TRPV (Vanilloid receptor like) and TRPM (Melastatin like). The human TRPM subfamily consists of eight members with partly unknown physiological function but possible involvement in human disease. Three isoforms TRPM2, TRPM6 and TRPM7 contain an carboxy-terminal enzymatic domain, a unique feature among ion channels. The founder TRPM1 (Melastatin) is discussed to be a tumor suppressor gene, due to the fact, that it is downregulated in highly metastatic melanoma cells (Duncan et al., 1998; Fang and Setaluri, 2000). Other TRPMs also seem to play a role in aspects of human disease: TRPM2 is involved in apoptosis (Hara et al., 2000), TRPM6 is crucial for magnesium homeostasis leading to hypomagnesemia with secondary hypocalcemia when mutated (Schlingmann et al., 2002) and TRPM8 has been shown to be upregulated in prostate tumors (Tsavaler et al., 2001). Recent publications (Perez et al., 2002 and Zhang et al., 2003) demonstrate that TRPM5 is expressed in certain gustatory cells and is responsible for the perception of bitter, sweet and amino acid (umami) tasting stimuli. In contrast to these publications we can demonstrate that TRPM5 is expressed as a 4.5 kb transcript not solely in the gustatory cells, but in a variety of fetal and adult tissues. Therefore we wanted to reveal the exact activation mechanism of TRPM5 to further elucidate the biological function of this protein. We have established stable transgenic HEK293 cell lines with the human TRPM5 and TRPM5-EGFP fusion constructs. The latter enabled us to localize the protein to the cell membrane, suggesting a functional channel. We then characterized the electrophysiological properties of the TRPM5 channel by using whole cell recordings and excised membrane patch-clamp analysis. TRPM5 displays characteristics of a CAN (Calcium-activated-nonselective) cation channel. It is directly activated by [Ca2+]i, but seems not to conduct Ca2+ ions. Instead it carries monovalent ions (Na+, K+) after receptor mediated cytosolic Ca2+ increase. Activated TRPM5 modulates the membrane potential (depolarisation) with effects in cells with non-excitable and excitable membranes. We could also show the specific electrophysiological properties of the channel in cells with endogenous TRPM5, supporting the proposed functional involvement in the conversion of a chemical stimulus into an electrical signal.

W7 02

The RING finger protein RNF4, a coregulator of transcription, interacts with the TRPS1 transcription factor

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The TRPS1 gene encodes a transcription factor protein which is known to function as repressor of GATA mediated transcription. The human TRPS1 is involved in the tricho-rhino-phalangeal syndromes. However, its precise molecular functions as well as the TRPS1 target genes are still unknown. In a yeast two-hybrid screen with the C-terminal 647 amino acids (aa) of the orthologous Trps1 as bait, we obtained 23 yeast clones, which code for six different Trps1 binding proteins. Three of these clones encoded two overlapping fragments of the 194 aa protein RINGfinger protein 4 (Rnf4). The overlap narrows down the Trps1-Rnf4-interacting region within Rnf4 to aa 6 through 65. This region is also known to interact with androgen receptors and other proteins. By using truncated Trps1 constructs in yeast in vivo beta-galactosidase assays, the Rnf4-interacting region in Trps1 could be narrowed down to aa 885 through 1084. This 200 aa region does not contain any predicted protein-protein interacting motif. We could verify the Trps1-Rnf4 interaction using the human orthologes in transfected and native eukaryotic systems by immunochemical precipitation experiments. The intracellular distributions of both proteins were analyzed by confocal laser scanning microscopy. RNF4 as well as TRPS1 are located in specific dot-like nuclear structures. The structures containing RNF4 are smaller and more numerous than those containing the TRPS1 protein. In luciferase reporter assays we could demonstrate that the repressional function of TRPS1 is inhibited by RNF4. RNF4 is known as a coregulator of various different transcriptional elements. Modulation of TRPS1 activity can now be added to this list of functions. Whether the RNF4-TRPS1 interaction has an implication in the development of the TRP syndromes remains elusive.

W7 03

Characterization of the zinc finger protein ZNF297B that interacts with TFNR - a subunit of TFIIIB

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The transcription factor-like nuclear regulator (TFNR) is a novel human gene that maps on 5q13, distally to the duplicated region which includes SMN1, the spinal muscular atrophy (SMA) determining gene. TFNR shows homology to yeast B" protein which is required for transcription of both TATA-less and snRNA-type RNA polymerase III promotors and thus is an essential factor of the basal RNA poymerase III transcription machinery. Northern blot and transcription start point analysis allowed us to determine a transcript length of ~ 10 kb. The TFNR transcript is highly expressed in cerebellum, cerebral cortex and weakly in all tissues tested. TFNR encodes a 2254 amino acids (aa) protein. The protein contains a SANT domain, and ssDNA- and dsDNA-protein interactions were shown. We identified one SMA patient with additional brain atrophy and a large deletion including the TFNR gene. Based on this observation, we postulated that TFNR may have an important role in the development of the brain. The N-terminal part of TFNR reveals a strong protein interaction to a novel zinc-finger protein identified by yeast two hybrid screens and co-immunoprecipitation. The zinc finger protein interacts with the first 640 aa of TFNR. It encodes a protein that contains three zinc finger domains (N-terminus) and one POZ/BTB domain (C-terminus). Northern blots revealed a transcript length of ~ 5,5 kb in all tested human tissues. An antibody generated in rabbit shows a stronger expression in human brain and cerebellum on western blots. In immunofluorescence stainings a colocalisation with tubulin stained structures can be seen. The construction of a TFNR hemizygous mouse is in progress.

W7 04

The variability pattern of the NF1 gene indicates a bottleneck in the European population less than 20.000 years ago

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The neurofibromatosis 1 (NF1) gene is an evolutionaryly highly conserved structurally and functionally defined genomic unit. The gene comprises 350 kb of GC poor DNA in an otherwise GC rich chromosomal band with sharp GC content boundaries at its 5´ and 3´ end in human and mouse. This structural unit has distinct functional properties. The NF1 gene is replicated late, the surrounding sequences early in S-phase. SNP analysis revealed a high degree of linkage disequilibrium, corresponding to an extremely low recombination rate, throughout the whole NF1 gene, whereas the neighbouring sequences show recombination rates comparable to the genomewide average. The SNP analyses and resequencing of intronic NF1 gene sequences further demonstrated the presence of only three common haplotypes of the whole 350 kb unit in the German population. The interhaplotype divergence amounts to approximately one variable site per 1300 nucleotides. The intrahaplotype divergence is low, with only one variable site per 50 kb and all these variants were observed only once in 96 chromosomes analysed. This pattern of sequence variation indicates a severe bottleneck in the history of the present European population resulting in the reduction to only three founder haplotypes at the end of the bottleneck when the population started to expand. The low frequency variants arose by mutations which occurred after the end of the bottleneck. From the frequency such variants (one per 50.000 nucleotides) it can be calculated, that the expansion of the European population began less than 20.000 years before present.

W7 05

Profiling the molecular signature of Patched- and p53-dependent rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood encompassing two major subtypes, embryonal and alveolar, which differ in clinical behavior and genetic markers. This tumor is associated with mutations in several genes, including the tumor suppressor genes Patched (Ptch) and p53. In mice, inactivation of one Ptch allele as well as inactivation of one or both p53 alleles results in a high incidence of RMS. Here, we report that loss of p53 dramatically accelerates the incidence and development of RMS in heterozygous Ptchneo67/+ mice. Our data indicate that mutations in p53 or malfunctioning of its pathway are not required for RMS formation in these mice. In comparison to p53-dependent RMS, RMS of heterozygous Ptchneo67/+ mice show a less aggressive growth and are histologically more differentiated. By means of cDNA microarray analysis, we demonstrate that there is diversity in gene expression among Ptch- and p53-dependent RMS, apparently reflecting the variation in tumor proliferation rate and differentiation state of the tumor. Whereas tumors of Ptch mutants predominantly express various myogenic markers like myosin, actin, myoglobin, and troponin, tumors in p53 mutants display higher expression levels of cell cycle-associated genes like cyclin B1, cyclin-dependent kinase 2, cyclin-dependent kinase 2-associated protein 1, and cell division cycle 2. Ptch-associated RMSs are also clearly distinct from those that are p53-dependent when hierarchical clustering is performed on the data set. These results demonstrate that, within one tumor type, different causative mutations lead to distinct molecular characteristics as revealed by transcript analysis. These differences may allow for identification of new, gene mutation- and expression-specific treatments of these tumors.

W7 06

X-linked cone opsin genotypes in patients with blue cone monochromacy: Further evidence for unequal homologous recombination in the opsin gene

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Blue Cone Monochromacy (BCM) is a rare X-linked retinal disorder characterized by a severe color vision defect, reduced visual acuity, photophobia and nystagmus. BCM results from the simultaneous functional loss of middle (green) and long wavelength sensitive (red) cone photoreceptors caused by mutations in the opsin gene cluster on Xq28. In normal subjects the opsin gene cluster is composed of a locus control region (which drives the expression of opsin genes), followed by a single long wavelength sensitive cone opsin gene (red opsin) and one up to five copies of the middle wavelength-sensitive cone opsin gene (green opsin). We have analysed the opsin gene cluster in 28 independent Caucasian BCM patients by means of PCR/RFLP analysis, deletion mapping and DNA sequencing. The mutant genotypes could be divided into two separate groups. Patients in the first group (n = 9) carried deletions of variable size that encompass the locus control region and parts of or the whole opsin gene cluster. A common feature in the second group of patients was the presence of the Cys203Arg missense mutation, either present on a single red/green hybrid gene (n = 12) or simultaneously present on each gene copy in subjects with mutiple opsin genes (n = 7). Sequence comparisons indicate that the formation of single hybrid genes carrying the Cys203Arg mutation have occurred multiple times. Analysis of 500 unselected male control subjects revealed a frequency of ~ 1.5% for the Cys203Arg mutation in the population. Yet in controls the mutation is present on only one of multiple opsin gene copies and thus has either no phenotypic effect (cryptic mutant allele) or may result in a simple red-green colorblindness. We argue that the formation of the most common BCM genotype - that is a single red/green hybrid gene carrying the Cys203Arg mutation - occurred multiple times through unequal homologous recombination involving a chromosome harbouring a cryptic mutant allele.

W8 01

Spectrum and functional impact of GLI3 mutations in different morphopathies

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Mutations in the gene for the human transcription factor GLI3 underlie a number of related but distinct dominant developmental disorders associated with polydactylies. They include Greig cephalopolysyndactyly syndrome (GCPS), Pallister-Hall syndrome (PHS), as well as isolated polydactylies like preaxial polydactyly type IV and postaxial polydactylies type A1 (A and B). The correlation between type and position of mutations with the distinct syndromes is poorly understood.Besides cytogenetic rearrangements affecting the gene locus in 7p14.1, about 50 point mutations were identified at our centre and deposited at the human gene mutation database (http://archive.uwcm.ac.uk). Deletion/translocation of the GLI3 gene was noted in cases that often manifest phenotypic features additional to the ones observed in typical GCPS, thus representing a contiguous gene syndrome. GCPS is caused by a wide spectrum of point mutations including splice site and missense mutations. However, truncating (nonsense/frameshift) mutations are by far more prevalent in GCPS (ca. 65%). In Pallister-Hall syndrome, they remain the sole mutation type identified to date. The high incidence of mutations leading to the introduction of premature termination codons in GLI3 associated morphopathies has prompted us to determine the relative levels of wildtype and mutant GLI3 mRNAs. The imbalance between allelic GLI3 transcripts in patient derived fibroblasts lines was assessed following RT-PCR. Transcripts with stop codons in early exons were consistently found to be present at lower levels (20-40%) compared with the allelic wildtype transcript. In contrast, transcripts with a premature stop codon located in the last GLI3 exon or carrying a silent amino acid exchange were present at equal levels compared with their wildtype counterparts. These data are in line with previous observations derived from different genes that only transcripts with stop codons upstream of the last intron-exon junction undergo nonsense mediated mRNA decay. Only part of GLI3 truncating mutations are located in the first 14 exons. Nearly 50% of the nonsense/ frameshift mutations were identified within the last exon. Thus nonsense mediated mRNA decay can only be invoked for ensuring GLI3 haploinsufficiency of a subset of mutations. The position of the mutations, the inferred occurence of nonsense mediated mRNA decay or retention of functional domains in the mutant products do not provide a satisfactory explanation for resulting phenotypic differences. Alterations in the second GLI3 allele or in other genes modifying GLI3 function may yet be a factor responsible for the apparent lack of genotype-phenotype correlation. Cases with or without known GLI3 point mutations were screened using multiplex amplifiable probe hybridization (MAPH) to reveal possible microdeletions that escaped detection in the cytogenetic or molecular analysis. For specific GLI3 mutations additional mechanisms were studied, like modulation of the complex activities of this posttranslationally modified protein.

W8 02

The Ror2 knock out mouse as a model for the developmental pathology of autosomal recessive Robinow syndrome

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ROR2 belongs to a small family of receptor tyrosine kinases, and mutations in ROR2 account for autosomal recessive Robinow syndrome (RS; Afzal et al., 2000; Borkoven et al., 2000) and dominant Brachydactyly type B (BDB; Oldridge et al., 2000; Schwabe et al., 2000). The function of Ror2 has been elucidated by the analysis of mice with inactivated Ror2 alleles (Takeuchi et al, 2000; DeChiara et al., 2000). In this study we have analyzed the temporospatial Ror2 expression pattern during murine development. We show that Ror2 is expressed during limb, somite and craniofacial development. In addition we present a detailed developmental study of Ror2 -/- mice. Ror2-/- mice exhibit short-limbed dwarfism characterized by abnormal morphogenesis of the face and external genitalia and vertebral segmentation defects. We describe the craniofacial phenotype of Ror2-/- mice characterized by shortening of the mandible, broadening of the frontonasal region, a cleft palate, additional bones of the skull region and defective auditory ossicles. The long bones of the appendicular skeleton are severely reduced in size with the zeugopod being affected strongest. This is due to defective chondrocyte differentiation, as seen by a reduction of Indian hedgehog (Ihh) expression in the growth plate. Analysis of somite development with marker genes demonstrates a small presomitic mesoderm and segmentation defects of somites with a reduction of the anterior portion of the somite. Similiar to Robinow patients Ror2-/- mice exhibit small external genitalia. Expression of Ror2 in the pituitary gland suggests that the hypogenitalsm in RS may possibly be caused by congenital hypopituitarism.

W8 03

CHILD-syndrome: A metabolic disease with impact on development

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CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform Nevus and Limb Defects, MIM 308050), is an X-linked dominant, male-lethal trait characterized by an inflammatory nevus that usually shows striking lateralization with strict midline demarcation as well as ipsilateral hypoplasia of the body. Recently, we were able to demonstrate that this trait is caused by mutations in in the gene Nsdhl (NAD (P)H steroid dehydrogenase-like protein) encoding a 3ß-hydroxy-steroid dehydrogenase functioning in the cholesterol biosynthetic pathway. NSDHL maps to Xq28. Here we present the results of mutational analysis in 25 familial and sporadic cases. The CHILD phenotype is distinct from, but shares various clinical and biochemical features with chondrodysplasia punctata (CDPX2, MIM 302960) associated with mutations affecting a Delta8-Delta7 sterol isomerase (EBP, emopamil binding protein, at Xp11.22 - p11.23) that functions downstream of NSDHL in a later step of cholesterol biosynthesis. EBP was unaffected in the patients analyzed by us demonstrating that CHILD syndrome and CDPX2 are not caused by allelic mutations. Two murine X-linked dominant male-lethal traits, bare patches (Bpa) and striated (Str) had previously been associated with mutations in Nsdhl. A third, phenotypically similar phenotype, tattered (Td) is caused by mutations in Ebp, the gene homologous to the one affected in CDPX2. Remarkably, all of these murine traits resemble human CDPX2. The developmental defects associated with lack of NSDHL in humans suggest an impact on the hedgehog signaling pathway during development. Bpa mice are used as a model to study this effect during early development.

W8 04

Detection of IRF6 mutations in patients with Van der Woude or popliteal pterygium syndrome

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Van der Woude syndrome (VWS; OMIM 119 300) and popliteal ptyerygium syndrome (PPS; OMIM 119 500) are allelic autosomal dominant disorders, caused by missense and truncating mutations in the Interferon regulatory factor 6 (IRF6) gene. Clinical features, common in both disorders, include cleft lip and/or cleft palate and characteristic lip pits. Additional features in patients with the rare PPS include webbing of the lower limbs, pyramidal skin folds of the hallucal nails, filiform adhesions between the eyelids and hypoplastic genitalia. Here we present the clinical data of 4 patients with VWS or PPS, respectively, and identified IRF6 mutation.Two large VWS families from the northern German region were identified to carry a heterozygous 1 bp insertion of an adenine residue in codon 54 of the IRF6 gene, resulting in the introduction of a stop codon at position 58 within the highly conserved DNA binding domain of the predicted IRF6 gene product. The presence of this identical, previously undescribed insertion in two pedigrees from the same area suggests a common ancestral mutation carrier. Another sporadic VWS patient was found to be heterozygous for a 2 bp insertion in exon 5 of the IRF6 gene, resulting in the introduction of a stop codon in codon 167 of the predicted IRF6 gene product.Mutation screening of a sporadic PPS proband revealed the presence of the common missense mutation Arg84Cys, originally reported in seven of the 15 PPS pedigrees, analyzed by Kondo et al. (2002). Although both disorders seem to be caused by distinct IRF6 mutations, the presence of a mild phenotype compatible with the diagnosis of VWS in some relatives of PPS families with identified IRF6 mutation confirms the broad clinical variability of both syndromes even between members of the same family. Genotype-phenotype studies of these two allelic disorders will define the mutation spectrum for both clinical entities and ultimately help to identify those VWS probands, which might be at increased risk for offspring presenting with PPS.

W8 05

Functional analysis of the heat shock protein Apg1 during the testis descent

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The insulin-like factor-3 (Insl3) was found to mediate the gubernaculum development during the testis descent. Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. We showed that the sequence between nucleotides -157 to +4 directs the transcription of the Insl3 gene.In this region, there are two conserved motifs (SF-1 and B1). Transient transfection assays revealed that the SF-1 binding site is required for SF-1 mediated stimulation of Insl3 transcription. In contrast, mutations in the B1 motif increase the transactivation of the Insl3 promoter suggesting that a potent transcriptional repressor bind to this motif. In order to identify the B-1 binding factors, we screened a yeast one-hybrid cDNA library derived from mouse testis. Two clones encoding a heat shock protein Apg1 were isolated. EMSA showed that the recombinant Apg1 specifically binds to the B1 motif. Mutations in this motif abolished the Apg1 binding. Northern blot analysis revealed that two Apg1 transcripts of 3.0 and 3.5 kb are abunhdantly expressed in the testis. In contrast to the heat response pattern of the Ins3 expression, which was found to be increased by a shift from 37 to 32°C, the 3.5 kb transcript was induced by a temperature shift from 32 to 37°C. To determine the function of the Apg1 during the testis descent, we have produced a knock-out mouse for Apg1. Preliminary result are reported.

W8 06

Assessment of association between TBX1 variants and haplotypes with manifestiation of congenital heart defects in 22q11.2 deletion patients

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Deletion 22q11.2, commonly associated with DiGeorge or velocardiofacial syndrome (DGS/VCFS; MIM 188400), is a major cause of congenital heart disease (CHD), accounting for about 5% of all CHD in live borns. However, presence of the deletion does not allow to predict the phenotype as patients with a 22q11.2 deletion usually show a broad clinical variability, despite of a common deletion size of 3 Mb. TBX1 was shown by several knockout mice experiments to play a role in congenital heart and other developmental defects and therefore constitutes a candidate gene for a modifier in deletion 22q11.2 patients, in terms of uncovering of recessive mutations by hemizygosity. Thus we sequenced the coding region of TBX1 in a total of 174 patients with 22q11.2 deletion and detected 16 different variants, 7 of which represent common SNPs. Up to 96 dizygous control persons were additionally typed for these 16 variants. A combined approach with pair wise LD calculation on 174 due to hemizygosity naturally given haplotypes and analysis of background haplotypes of rare variants in a total of 366 chromosomes allowed us to define a reliable haplotype structure consisting of 3 LD blocks within the coding region and a further LD block including the 5'UTR exon 1. 14 of the 16 variants as well as the common haplotypes were detected at equal frequencies in deletion patients with and without CHD as well as in healthy controls. Thus we excluded common TBX1 variants or haplotypes as modifiers of expression of congenital heart defects in 22q11.2 deletion patients.Nevertheless, one 3 amino acid deletion was not detected in normal controls and segregated with VSD in one family and might therefore constitute a causative factor for CHD in this family, as might apply for one further variant in one patient with PA-VSD, which was not detected in normal controls and which was the only variant to affect a conserved nucleotide.

W9 01

Array CGH identifies 4 small deletions in a case with a complex translocation involving 5 chromosomes

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We report on a two year old child with mental retardation, microcephaly, microsomia, coloboma,

optic atrophy, cleft palate, PDA, laryngostenosis and stenosis of the right auditory channel. GTG- and GBG-banding analysis revealed a complexly rearranged karyotype. For further clarification we first performed a 7-fluorochrome M-FISH analysis (Azofeifa et al. [2000] Am J Hum Genet 66:1684-1688). The resulting karyotype could be described as 46,XX,der (5)t (5;14),der (6)t (6;8),der (8)t (5;8;5;8;6;2),der (14)t (8;14). However, we were unable to identify any genomic imbalance, which was expected because of the phenotype of the patient. Chromosome-CGH showed a normal, balanced female ratio profile. To increase the resolution to detect even small imbalances, we repeated the CGH-experiment on the recently developed 1 Mb large insert clone array (Fiegler et al. [2003] Genes Chrom Cancer 36:361-374). This array consists of about 3,500 clones selected from the published Golden Path which are spaced at approximately 1 Mb intervals across the genome. Array-CGH readily identified 4 different deletions, each with a size of smaller than 7 Mb. This is clearly below the resolution limits of chromosome-CGH. These deleted fragments contain 14 different known genes which probably contribute to the patient's phenotype. This case demonstrates that high-resolution screening tools, such as array-CGH, for detecting small imbalances will become an indispensable tool in clinical genetics. The application of these technologies will unravel the cause of many currently unexplained cases with mental retardation and dysmorphic features.

W9 02

Cytogenetic and molecular analysis of a familial translocation with breakpoints at 12p11 and 8q13 cosegregating with brachydactyly type E

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Brachydactyly E associated with severe hypertension inherited in an autosomal dominant fashion has been described in a large Turkish kindred. Linkage analysis of this pedigree localized the critical region to an interval of 6 cM on chromosome 12p11 (Schuster et al. 1996). We previously described a translocation with breakpoints on 12p11 and 8q13 cosegregating with typical features of brachydactyly E in four generations. Cytogenetic and molecular studies revealed that the breakpoint on chromosome 12 is located 2 Mb proximal to the critical interval for brachydactyly E and hypertension. Breakpoint spanning YAC and PAC clones were identified. Partial sequencing of clones allowed us to extract a complete sequence of 75 kb spanning this breakpoint. By using Southernblot analysis we could demonstrate that the open reading frame of PTHLH is not disrupted. Interestingly, FISH experiments with YACs of chromosome 8q13 and database analyses revealed gene disruption of a potassium-voltage gated channel between exon 1 and exon 2 caused by the translocation. Further experiments to prove gene expression of this gene during skeletal development are ongoing.

W9 03

Analysis of Ovarian Borderline tumors by CGH with DNA obtained from paraffin embedded material

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Ovarian carcinoma is the commonest cause of death from gynecological malignancy in most of the Western world. Because of lack of early symptoms and reliable screening tests, only one fourth of the cases are diagnosed as stage I. Furthermore, ovarian cancer differs from other common human cancers in having greater disease heterogeneity, poorly understood progression, and the absence of definite precursor lesions. Further analysis of genetic abnormalities may delineate the relationship between borderline tumors and carcinomas better, and will hopefully lead to a unifying hypothesis as to the origin of these important ovarian lesions. The few cases of early stage disease and borderline tumors available for analysis are mostly paraffin embedded material. The quality of DNA from this source depends mainly on the duration and the kind of fixation. Comparative analysis with fresh and simultaneously paraffin embedded material with different time of fixation revealed that CGH results from material with a fixation time longer than 4 hours should be interpreted with caution. The study included 34 from pathologists classified tumors with low malignant potential. In about one third of the cases 3 to 18 alterations per tumor, another one third 1 to 2 and the remaining one third none alterations could be detected by CGH. In 5 cases with the highest chromosomal imbalances pathological reapplication revealed areas with invasive tumor growth and were reclassified as stage I serous ovarian carcinomas. Therefore CGH is a valuable additional tool to the pathologists for classification of borderline lesions. The most frequently gains in descending order were observed at chromosomes 7q, 8q, 12 and losses at 4q, 22q and 1p. These results resembles the frequent distribution of gains and losses found by CGH in advanced stage ovarian carcinomas.The findings suggest, that there exists two types of borderline tumors of which one is indeed a potential precursor to ovarian carcinoma. This is also in agreement with the observation, that some of the borderline tumors do progress and become lethal.

W9 04

Molecular cytogenetic analysis of endometrioid ovarian cancer

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Ovarian cancer is the fifth most frequent cancer in women worldwide and is characterized by a high mortality rate. The majority of ovarian carcinomas originate from the mullerian epithelium of the ovaries and are histologically classified into serous, endometrioid, and mucinous subtypes. Endometrioid tumors represent approximately 15% of all ovarian carcinomas. Little is known about the genetic alterations involved in the carcinogenesis of endometrioid ovarian cancer and, moreover it is still unclear whether bilateral tumors represent metastasic disease. To investigate recurrent chromosomal imbalances involved in the tumorigenesis of the endometrioid ovarian carcinoma we have applied comparative genomic hybridization (CGH) to analyze tumor DNA extracted from formalin fixed, paraffin-embedded tissue samples from 21 patients (11 patients with unilateral tumors, 10 with bilateral tumors). Our results demonstrate a higher frequency of gains compared to losses. Gains cluster to chromosomes 8q, 1q, 6p, 19, 11q, 3q, 10q, and 21q22-qter while losses most commonly affect chromosome arms 4q, 13q and 6q. These data present a high number of chromosomal aberrations which may be involved in the pathogenesis of endometrioid ovarian cancer.

W9 05

Multicolor interphase FISH for the diagnosis of IGH translocations in plasma cell disorders

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Translocations involving the immunoglobulin heavy chain (IGH) locus at 14q32, or its variants, are among the most common chromosomal abnormalities in multiple myeloma (MM). These translocations juxtapose IG regulatory sequences next to various oncogenes, whose expression is subsequently altered. IGH partners in MM targeted by such translocations are CCND1 (11q13), FGFR3/MMSET (4p16), MAF (16q23), CCND3 (6p21), IRF4 (6p25), MAFB (20q11) and IRTA1/2 (1q21). Some of these translocations affecting IGH in MM are difficult to detect by conventional chromosome analyses because they involve subtelomeric regions on both affected chromosomes, e.g. t (4;14)(p16;q32), t (14;16)(q32;q23) or t (6;14)(p25;q32). This limitation can be overcome by fluorescence in situ hybridization (FISH) using suitable locus-specific probes. Here, we present new multicolor interphase FISH (MI-FISH) assays for the rap-

id and simultaneous detection of IGH translocations in MM. Probes flanking the chromosomal breakpoints of the most common translocation partners were designed and labeled in a dual-color fashion. These probes were validated in healthy donors and the cut-off for false-positive results was calculated. Their suitability to detect their respective chromosomal breakpoints was proven by using cytogenetically-positive controls. After this thorough validation process, the probes for each gene were pooled and differentially labeled with DEAC, Spectrum Orange, Texas Red or Cyanine 5. The first multicolor assay was made of a probe for the IGH locus labeled in Spectrum Green together with probes for the most frequent gene partners: CCND1, FGFR3/MMSET, MAF and CCND3. By means of this assay, the t (11;14)(q13;q32), t (4;14)(p16;q32), t (14;16)(q32;q23) and t (6;14) (p21;q32) translocations can be detected in a single experiment. The second multicolor assay included a probe for the IGH locus together with the new probes for the less frequent IG gene partners: IRF4, MAFB and IRTA1/2 to detect the t (6;14)(p25;q32), t (14;20)(q32;q11) and t (1;14)(q21;q32) translocations. These MI-FISH assays were tested in negative and positive controls and hybridized in a small series of MM cases with normal karyotypes where IGH breakpoints were previously detected. Thus, we could validate the capacity of the new probe sets to simultaneously detect the most common translocations in MM. Furthermore, the modular probe design allows easy combination with probes for IGL or IGK instead of IGH to detect variant translocations. In the near future, in order to increase the sensitivity of the MI-FISH approach, multicolor combined immunophenotying and FISH assays will be established. This technique will pave the way for the accurate study of low infiltration with chromosomally altered plasma cells, as it occurs in monoclonal gammopathy of unknown significance (MGUS).

W9 06

Acute myeloid leukemia (AML) with complex aberrant karyotype are characterized by a distinct pattern of gains and losses of chromosomal regions identified by using 24 color FISH and comparative genomic hybridization (CGH)

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AML with complex aberrant karyotype accounts for 10%-20% of AML and is associated with the most unfavorable prognosis of all AML subtypes. Despite intensive treatment including allogeneic stem cell transplantation long term survival is achieved in less than 10% of these patients. So far only little is known about patterns of cytogenetic abnormalities in AML with complex aberrant karyotypes. It was the aim of the present study to analyze the cytogenetic abnormalities in this AML subgroup in detail to elucidate the underlying pathogenetic mechanisms. A series of 250 AML with complex aberrant karyotype had been studied with conventional chromosome analysis and 24-color-FISH. Loss of chromosomal material occurred most frequently on 5q, 7q, 17p and 12p. The gain of chromosomal material most often involved chromosomes 8 and 11. In order to describe the gained and the lost regions in more detail comparative genomic hybridization (CGH) was carried out additionally in 41 patients. Ten regions were identified which were aberrant in more than 20% of cases. These were: loss of 5q31.1-q31.3 (n=37), 17p13 (n=25), 7q32-q35 (n=23), 18q21q22 (n=14), 12p13 (n=12), 16q22q24 (n=12), 13q14 (n=9); gain of 11q23q25 (n=13) with high level amplification in 5 cases, gain of 1p33p36 (n=10) and gain of 8q22q24 (n=8) with high level amplification in one case. The median number out of the 10 most frequent abnormalities involved per patient was 4 (range 1-8). No specific pattern of combinations between the different abnormalities was observed. In conclusion, the most frequently involved regions identified by 24 color FISH were confirmed by CGH. Furthermore the involved regions were narrowed down and 4 additional regions were identified (loss of 18q21q22, 16q22q24, 13q14, gain of 1p33p36). Thus, AML with complex aberrant karyotype is characterized predominantly by loss of genetic material of 7 distinct regions and gain of 3 regions. As a large variety of different combinations of the 10 most frequent aberrations occurred, it can be speculated that the accumulation of these abnormalities is more important than the occurrence of a specific defect itself.

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W10 02

Tep22 is involved in the biogenesis of the acrosome and the midpiece of the murine sperm tail

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Spermatogenesis in mammals is an excellent model system to study cellular and developmental processes. Here, we report the identification of a new gene named Tep22 (Testis expressed protein of 22 kDa), which is translational regulated and is involved in the development of male germ cells of mice. The gene encoding Tep22 consists of three exons and is localized in the telomeric region of mouse chromosome 12. Sequence and computer analyses of Tep22 revealed no significant similarity to any known gene. Specific antibodies against Tep22 detected an approximately 22 kDa band in testicular protein extracts which was first observed in 18-day-old mice. Indirect immunofluorescence and immunoelectron microscopy experiments demonstrate that Tep22 is localized in the acrosomal region of early elongating spermatids, while the surrounding cytoplasm is barely labeled. During further germ cell development the intensity of the staining in the acrosomal region decreases and is not longer detectable in late stages of elongating spermatids, whereas the amount of the Tep22 protein increases in the cytoplasm. Finally, Tep22 is incorporated into the midpiece of spermatids and is also present in the mitochondrial sheath of mature spermatozoa. Taken together, our results indicate the involvement of Tep22 in the biogenesis of the acrosome and the midpiece of the sperm tail as well as in the function of mature murine spermatozoa. To elucidate the function of Tep22 during male germ cell development we have started to generate Tep22-deficient mice using the „knock-out" approach.

W10 03

Mosaicism of sex-chromosomes in the gonads of patients with gonadal dysgenesis and normal female or male karyotypes in lymphocytes

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Currently, most cases of gonadal dysgenesis remain unexplained. SRY gene mutations and deletions can be detected in about 20 - 30% of patients with XY-gonadal dysgenesis. Mutations of other known genes, such as SF1, WT1, SOX9, DMRT1 and DAX1, altogether contribute to a very small portion of patients with XY-gonadal dysgenesis. On the other hand, mutations of the FSH receptor gene are a very rare cause of XX-gonadal dysgenesis. In this study we have searched for mosaicism of gonosomes in streak gonads of patients with gonadal dysgenesis and normal female (2 patients) and male (2 patients) karyotypes in lymphocytes. Cytogenetic and FISH analyses of the gonads demonstrated in three patients a sex-chromosomal mosaicism. The gonadal tissue of the forth patient confirmed the result of the lymphocytes by cytogenetic analysis with 46,XX, but FISH analyses revealed in 3% of cultured cells and in 17% of uncultured cells only one X-chromosome. In summary, our data indicate that gonosomal mosaicism in gonads may be a frequent cause of gonadal dysgenesis in patients with normal male or female karyotypes in lymphocytes. Therefore, cytogenetic and molecularcytogenetic analysis of gonadal tissue can provide important information in unexplained cases of gonadal dysgenesis.

W10 04

Polar body diagnosis for Norrie disease using minisequencing and linked markers

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Norrie disease is an X-linked neuroectodermal disorder, characterized by blindness, deafness and mental retardation (OMIM 310600). Systematic mutation analysis allows the detection of intragenic deletions and missense mutations of the Norrie (ND) gene in more than 90% of clinically characterized pedigrees and the identification of female mutation carriers. Here we report the results of two ICSI cycles with polar body diagnosis for a 33 year old female carrier of the ND missense mutation K104Q. After laser-assisted biopsy of the first and second polar bodies 1 hour and 10 hours after ICSI respectively, cell lysis was performed using Proteinase K. In a single multiplex PCR reaction a PCR product containing the ND mutation and two additional PCR products of closely linked fluorescence-labelled markers were amplified. One sample of each reaction was separated on a Beckman CEQ8000 to analyze the allele distribution of the

microsatellite markers. A minisequencing reaction was performed with another aliquot of each reaction using the SNaPshot Multiplex Kit (ABI). Separation of these reaction products on an ABI 310 allowed direct detection of the disease causing nucleotide changes on both strands of wildtype and mutant allele for each sample.In two ICSI cycles 15 oocytes were obtained. From these the first polar body could be removed from all 10 oocytes presenting a first polar body and biopsy 10 hours after ICSI was also successful for all 9 oocytes presenting a second polar body. Three and two oocytes, respectively, were predicted to carry the normal ND allele in the first and second ICSI cycle based on concordant results of the combined detection of mutation and linked markers in first and second polar body. Transfer of these embryos, identified to carry the normal allele, so far resulted in one biochemical pregnancy. An optimized protocol was established for parallel detection of a missense mutation by minisequencing and of the allele distribution of closely linked informative markers for a family with a known ND mutation. This allowed the sequential analysis of first and second polar body and prediction of the mutation status of the corresponding oocytes within the time frame of 24 hours after oocyte biopsy in concordance with the German embryo protection law.

W10 05

Diagnosis of the genetic origin of complete hydatidiform moles by microsatellite analyses in archival material

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Complete hydatidiform moles (CHM) are the most common form of gestational trophoblastic disease and a frequent antecedent to choriocarcinoma. Cytogenetic investigations have shown that they arise by unusual fertilisation events. CHM may be either monospermic if it arises from the doubling of a haploid sperm (homozygous mole), or dispermic if it arises from two haploid sperms (heterozygous moles). In this study we used microsatellite analyses using highly polymorphic markers to examine the genetic origin of 15 CHM retrospectively. DNA was extracted from formalin fixed, paraffin embedded tissue after thorough separation of chorionic villi and decidua. A total of 10 highly polymorphic microsatellites of different chromosomes (5, 9, 16 and X) was used. Analyses of the PCR products were performed by means of 8% polyacrylamide gel electrophoresis (PAGE) and silver staining. With informative microsatellites, diagnostic patterns of amplification were obtained. Complete moles should either yield one or two microsatellites which differed from both maternal (decidual) microsatellites. Complete mole would be excluded by all the microsatellites showing alleles identical with those in maternal DNA. Our results confirm the androgenetic origin of the CHM having only paternal alleles. Furthermore in our sample, CHM were always due to a monospermic mechanism implicating that most CHM were caused by fertilisation of an empty egg by a duplicated haploid sperm. A higher malignant potential in heterozygous CHM is discussed. Therefore, it can be important to distinguish between both forms of CHM to assess the relative risk. However, the number of published cases is small and additional studies are required to determine conclusively whether the heterozygous form is potentially more aggressive.

W11 02

An autosomal genomic screen for interspecific hybrid placental dysplasia in the genus Mus: Linkage and candidate gene analysis

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Introduction: It has been shown previously that abnormal placental growth occurs in interspecific hybrids between different species of Mus , i.e., M. musculus, M. spretus, M. macedonicus, and M. spicilegus. Loci involved in the generation of these placental phenotypes were mapped to the X-chromosome.Objective: To identify the putative autosomal loci whose abnormal interaction with the X-linked loci or their gene products causes abnormal development in the interspecific context. METHODS: More than 100 (M. musculus x M. spretus) x M. musculus backcross conceptuses were prepared. Placentas were weighed and genomic DNA of the fetuses was examined by analysis of polymorphic markers. Results: This analysis identified four regions, one of them subject to genomic imprinting, on chromosomes 2, 9, 13, and 14 that exhibited linkage to placental dysplasia. However, for three of these regions sex-specific linkage was detected. Thus, the regions on chromosomes 9 and 14 exhibited linkage only in male placentas, whereas the imprinted distal chromosome 2 region exhibited linkage in female placentas. In addition, for chromosome 13 linkage was dependent of the M. spretus strains used for analysis.Conclusions: These results suggest that (an) imprinted gene (s) may be involved in murine placental hybrid dysgenesis. In addition, they indicate an unexpected degree of complexity due to influences of sex and geographical derivation.

W11 03

Lessons from cation-cotransporter deficient mice

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K-Cl cotransporters are encoded by four homologous genes and may have roles in transepithelial transport and in the regulation of cell volume and cytoplasmic chloride. KCC1, a widely expressed, swelling-activated cotransporter, has been implicated in many transport processes, including the regulatory volume decrease of erythrocytes. KCC2 is neuron-specific and mediates transport under isotonic conditions. KCC2-/- mice reveal a pivotal role of KCC2 in regulating the intraneuronal Cl- concentration and thus synaptic inhibition. Loss of KCC4 in mice leads to deafness and renal tubular acidosis, presumably due to a diminished removal of K+ after its exit from sensory outer hair cells and to an impaired Cl—extrusion from renal acid secreting cells, respectively. KCC3 is expressed in the central nervous system as well as in numerous other tissues such as kidney, lung, pancreas, and heart. In humans, mutations in KCC3 (encoded by the SlC12A6 gene) lead to a peripheral neuropathy that is associated with an agenesis of the corpus callosum, mental retardation, and seizure susceptibility (ACCPN or Anderman syndrome) (Howard et al., Nature genetics, 2002, 32: 384-92). As in the human disease Kcc3-/- mice show severe motor abnormalities that correlate with a progressive neuronal degeneration in the peripheral and central nervous system. The analysis of the Kcc3-/- mouse model has revealed important cellular and systemic functions of KCC3 and is highly relevant for Anderman syndrome.

W11 04

Detailed imaging of brain-specific expression patterns in Tet-off promoter mice

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The "Tet-Off-System" developed by Dr. H. Bujard (Heidelberg) allows the generation of inducible transgenic mouse models. This system is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Tetracycline which allosterically inhibits the tTA protein. In order to assess whether a specific promoter mouse line is suitable for the generation of a disease model the knowledge of the brain regions in which the transgene will be expressed is indispensable. The expression pattern of the promoter mouse line states whether the transgene will be targeted to the desired brain regions and in which brain regions a phenotype or pathology is to be expected, respectively. For this reason we studied the expression pattern of available Tet-Off promoter mouse lines with known expression in the brain (Prion protein (Prp) promoter, Ca2+/Calmoduline-dependent protein kinase II (CamKII) promoter). We crossbred these mouse lines with responder mice transgenic for the lacZ reporter gene. The expression of beta-galactosidase in brain regions with promoter activity was detected using X-Gal as beta-galactosidase substrate resulting in a blue staining. For an overall view entire mouse brains were stained. Brains were sectioned for a detailed analysis of the localization of beta-

galactosidase, and composite images of whole sections were transferred to a 3-D digital atlas. The visualization of this expression data in a 3-D brain atlas facilitates the future goal-directed generation of inducible mouse models using the Tet-Off-System.

W11 05

Autosomal Recessive Polycystic Kidney Disease (ARPKD): pkhd1-deficient mice with typical hepatic fibrosis but without renal phenotype

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Autosomal recessive polycystic kidney disease (ARPKD/PKHD1) is one of the most common causes of renal- and liver-related morbidity and mortality in childhood. We have recently shown, with others, that mutations in the PKHD1 gene encoding a large multidomain transmembrane protein underlie ARPKD. In this study, we have used knockout mice habouring a truncation mutation in exon 41 to analyse developmental defects resulting from pkhd1 inactivation. These mice develop severe malformations of intrahepatic bile ducts but surprisingly no renal cysts. Cholangiocytes maintain an immature, proliferative phenotype and continue to secrete TGF-ß1. Subsequently, mesenchymal cells within the portal tracts continue to proliferate and synthesize collagen types I and III resulting in progressive portal fibrosis and cystic bile duct transformation. Careful comparison with human ARPKD patients of different prenatal and postnatal stages indicated that the mechanisms leading to progressive portal fibrosis parallel precisely disease progression in human liver. Immunostaining in patients with missense mutations revealed accumulation of mutant PKHD1 protein in the intracellular secretory pathway. Taken together our data indicate that PKHD1 provides a receptor-like function on the surface of embryonic cholangiocytes. Subsequent to formation of the embryonic ductal plate PKHD1 signalling is required for terminal differentiation of cholangiocytes. Defective transmembranous positioning of PKHD1 results in failure to downregulate proliferation and matrix synthesis of the portal tract mesenchyme.

W12 02

Evaluation and Improvement of For-Client Letters in Genetic Counseling

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Ensuring the quality of the genetic counseling process presently appears in the same order of importance as ensuring the quality of the technical and scientific procedures that are employed in genetic diagnostics. However, the rapidly growing knowledge in the field of genetics makes the counseling process more and more cognitively complex and increasingly leads to its fragmentation and scattering over time. The concept of informed consent and the intention to help clients by genetic counseling to arrive at valid decisions obviously come under pressure. Do the actually dialogical phases of the process provide sufficient integration? Or will the medium of speech – the counseling session - soon have to be supported by other media? For-Client Letters ought to serve as a medium for recording the actual state of knowledge as well as its individualized interpretation, facilitating clients' deliberation beyond the temporal limitations and other constraints of the actual counseling session. The present investigation studies the effectiveness, the scope and the ethical significance of enhancing the flow of communication by for-client letters. An established standard format for such letters is varied and enriched systematically. One half of the clients receive a standard letter, the other an enriched letter in a randomized, double blinded mode. For this purpose information gained by tape-recording the whole counseling session is used. Comprehensibility and use-value of the standard format and the enriched format are empirically compared, both by quantitative (interview and questionnaire with a 20 point score respectively) and qualitative methods. The cases come from three diagnostic conditions in which increases in diagnostic complexity and predictive power translate into individual persons' practical problems of valid decision-making: Counseling concerning i) suspected hereditary cancers, ii) anomalous findings in prenatal diagnosis and iii) infertility disorders. Altogether we counseled 132 families/couples with 161 clients, 102 families with suspected hereditary cancers (hereditary breast and ovarian cancer, colorectal cancer and thyroid cancer), 10 couples with anomalous findings in prenatal diagnosis and 20 couples with infertility disorders. Analysis in the 102 families counseled because of suspected hereditary cancer revealed that clients receiving the enriched format had a significantly better knowledge and comprehension of relevant facts than clients receiving the standard format (Kruskal-Wallis One-Way Analysis of Variance: $p < 0{,}01$). This indicates that for-client letters can improve client´s knowledge and comprehension of relevant facts and that form and content of the letter influence this effect. Evaluation of the other two groups is under way. Supported by BMBF, FKZ 01KU9904

W12 03

Attitudes towards genetic testing: A comparison between geneticists and the general population

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Objective: This study has aimed to explore general attitudes towards genetic testing in professionals in human genetics, and to compare geneticists` attitudes with attitudes held by the general population. Method: Attendants of the 13th Annual Meeting of the Deutsche Gesellschaft für Humangenetik, which took place 29.09.-02.10.2002 in Leipzig, comprise the sample of experts. They received a self-report questionnaire on attitudes towards genetic testing at the registration desk. The questionnaire comprises 13 items pertaining to approval, disapproval, and concern for genetic testing. The same questionnaire was used in a multiple-issues survey in a sample representative of the German population. Of the 753 German conference attendants who received the questionnaire, N = 97 (12.9 %) took part. The mean age of the 41 men (41.3 %) and 56 women (47.7 %) was 39.3 years. For statistical comparison, a matched sample (N = 97) was drawn from the representative sample, matching criteria were age, gender, educational level and religious affiliation. Results: The results reveal that geneticists hold a more favourable view of gene tests than the general population. There was a significantly higher rate of agreement with approval items (e.g. „reducing the costs of health care") and lower rate of agreement with disapproval items (e.g. „discrimination of disease gene carriers"). Further, professionals in human genetics showed less concern for genetic testing (e.g. „misuse of test results for scientific purposes") than persons from the general population. Discussion: Professionals in human genetics a more in favour of genetic testing than persons from the general population. This may be due to limited knowledge about gene tests, as well as fears regarding the further advancement of biotechnology in the general population. This illustrates the continuing need to educate the public about the possibilities – as well as limitations – of genetic testing. Furthermore, genetic counsellors should be cognizant of a potential positivity bias in their attitudes toward gene tests as compared to their consultants.

W12 04

Use of the Internet as a Source of Information by Genetic Counselees and General Practitioners in Germany

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Objective: Detailed knowledge on specific diseases is increasingly achieved from the internet. We assessed web search as a source of information on genetic diseases by counselees and general practitioners.Methods: 152 individuals referred for genetic counselling were interviewed at the beginning of the appointment assessing sociodemographic data, ways used to get the desired information, and the results of the individual search. Forty questionnaires had been distributed to family doctors evaluating their attitude towards the Internet as an informational tool.Results: 78% (119/152) of the counselees have access to the Internet. Of those, 45% (53/119) had searched it for information before the appointment. 60% were satisfied with the information they found. English web sites had been read by 16/53.90% of the practitioners approve of their patient`s Internet search but only 43% (17/40) used the Internet themselves to receive information on genetic conditions. The majority of those (82%) found the retrieved information sufficient. English language sites had been used by only 29%.Conclusion: The study revealed that in 2002 about half of all individuals had looked for information outside the professional health system and every third counselee had used the Internet for personal information before genetic counselling. The actual number of those properly informed remained low, though.On the other hand only just 40% of the family doctors are using the Internet to retrieve information on genetic diseases.Although the Internet is largely accessible and its use by patients is generally supported by health professionals the effectiveness is limited. Personal and

individual counselling will still be necessary in most cases.

W12 05

The molecular genetics of phenylketonuria in Europe

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Phenylketonuria (PKU) is heterogeneous, with more than 400 different pathogenic mutations identified in the phenylalanine hydroxylase (PAH) gene. Comprehensive mutation data are now available for most European countries. In a systematic review we identified 29 mutations that may be regarded as the prevalent PKU-mutations in Europe. Common mutations include R408W on a haplotype 2 background in Eastern Europe, IVS10-11g>a in the Mediterranean, IVS12+1g>a in Denmark and England, Y414C in Scandinavia, I65T in Western Europe and R408W on haplotype 1 in the British Isles. The degree of heterogeneity differs markedly between populations. PKU in Germany is more heterogeneous than in other, particularly Northern and Eastern European countries, with homogeneity („homozygosity") at the PAH locus approaching that of Sicily or North America (0.06). Factors that have influenced the regional mutation spectrum include founder effect, range expansion, migration and genetic drift. In addition, the mutation data indicate that overdominant selection (heterozygote advantage) has contributed to the high incidence of PKU in Europe. Differences in the severity of mutations explain differences in the predominant clinical phenotypes between countries. The molecular data are useful for the understanding of both the clinical features and the population genetics of PAH deficiency in Europe.

W12 06

The origin of mutations in the lethal X-linked recessive Myotubular Myopathy MTM1

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MTM1 (XLMTM, MIM# 310400) is a congenital myopathy characterized by severe hypotonia (floppy infant syndrome) and generalized muscle weakness in affected males.Most patients die within the first year of life from repiratory failure. Some patients survive for several years and may show improvement of the respiratory function. Female carriers rarely express significant clinical symptoms, not clearly depending on skewed X-inactivation. The disease is caused by mutations in the myotubularin gene, a potent phosphatidylinositol 3-phosphate phosphatase exerting its effects during myogenesis. Till today about 200 hundred different mutations which are widespread throughout the gene were found.Taking together the 238 published informative families harbouring a mutation and our own mutational data (15 new cases) it is possible to clarify the origin of mutation in oogenesis or spermatogenesis.Due to the high number of different mutations it is possible to give an account of missense/stop mutations, small insertions/deletions and splice mutations separately. With the knowledge of the individual familiar mutation and the carrier status of the mother an estimation of the k-value (mutation rate in males/mutation rate in females) is straightforward.

Number of informative MTM1-families (without large deletions) = 253

mutation type	number	mother not carrier	proportion of new mutations	k-value
missense / nonsense	132	19	0.14 (0.09-0,22)	4.9 (2.6-9.2)
small deletions / insertions	70	9	0.13 (0.06-0.23)	5.8 (2.314.5)
splice	51	5	0.10 (0.03-0.21)	8.2 (2.7-28.7)
all	**256**	**33**	**0.13 (0.09-0.18)**	**5.7 (3.6-8.9)**

There is no significant difference between the proportion of new mutations values and k-values of different mutation types. The results for all types of mutations in MTM1 are comparable to those in Lesch-Nyhan disease, Hemophilia A and DMD (if the large deletions, arising particularly in oogenesis, are omitted).All these data demonstrate that the classical hypothesis of Haldane, one third of all affected boys are new mutations in lethal X-linked recessive diseases, is wrong, because the mutation rate in females and in males are different (k unequeal 1).

W13 02

Spondyloepiphyseal Dysplasia Omani Type - a New Recessive Type of SED with Progressive Spinal Involvement – is Caused by Mutation in the Sulfotransferase CHST3

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We report a large inbred kindred from Oman with a distinct type of spondyloepiphyseal dysplasia. We evaluated 8 individuals from two consanguineous sibships, 1 male and 7 females between the ages of 2 and 22. The pedigrees strongly suggest autosomal recessive inheritance and both families are likely to be related through distant consanguineous loops. The clinical features include near to normal length at birth, short stature with final height of 110-130 cm, shortening of the upper segment due to severe progressive kyphoscoliosis, severe arthritic changes with joint dislocations, rhizomelic limbs, genu valgum, cubitus valgus, mild brachydactyly, camptodactyly, microdontia and normal intelligence. During the first year of life the vertebral bodies are of normal height but the endplates are irregular and intervertebral space is narrow. With age, the vertebral endplates become increasingly irregular, the intervertebral space diminishes further and individual vertebrae start to fuse resulting in a severe short trunk dwarfism with kyphoscoliosis. The epiphysis are small and precocious osteoarthropathy was observed involving small and large joints. Osteoarthropathy and spinal involvement resulted in physical handicap in early adulthood. Comparison of these patients with other skeletal dysplasias suggests that they represent a previously undescribed variant of spondyloepiphyseal dysplasia.The two families were mapped to a 5 Mbp interval on chromosome 10q22 containing approx. 50 genes. A nearby locus containg the gene ATPSK2 which is mutated in SEMD Pakistani type was excluded from the interval by two recombinants. The interval contains the gene for the carbohydrate chondroitin 6 sulfotransferase 3 (CHST3), an enzyme involved in the sulfatation of proteoglycans. We identified a G1351A point mutation in exon 3 of the CHST3 gene changing a Arg to Gly. The mutation is within a highly conserved region of the enzyme that has been implicated as a putative PAPS binding site. Functional studies of the mutation are currently being performed. The clinical overlap of Omani type SED with other dysplasias caused by defects in sulfate metabolism suggests a common molecular pathway in these conditions.

W13 03

COMPLEX REARRANGEMENTS CAUSE HYPERTENSION AND BRACHYDACTYLY ON THE SHORT ARM OF CHROMOSOME 12

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Hypertension is a major risk factor for many common causes of morbidity and mortality in industrialized societies, including stroke, myocardial infarction, heart failure, and endstage renal disease. Monogenic forms of hypertension provide a unique opportunity for studying the effects of single genes and identifying single pathways and mechanisms leading to blood pressure elevation. In 1973, Bilginturan described a Turkish family with autosomal-dominantly-inherited severe hypertension, cosegregating 100% with brachydactyly. The family was revisited and the responsible gene locus mapped on the short arm of chromosome 12. The skeletal phenotype is characterized by brachydactyly type E and approximately 10 cm shorter stature of the affected compared to the nonaffected family members. To learn more about the hypertension, extensive clinical studies have been performed in this family. They resemble patients with primary hypertension, although they show a marked failure in baroreflex blood pressure buffering. We are aware of four families and one spontaneous case of the syndrome.We used newly identified and database SNPs for further mapping of the disease locus and reduced the region about 1.2 Mb to the minimum size of 3.15 Mb. At least 22 genes lie within the genomic DNA interval for the hypertension brachydactyly locus. Fourteen have a known function. The others are predicted genes with mRNA and/or EST evidence. Their function is unknown. We sequenced 11 genes; however, we found no mutations. Two candidates were genes for the subunits of an ATP-

sensitive potassium channel: Kir6.1 (KCNJ8) and SUR2. Sequencing and SNP mapping ruled out the most promising candidate gene for the skeletal phenotype "long" SOX5.With Interphase FISH experiments using PACs and BACs, we have discovered a complex chromosomal rearrangement in the Turkish family. In an additional spontaneous case rearrangements in the same chromosomal regions have been detected. Work to define the chromosomal breakpoints and the influenced genes is ongoing. Chip experiments and TaqMan assays are being conducted to realize expression differences of the candidate genes due to aberrations in regulatory elements. Thus, autosomal-dominant hypertension is a novel gene rearrangement syndrome.

W13 04

FGF signaling in achondroplasia

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Several mutations in the Fibroblast Growth Factor (Fgf) receptor 3 gene lead to the short-limb dwarfism syndrome achondroplasia in human. Other signaling systems, such as Indian hedgehog (Ihh), Parathyroid hormone-like peptide (Pthlh), and Bone Morphogenetic Proteins (BMP), regulate skeletal development as well. We have analyzed the interaction of FGF signaling with the Ihh/Pthlh and the BMP pathways using an organ culture system of embryonic mouse limbs, which was supplemented with activators or inhibitors of each pathway. In addition we used a mouse model for human achondroplasia carrying an activated Fgf receptor3 (Fgfr3ach mice). We have shown that FGF signaling regulates the onset of hypertrophic differentiation upstream of the Ihh/Pthlh pathway. Additionally, we found that FGF signaling advances terminal hypertrophic differentiation and reduces chondrocyte proliferation in an Ihh-independent manner. We interpreted the role of FGF signaling in regulating chondrocyte differentiation in a new way: instead of delaying hypertrophic differentiation, FGF signaling actually accelerates this process. As the FGF-induced phenotype resembles the phenotype of blocking BMP signaling, we performed co-culture experiments with FGF2 and BMP2. We found that both pathways act in an antagonistic relationship. Moreover, in a limb culture system BMP signaling can rescue the dwarfism phenotype characterized by reduced zones of proliferating and hypertrophic chondrocytes in Fgfr3ach mice. Therefore BMP signaling might be a promising approach to improve the achondroplasia phenotype.

W13 05

Selective screening for cartilage/bone specific genes

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Our group is focusing on the functional characterisation of cartilage and bone related genes in mouse and chicken. Current studies going on in our lab aim to identify new genes involved in skeletal development. For this purpose, we are currently screening four different skeletal mutants (Shh-short-digits (Dsh), Gdf5-brachiopodism, Ror2-KO, Runx2-KO) by hybridizing cDNA to Affymetrix arrays representing approximately 36000 genes and ESTs. The choice of these mutants allows the follow up of skeletogenesis from early patterning events (Dsh), early cartilage formation (Gdf5, Ror2) to later cartilage differentiation and bone formation (Ror2, Runx2). Genes showing differential expression between mutant and wild type are corroborated by real-time PCR. These genes might play a role in cartilage or bone development and thus are interesting candidates for further analysis. Hybridizations for Runx2, Dsh and Gdf5 are completed, Ror2 is on the way. Expression profiling of the differentially expressed genes on mouse tissue will be done by automated in-situ hybridization using a TECAN Genesis ISH system. First results from real-time PCR for the Runx2 mutant indicate, that approximately 80% of genes resulting from the chip hybridizations can be confirmed as being differentially expressed. In the case of the Runx2 mutant we use computational methods for further selection of directly by Runx2 regulated candidate genes by screening promoter regions for conserved Runx2 binding sites in human and mouse promoter regions. Genes that passed this selection will be tested in the micromass culture system an in vitro model for cartilage differentiation.For this assay limb buds from chicken at Hamburger Hamilton stage 22-24 are dissected and trypsinated to get a single cell suspension of mesenchymal cells. If cells are plated in high density they differentiate into chondrocytes and undergo all steps of cartilage differentiation.Differentially expressed genes from chip hybridization will be transfected with an avian retrovirus (RCAS) containing the specific gene of interest. Afterwards their influence on cartilage differentiation is monitored by staining of the extracellular matrix of chondrocytes with Alcian blue or quantification of marker gene expression by real-time PCR. Genes which have an influence on chondrocyte differentiation can be analysed in vivo by injecting the RCAS virus into the limb fields of 1.5 day old chicken embryos.

W14 01

FUNCTIONAL ANALYSES OF THE TRPS1 TRANSCRIPTION FACTOR

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The tricho-rhino-phalangeal syndrome is characterized by craniofacial and skeletal abnormalities. Mutation analyses revealed that the TRPS1 gene on human 8q24.1 is the major locus for this disease. It is highly conserved between human and mouse and encodes a transcriptional repressor for hitherto unknown GATA-regulated genes.To understand the role of TRPS1 in chondrocytes during skeletal development, we generated TRPS1 transgenic mice. The transgene contains the entire human TRPS1 coding region under the control of a mouse Col2a1 promoter and enhancer that is restricted to expression in chondrocytes. It carries a T901P missense mutation in the GATA-type zinc finger (zf) which has been found to cause severe brachydactyly and short stature (TRPS type III) in a human patient, presumably as the result of a dominant negative effect. Proven by PCR and Southern blot analyses we obtained four independent lines of transgenic animals from two male and two female founders. Unexpectedly, the adult animals do not show the TRPS typical brachydactyly or growth retardation. The expression of the transgene is currently being assessed by RT-PCR using RNA from the limbs of 12 d.p.c. embryos.The Trps1 protein is detectable in mouse embryonic stem cells (ES). Therefore, we used this cell type to identify potential target genes of TRPS1. To avoid contamination with feeder layer cells, the ES cells were purified by FACS. In microarray analyses, we compared the expression profiles of wild type ES cells (wt-ES) with those of ES cells in which the GATA-type zf coding exon 5 has been knocked out in frame in one allele of Trps1 (k.o.-ES) by Malik et al. (Mol Cell Biol, 2002, 22, 8592-8600). We confirmed the expression of both Trps1 alleles in k.o.-ES cells by RT-PCR and western blot analyses. Because Trps1 is a repressor of transcription, we expect an overexpression of Trps1 target genes in the k.o.-ES cells. Our preliminary results reveal only few genes differentially expressed between wt- and k.o.-ES cells. Among these genes some encode proteins involved in degradation of the extracellular matrix and one is an osteoblast specific gene. Currently, additional microarray experiments using three independent wt-ES and two independent k.o.-ES cell lines are performed to confirm and/or extend our results.

W14 02

Characterization of a novel gene located in 10q24 encoding a centrosome-associated protein

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We are studying a female patient who presented with a complex phenotype including stato- and psychomotor retardation, bilateral cleft lip and palate, facial dysmorphic signs, agenesis of corpus callosum, enlarged ventricles, diffuse reduction of white matter, rotatory nystagmus, and abnormalities in the retinal pigment epithelium. Cytogenetic analysis revealed a de novo and apparently balanced reciprocal translocation, 46,XX,t (10;X)(q24;p22). By molecular characterization of the X;10 translocation, we found 26 PAC clones overlapping the breakpoint region in 10q24. These clones are arranged in a contig together with further 21 PACs. The identification of numerous breakpoint spanning clones suggests the presence of a complex rearrangement in the patient. By exon-trapping, we identified a novel gene on one of the breakpoint spanning PACs that consists of 9 exons and spans a genomic region of 32 kb. Northern blot analysis showed the existence of a ~2800-bp transcript in thymus, colon, placenta, and small intestine. By 5-RACE and RT-PCR, we assembled a 2634-bp cDNA sequence that comprises an open reading frame of 1395 bp and encodes a protein of 464 amino acids. The protein showed no significant homology to other proteins in the database. To

gain insight into the biological function of the protein, we transiently expressed it as an EGFP fusion protein in PC12 and CHO-K1 cells. During interphase, green fluorescence was detected in one or two small dots near the nucleus. In contrast, during telophase, the green fluorescent signal was located in the midbody of dividing daughter cells. Immunocytochemical analysis using specific antibodies revealed that the protein co-localized with gamma tubulin, a specific marker for centrosomes, during interphase, appeared at the cleavage furrow in the early telophase, and remained in the midbody at late telophase. Neither the treatment of transfected cells with taxol, which stabilizes microtubules and detaches them from the centrosome, nor nocodazole, which depolymerises microtubules, did significantly alter the centrosomal localization of the protein. These data show that the protein described here undergoes cell cycle-depended shuttling between the centrosome and the midbody suggesting that this novel protein may have important functions during mitosis.

W14 03

GLI3 subcellular localization depends on microtubule-associated PP2A activity

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MID1 is mutated in patients with Opitz Syndrome (OS), which is characterized by malformations of the ventral midline, such as hypertelorism and hypospadias. Recently we have shown that the MID1-protein, a member of the RING finger protein family, has E3 ubiquitin ligase activity and targets microtubule-associated phosphatase 2A (PP2A), a central cellular regulator, for degradation. The target proteins of the microtubule-associated PP2A are not known so far. Based on a striking phenotypic similarity between patients with OS and patients with Greig syndrome we have defined the GLI3 transcription factor, mutations in which are responsible for the development of the Greig phenotype, as a putative target of microtubule-associated PP2A. Moreover, Ci, the drosophila homologue of GLI3, is a microtubule-associated protein, the intracellular localization and activity of which is regulated by serine/threonine phosphorylation. In immunofluorescence experiments we could show, that the intracellular localization of the GLI3-protein is regulated by the PP2A/MID1 complex. Dominant negative effects blocking ubiquitin specific modification of microtubule-associated PP2A resulted in a clear shift of the GLI3 localization, which could be blocked by specific inhibition of PP2A activity. In further experiments we showed, that the phosphorylation of human Fused, an interaction partner of cytosolic GLI3-protein, depends on the PP2A/MID1 complex. In drosophila, Fused plays an important role in maturation of the Ci protein. These results clearly show a functional interaction between the PP2A/MID1 complex and the SHH signaling pathway.

W14 04

Two phylogenetically conserved elements located 28 kb and 250 kb 5´ to SOX9 account for many aspects of endogenous Sox9 expression

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Campomelic dysplasia (CD), a human skeletal malformation syndrome with XY sex reversal, is caused by heterozygous mutations in and around the SOX9 gene. SOX9 has an extended 5´ control region as indicated by CD translocation breakpoints scattered over 1 Mb proximal to SOX9, and by expression data from mice transgenic for human SOX9-spanning YACs (Wunderle et al, 1998). By phylogenetic footprinting comparing the genomic sequences of the SOX9 regions of human and pufferfish, we had previously identified five conserved sequence elements, E1-E5, up to 290 kb 5´ to human SOX9, and had shown that 8 of 10 CD translocation breakpoints separate some or all of these elements from SOX9. To assay their regulatory potential, we examined these sequence elements in transgenic mouse lines. We found that elements E1 and E3 activate the lacZ reporter gene in a manner that accounts for many aspects of the endogenous Sox9 expression pattern. Specifically, element E1, located 28 kb 5´ to SOX9, directs a high level of reporter gene expression in the node, notochord, gut, pancreas and in the bronchial epithelium, while element E3, located 250 kb 5´ to SOX9, controls lacZ gene expression in the otic vesicle and in cranial neural crest cells. Conserved binding sites for HNF3ß and TCF/LEF1 within element E1 and element E3, respectively, indicate that SOX9 might be a direct target of these transcription factors in the respective tissues. No reporter gene expression could be detected with any of the elements in the chondrocytes, testis, brain, kidney and in the heart. Enhancers for SOX9 expression in these tissues still need to be identified.

W14 05

Many substrates and functions of ATM: Microarray analysis of ATMtm1Awb mutant mice

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Ataxia-telangiectasia is a rare, autosomomal-recessive inherited disorder with a complex clinical phenotyp. The gene responsible, ATM, encode a 370 kDa protein kinase. ATM belongs a familiy of proteins, conserved from yeast to human, that regulate cell-cycle checkpoints and are involved in DNA repair and recombination. This fits with the clinical phenotyp of ataxia telangiectasia; cells from patients show abnormal responses to ionizing radiation, Checkpoint alterations in cell cycle, and increased chromosomal breakage and telomere end fusions. But various other abnormalities in patients have raised the possibility that ATM may also be involved in other cellular processes. For a detailed insight about the functions of ATM we analysed the ATM mutant mice ATMtm1Awb with Affymetrix microarray technology. RNA for microarray analysis was isolated from total mouse brain of homozygous mice. Because C57Bl6 mice is the genetic background of the ATM mutant mice, we used this mouse strain as the baseline. Three 3 month-old males were analysed both for ATM-mice and the wildtyp-mouse strain with the Affymetrix U74A array analysing approximatly 12000 transcripts. For data analysis of the microarray results we used the Affymetrix software tools (MAS 5.0, MicroDB, DMT 3.0), the Internetportal of NetAFFX, and pathway software like GenMAPP. We found 154 genes differently regulated which we could divide into 12 functional groups. Only those genes, which were found in at least 7 of 9 comparisons similarly adjusted and with a signal log ration of more than 0.5, were defined as differently expressed genes. The results reflects the relativly large number of substrates and functions of ATM. We will present different biochemical pathways, which are dysregulated in ATM mutant mice to get more informations about the pathogenesis of ataxia telangiectasia diseases in humans.

W14 06

Alteration of bone density by mutations in key factors of the osteoclast acid secretion machinery

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ClC-7 is a ubiquitously expressed chloride channel that is mainly localized in late endosomes and lysosomes. Clcn7-/- mice show a severe osteopetrosis (marble bone disease) that becomes apparent shortly after birth. Although osteoclasts are present in normal numbers, they fail to resorb bone. In osteoclasts, ClC-7 is highly expressed in the ruffled membrane that is formed by the fusion of H+-ATPase-containing late endosomal vesicles. We could show that chloride ions conducted by ClC-7 provide the necessary countercharge to allow the ruffled border H+-ATPase to efficiently pump large amounts of protons into the resorption lacuna. The phenotype of Clcn7-/- mice closely resembles human infantile malignant osteopetrosis. A screening for mutations in the human gene, CLCN7, was performed in 18 patients suffering from this disease. Two patients were compound heterozygous for a nonsense mutation and two different missense mutations. A combination of two mutations lead to a complete loss of the ClC-7 protein in fibroblasts from one patient. Therefore, the most likely disease mechanism is a loss of function, which corresponds to the Clcn7-/- mouse model. The majority of recessive osteopetrosis cases, however, were positive for mutations in TCIRG1, which encodes the osteoclast-specific H+-ATPase a3 subunit. This proves that ClC-7 and the osteoclast proton pump are tightly linked in the resorption process. Recently, mu-

tations in CLCN7 were found to cause also the much milder autosomal dominant form of osteopetrosis (ADOII). Investigation of primary osteoclasts from several ADOII patients revealed that ClC-7 levels and subcellular localization are not detectably altered. Nevertheless, their resorptive activity is diminished. This implies that dominant mutations most likely perturb the ion channel function of ClC-7 and that alterations of the chloride conductance of the ruffled membrane are able to regulate bone resorption and bone density.

W15 01

SNP and haplotype analysis in 13 lipid metabolism relevant genes

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Aims: To study the association between SNPs and haplotypes in thirteen lipid metabolism relevant genes with plasma lipid levels. Genes & genetic diversity: We genotyped >90 SNPs and >20 microsatellite markers in and around genes coding for apolipoproteins (AI, AIV, AV, B, CII, CIII, E), ABC transporter A1 (ABCA1), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), hepatic triglyceride lipase (HL), low density lipoprotein cholesterol receptor (LDLR), and lecithin:cholesterol acyltransferase (LCAT) genes. Study cohort: 1054 individuals from 250 German families were genotyped and phenotyped for total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL). Statistical genetic analysis: Haplotypes were constructed based on nuclear family information. The genotype-phenotype relationship based on founder individuals, nuclear families, men and women, was studied using variance and regression based methodsResults: In an earlier study, in which we evaluated only 5 genes, we could explain a substantial part of the genetic variance. We have strong evidence that the additional genes also exert strong influence. While some genes contribute substantial influence on lipid phenotypes, e.g., ApoB, ApoE, CETP, ABCA1, ApoA5, other genes contribute little, e.g., LIPC, LPL, LDLR, ApoA1, ApoA4, ApoC2, ApoC3 or are invariable, e.g., LCAT. * both authors contributed equally to this work

W15 02

Meta-analysis of linkage genome scans for complex phenotypes with the example adult height

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Linkage genome scans are a popular approach to indentify genes underlying monogenic or complex traits. For complex traits that are influenced by several genetic and environmental factors, the power of the usual sample sizes is low and a single study rarely reaches statistical significance. For many common diseases, several genome scans from different groups are already published, often with conflicting results and seldom with replication of suggestive linkage findings. Meta-analysis is a way to combine all available information and achieve the necessary sample sizes for genes with smaller effects. Meta-analysis should account for differences among primary studies in sample size, family structures, study design, ascertainment schemes and others. Even though the phenotype height is not of clinical relevance per se, its extremes short stature or growth retardation are a common cause of pediatric concern. Adult height is a classic example of a polygenic quantitative trait, but segregation analysis has also yielded evidence of major genes regulating final height. To date, 8 genome scans for adult height have been published, comprising a total of more than 16000 individuals from population based samples or families selected for other phenotypes. Regions on chromosomes 5, 6, 7 and 9 show LOD scores greater than 2 in at least two scans, but in general there is no consistent replication of linkage regions. Using only published results and not individual genotype data, a meta-analysis of these scans is presented.

W15 03

DNA damage in peripheral blood lymphocytes detected by the Micronucleus test is a genetic marker for breast cancer risk

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Background: An increased micronucleus frequency compared to normal controls has been observed in irradiated peripheral lymphocytes of BRCA1 and 2 mutation carriers by the G0 micronucleus assay (G0MNT). The same observations have also been reported for a minor portion in sporadic breast cancer patients indicating a related cellular phenotype that predisposes for radiosensitivity in such breast cancer cases. Methods: We used the G0 MNT to analyze unselected sporadic breast cancer patients (n = 140) and controls (n = 149) by measuring total induced (2 Gy) MN frequency, frequency of cells containing two MN as well as spontaneous MN frequency with the 75th percentile of the controls as cutoff point. Twenty controls and 20 patients were tested prospectively using image analysis with the threshold obtained in the first analysis. Image analysis on a subsample of the patients proved to be superior over manual counting. Other cancer samples were investigated by the same approach. Results: Every single endpoint discriminates between patients and controls with an odds ratio (OR) of 5 to 10 ($p<10^{-4}$). Confirming results were obtained by the automated image analysis (OR = 22.9 p< .0001). A combined evaluation of the different endpoints demonstrated improved discriminative power. Furthermore, discrimination between breast cancer and controls was seen in prospective samples (OR=13.2 p=0.001). While prostate cancer patients did not differ from controls (p=0.7), ovarian cancer patients behave like breast cancer.Conclusions: The G0 MNT assay may be able to detect individuals having a risk for sporadic breast cancer. Our results demonstrate that this assay discriminates the susceptibilty for chromosomal instabilities in patients versus controls with high certainty. We hypothesize that the increased sensitivity for irradiation is caused by an intrinsic impairment of the DNA repair pathway and is genetically determined.

W15 04

Melanocortin-4 receptor gene polymorphism V103I protects from obesity as based on evidence from association and transmission disequilibrium studies and a meta-analysis

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Background: Many studies on obesity have analyzed the melanocortin-4-receptor gene (MC4R), which belongs to the melanocortinergic pathways controlling energy homeostasis and plays a role in the regulation of appetite and body weight. Several of the observed mutations are associated with obesity. For the missense variant V103I almost all study groups have reported lower frequencies of the I103 allele in obese individuals than in non-obese individuals but the sample sizes were inadequate for statistical analyses. In the context of a study on MC4R mutations (Hinney et al., J Clin Endocrinol Metab, 2003) we performed a TDT with 520 obese individuals and their parents, resulting in 10 transmissions and 25 non-transmissions for the I103 allele (p=0.017). The respective genotype relative risk of obesity for heterozygous carriers was 0.40 (95%CI: 0.17-0.86). Therefore, we initiated two new case-control studies, a meta-analysis, sequencing of the promoter region and further functional studies for this polymorphism. Results: Based on the TDT results, we decided to screen two large study groups and to combine all available information on obese and non-obese individuals in a meta-analysis. Our new studies comprised samples from the regions of Augsburg (KORA, 1976 non-obese and 383 obese individuals between 25 and 74 years) and Essen (489 obese and 172 non-obese individuals between 18 and 65 years). The two studies showed consistent results with odds ratios of 0.58 (95%CI: 0.29-1.16) and 0.69 (95%CI: 0.29-1.65), respectively. For our meta-analysis, we included all studies reporting carrier status in obese and non-obese individuals (total N=7738). Eleven of the 14 studies display a higher percentage of I103-carriers in the non-obese group. Meta-analysis results in an odds ratio for obesity of 0.69 (95%CI: 0.50-0.96) with a p-value of 0.03, providing evidence for a protective effect of the I103 allele against obesity. So far, in functional studies no differences were observed between carriers of the polymorphism and homozygous wild-type carriers and further studies of our group are still going on. Additionally, we sequence both the 5´ and 3´ non-coding regions in carriers of the I103-allele. The newly detected SNPs will be analysed to find out if a variant in

LD with V103I or a haplotype explains the negative association of the I103 allele with obesity.

W15 05

Genotypic association of LGI4 exonic polymorphisms and childhood absence epilepsy

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Autosomal dominant lateral temporal lobe epilepsy (ADLTE) is caused by mutations in LGI1 (leucine-rich repeat LGI family, member 1), the first gene in idiopathic epilepsies not coding for an apparent ion channel. We recently cloned several genes homologous to LGI1, demonstrating that LGI1 belongs to a new subfamily of leucine-rich repeat-proteins (Gu et al., FEBS Letters 2002). One of the new genes, LGI4, maps to 19q13.11, a chromosomal region linked to benign familial infantile convulsions (BFIC)(Giupponi et al., HMG 1997). The same region showed evidence for a susceptibility gene for idiopathic generalized epilepsy (IGE) in a genome scan including 130 IGE multiplex families (Sander et al., HMG 2000). In an extended sample of 176 IGE-multiplex families, the maximum LOD score under heterogeneity increased up to 3.25 at D19S414, assuming an autosomal recessive mode of inheritance and a proportion of 34% linked families (Sander et al., unpublished data). The predominant IGE subtype in the families supporting evidence for linkage was childhood absence epilepsy (CAE). These linkage findings indicate a susceptibility gene for CAE/IGE close to markers D19S414/D19S225, which define an interval of 2 cM that harbours the LGI4 gene. We therefore investigated whether genetic variation of the LGI4 gene confers susceptibility to BFIC and CAE. Screening of the LGI4 coding region in BFIC and childhood absence epilepsy (CAE) revealed several frequent exonic polymorphisms. A genotypic association was found for the c.1914GC/AT polymorphism in 42 CAE patients compared to 110 population controls (shi square = 6.66, df = 1, P = 0.01), providing evidence for a so far undetected susceptibility allele for CAE in the LGI4 region. The evidence is predominantly based on the increase of c.1914GC/GC homozygotes in CAE patients compared to controls. The excess of c.1914GC/GC homozygotes in CAE patients suggests an autosomal recessively acting susceptibility effect, which is supported by the original genome scan that indicated a susceptibility gene for CAE/IGE in the chromosomal region 19q13.1 under an autosomal recessive mode of inheritance 5. The differences in genotype frequencies are more obvious in the CAEfam sample than in the sample of sporadic CAE patients. This finding is consistent with the expectation that the genetic load and the chance for major gene effects are increased in CAE patients with familial IGE.

W15 06

Analysis of a complete genome scan in bipolar affective disorder after subdivision into early- and late-onset families

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Gene identification in common disorders such as Alzheimer disease and breast cancer has greatly profited from the use of age of onset as criterion to delineate subgroups of disease characterized by different inheritance patterns. In bipolar affective disorder, where the majority of linkage studies have produced conflicting results, studies reporting clinical characteristics and familial occurrence of disease have suggested that age of onset might serve as an indicator for identifying more homogeneous subgroups of disease. In a recent study, Grigoroiu-Serbanescu et al. (2001) examined this hypothesis in a large sample of bipolar I probands and their first- and second-degree relatives by the means of segregation analysis which they subdivided into an early onset (age of onset < 25 years) and late onset (age of onset > 25 years) group. Segregation analyses favored a model including a non-Mendelian major gene with a polygenic component in the early onset group while the data in the late onset group were compatible with a multifactorial model.Based upon these results, we re-analyzed our recently performed genome scan in a large sample of 75 families with bipolar disorder (Cichon et al., 2001). We subdivided the families in two early onset (<= 20y., <= 25y.) and two late onset groups (> 20y., > 25y.) and performed parametric and non-parametric linkage analyses. We regarded those linkage findings in the subgroups as interesting that were substantially higher or as high as results obtained from the analysis of the complete genome scan. In the early onset groups, the strongest signals were seen on chromosomes 13q (GENEHUNTER LOD > 4.0 for the <= 25y. group), 4p, 4q ,8p, and 19q (GENEHUNTER NPL scores of about 3.0 for the <= 20y. group). In the late onset groups, only region 11q gave a prominent linkage signal with an GENEHUNTER NPL score of >3.0. Thus, our results provide molecular support for the hypothesis that different genetic mechanisms may be involved in early and late onset forms of bipolar disorder.

W16 02

Survey of brain-expressed genes in a 7.3 Mb region on proximal Xp involved in non-syndromic X-linked mental retardation

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Severe mental retardation (MR) affects about 0.5% of the population in Western countries. Genetic disorders account for roughly two thirds of these cases, and 25% of these are thought to be due to mutations in X-linked genes. More than 130 different syndromic forms of X-linked mental retardation (S-XLMR) have been defined, and the molecular defect has been found in >30 of these. Non-syndromic XLMR (NS-XLMR) is more frequent than S-XLMR, but due to its extensive heterogeneity, elucidation of the underlying genetic defects has lagged behind. Analysis of linkage intervals in 125 families with NS-XLMR has shown that >30% of these gene defects are clustered on proximal Xp and in the pericentric region (Ropers et al. 2003). In this study we have employed DHPLC to screen 30 families with overlapping linkage intervals for mutations in 49 brain-expressed genes, all located within a 7,3 Mb segment of Xp11 flanked by ELK1 and ALAS2. So far, a total of 81different sequence variants were found in the coding regions and splice sites of these genes, and 16 of these were not found in >94 healthy male controls. In three genes, multiple mutations were seen which involved evolutionarily conserved or splice sites or gave rise to frame shifts, and in two other genes, single but apparently relevant mutations were found. Together, these mutations account for 12 of the 30 families examined. Screening of 20 additional brain-expressed genes from this region is in progress and may shed light on the molecular causes of up to one third of all cases with NS-XLMR.

W16 03

Screening for candidate regions prone to genomic rearrangements involved in non-syndromic X-linked mental retardation

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Genomic rearrangements play an important and hitherto underestimated role in the etiology of human genetic disease. Very often, such re-

arrangements are mediated by non-allelic homologous recombination (NAHR) between low-copy repeats (LCRs). Many of these LCRs appear to have arisen during primate speciation via paralogous segmental duplication. Analyses of the recently completed human genome sequence have shown that LCRs may account for 5% of the entire genome.In a recent study aiming at the detection of all causative mutations in families with non-syndromic X-linked mental retardation (NS-XLMR) that map to proximal Xp11, functionally relevant gene mutations were only found in 12 out of 30 families tested (see abstract Jensen et al.). While it is conceivable that some of the missing mutations hide in promoter and intronic regions which were not examined, some of these mutations may not be detectable by point mutation screening, such as many submicroscopic rearrangements due to NAHR between closely related repeated sequences.This has prompted us to screen the human X-chromosome for region-specific LCRs. Using the software REPuter (Kurtz et al, Nucleic Acids Res. 2001), we have compared the whole sequence of the X chromosome with itself and that of all other chromosomes. The genomic sequence was downloaded from ENSEMBL (www.ensembl.org). Setting the sequence parameters to a minimum length of 5kb and to a minimum identity percentage of 98%, respectively, we have found 435 duplication events on the X chromosome. Preliminary analyses suggest that the distribution of these duplications on the X is very similar to the distribution of mutations in families with NS-XLMR (Ropers et al, Trends Genet. 2003). However, some of the computationally identified duplicated segments may be artifacts resulting from the incompleteness and incorrect assembly of genomic sequences. Therefore, we have begun to verify these results by PCR amplification and sequencing of putative (direct) duplications. In these experiments, apparent heterozygosity in male DNA will indicate sites of sequence divergence between duplicated segments on the human X-chromosome and prove that the putative duplications are real.

W16 04

Expression and mutation analysis of PLXNB3, a candidate gene for neurologic disorders

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We characterized genomic structure, cDNA, and expression of PLXNB3 located on Xq28. PLXNB3 encodes neuronal adhesion molecule plexin B3 and is a promising candidate gene for X-linked neurological disorders (see also poster of C.Hartwig et al.). It spans more than 15 kb on Xq28, contains 36 coding exons, and displays a preferential expression in human and murine neuronal tissue. We performed PCR and SSCP analysis (SSCA) of the complete coding region of PLXNB3 in 191 male patients with suspected or demonstrated X-linked mental retardation (MR) including syndromic and non-syndromic MR. Each SSCA was run under two conditions (± 10% glycerol). In two patients we found a mutation in exon 10 (Glu670Lys) that, however, did not co-segregate with the phenotype. Furthermore, we found several unique single intronic and silent nucleotide changes in a few patients but not in controls. The significance of these changes remains unclear. In addition we found four intronic and eight exonic SNPs, some of the latter ones predicting an amino acid exchange. We identified 11 haplotypes based on five SNPs located in exons 9, 20, 27, 28, and 29 of PLXNB3 in 118 males. Four of the five SNPs predict changes of evolutionary conserved amino acids. This allowed us to determine the ancestral haplotype based on mouse PlxnB3 genomic and predicted protein sequences. These haplotypes will be suitable for association studies to investigate possible associations of structural changes in plexin B3 with quantitative neurological traits. [Supported by DFG, SFB444, C3; correspondence: finckh@uke.uni-hamburg.de]

W16 05

Evidence for implication of alphaPIX (ARHGEF6) in integrin-mediated signaling and cell spreading

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Non specific X-linked mental retardation (MRX) has been shown to result from mutations of genes encoding various components of the Rho signaling pathway. Previously, we reported that mutations in ARHGEF6, encoding the Rac1/Cdc42 guanine exchange factor alphaPIX, were found in patients with MRX. Rho GTPases, like Cdc42, Rac1, and Rho, participate in the reorganization of the actin cytoskeleton that leads to various forms of polarized outgrowth, including formation of filopodia, lamellipodia, and stress fibers. These small GTPases are also believed to be crucial for neuronal morphogenesis and connectivity. In an attempt to get insight into the biological function of ARHGEF6/alphaPIX and the upstream signaling cascades leading to its activation, we used the full-length coding region of ARHGEF6 as bait in yeast-two hybrid screens. The small regulatory subunit of both m- and µ-calpain, calpain 4 (CAPNS1), was identified as a novel binding partner. We confirmed the interaction by co-immunoprecipitation and GST pull-down experiments. By immunofluorescence, we showed that alphaPIX and calpain 4 co-localize at the cell periphery to lamellipodia and ruffles. Recent studies provide evidence that integrin-dependent cell adhesion to the extracellular matrix, e.g. to fibronectin, regulates the activity of Rho GTPases that finally leads to cell spreading. The protease µ-calpain has been implicated in integrin-mediated reorganization of the actin cytoskeleton and cell spreading. By overexpressing alphaPIX and calpain 4 in CHO-K1 cells and replating the cells on fibronectin to induce integrin-dependent cell spreading, we found that alphaPIX co-localizes with calpain 4 as well as with endogenous ILK at the leading edge of actively spreading cells. Inhibition of calpain in CHO-K1 cells caused a marked reduction in cell spreading suggesting that this integrin-dependent process is mediated by calpain. Expression of wild-type alphaPIX in the presence of calpain inhibitors still allowed the cells to spread suggesting that calpain acts upstream of alphaPIX during cell spreading. Taken together, we assume that calpain 4 provides a link between integrin-mediated signaling and activation of Rac1/Cdc42 through alphaPIX to induce reorganization of the actin cytoskeleton during cell spreading. It is tempting to speculate that the process of cell spreading in fibroblasts is similar in certain aspects to the sprouting and extension of neurites during neuritogenesis.

4. Posterpresentations

P 001

Genetic Instability in a Transgenic Mouse Model for Philadelphia Chromosome-Positive ALL

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Aims: The retrovirally induced expression of fusion proteins in human and mouse cells is a common tool to understand the development and progression of hematologic malignancies. Recently, it has been shown that chromosome aberrations, synthenic to those appearing in human leukemias, appeared in a double transgenic mouse model for AML M3. We therefore aimed to investigate a possible genetic instability and karyotypic evolution after transfection of BM185wt cells using Spectral Karyotyping (SKY) and fluorescence in situ hybridization (FISH). This mouse pre-B-cell line is characterized by the retrovirally induced expression of the p185 BCR-ABL fusion protein and thus represents an in vitro model for Philadelphia chromosome-positive acute lymphocytic leukemia (ALL).Methods: Chromosome preparations were made from BM185wt cells and two derivative cell lines. BM185wt cells were retrovirally transduced to express either GFP or ovalbumin-GFP, respectively. Hybridization with the SKY probe mixture was carried out according to manufacturer`s instructions (Applied Spectral Imaging). Image acquisition was performed with the Spectra cubeTM system and for analysis the SKY ViewTM software was utilized. To determine the quantity of cells with Der (12)T (XE?;12D), a FISH probe was generated from the BAC clone RP23-382J5, hybridizing to region F2 of the X chromosome. Results: Cytogenetic results of BM185 wild-type cells and transfected cells using SKY and quantification of cells carrying the Der (12)T (XE?;12D) by FISH analysis are shown in Table1.

Table1. Cytogenetic results of BM185 wild-type cells and transfected cells using SKY and quantification of cells carrying the Der (12)T (XE?;12D) by FISH analysis.

investigated cell lines: BM185wt41,
karyotype: XY,+5[8]//40-44,XY,idem,-12+18,+Der (12)T (XE?;12D),+mar[cp7]
percentage of cells with Der (12)T (XE?;12D): 15%

investigated cell lines: BM185GFP
karyotype: 41XY,+5[8]//41-43,XY,idem,+12+18[cp4]//37-41,X,-Y,-1,-2,+5,-8,+18[cp3]
percentage of cells with Der (12)T (XE?;12D): none

investigated cell lines: BM185ovalbumin-GFP
karyotype: 41,XY,+5[8]//39+40,X,-Y,+5[8]//36,XY,-1,+5,-7,-7,-9,-11.+Der (12)T (XE?;12D),-13,-15,+18[1]

percentage of cells with Der (12)T (XE?;12D): 12%

Conclusions: The chromosomal instability observed in this transgenic mouse model for Philadelphia chromosome-positive ALL might be induced by BCR-ABL expression. Our findings may help to find chromosomal regions involved in leukemogenesis and tumor progression.

P 002

Phenotypic variability in a family with familial adenomatous polyposis: consequences for genetic testing

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Introduction: Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome caused by germline mutations in the tumor suppressor gene APC. The penetrance of FAP was found to be close to 100% at the age of 40 years. Clinically this syndrome is characterized by > 100 colonic adenomas. If the polyps are not removed the risk of cancer increases up to 100%. Therefore, in case of polyposis prophylactic surgery of the colon is necessary. If colonic polyps are not detectable annual sigmoidoscopy is recommended up to the age of 40 years. Methods: Here we present a family with FAP showing a highly variable colorectal phenotype of FAP. Results: Two of three sisters presented with extensive polyposis of the colorectum, the classical feature of FAP. Genetic testing revealed the mutation c.3581C>G, S1194X in exon 15 of the APC gene. The third sister was referred for genetic counseling and risk assessment. She had had colonoscopy at the age of 35 years, no adenomas were detected. Four years later genetic testing revealed mutation carrier status. Afterwards a second colonoscopy was performed (at the age of 39 years) and three small adenoma with low grade dysplasia were found. Discussion and conclusion: This excessive intrafamiliar phenotypic variability could be due to modifying genetic factors. The family illustrates the importance of genetic testing in FAP families to evaluate the mutation carrier status. Exclusive clinical examination can cause false reassurance about cancer risk in patients and doctors. In addition, knowledge of the positive mutation carrier status may increase compliance with screening recommendations. Otherwise a negative mutation carrier status clearly rules out the necessity of long-term screening via endoscopy.

P 003

Consortium for Hereditary Breast and Ovarian Cancer (Deutsche Krebshilfe): A report of the Würzburg Center for the years 1996-2003

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In 1996, the Deutsche Krebshilfe established 12 Centers for Hereditary Breast and Ovarian Cancer in Germany. The major goal was to develop standards for interdisciplinary counselling, genetic testing, patient care and therapy in families with hereditary breast and ovarian cancer. The Würzburg Center brings together genetic counsellors, molecular geneticists, gynecologists and onco-psychologists. During the seven years of operation, a total of 422 individuals from 356 hereditary breast and ovarian cancer families requested consultation and of which 312 (264 families) were counselled in an interdisciplinary and simultaneous setting. A total of 301 counsellands were female, 11 were male. 118 persons were affected with breast and/or ovarian cancer. Approximately 60 % have been informed about the services in our Center by their doctors, another 19 % learned about our Center from the media. Questioned about their motivation for counselling, 42 % were especially interested in prevention, 23 % in a modification of their personal cancer risk, and 44 % were concerned about their offspring. After pedigree analysis, 226 of the families fulfilled the criteria of the Consortium for analysis in BRCA1 and BRCA2 and requested the testing. In 55 families (24 %) a disease-associated mutation was identified in BRCA1 or BRCA2. In 21 families (9 %) a so-called unclassified variant with unknown pathogenicity was identified. Out of the 55 families with a known BRCA1 or BRCA2 mutation, 31 healthy family members requested predictive testing. Conspicuous early onset of breast cancer in addition to additional cancers has led in two families to the testing of the TP53 gene. In each case, this revealed germline mutations in affected patients establishing a Li-Fraumeni syndrome in these families. Consequently, a close clinical surveillance modified from the protocol used for BRCA1/BRCA2-positive patients is strongly recommended.

P 004

Rad51 promoter variant 135G>C: increased Rad51 expression, but no general association with breast cancer

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The Rad51 promoter variant 135G>C is a sequence alteration of unknown functional significance that in two studies has been associated with breast cancer risk in BRCA2 mutation carriers (Levy-Lahad et al. 2001, Wang et al. 2001). In order to test whether this polymorphism is generally associated with breast cancer, we screened 120 patients with bilateral breast cancer, 120 patients with unilateral breast cancer, 120 random individuals, and a small group of 7 unselected male breast cancer patients for the 135G>C variation. No significant difference was observed for the allele frequencies between the breast cancer cohorts (0.06 and 0.08) and the comparison group (0.06). A non-significant trend towards a higher frequency of the rare allele was noted for the male breast cancer patients (0.43, n=7). In order to evaluate whether the 135G>C substitution results in an altered expression of Rad51, we established lymphoblastoid cell lines from three heterozygous carriers for functional studies. In all heterozygous lines, expression of the rad51 mRNA transcript harbouring the 135G>C substitution was increased about 2.5 fold compared to wild-type mRNA. In line with this observation, Rad51 protein was elevated 2-3 fold in cell lines from heterozygotes compared with non-carriers as judged from Western blot analyses using two different antibodies. To analyse the functional significance in vitro, reporter constructs were created encompassing nucleotides -543/+204 or -5/+204 of the rad51 gene to drive luciferase activity from the pGL3 vector. Using site-directed-mutagenesis positions +135 (G) and +172 (G) of the wild-type constructs were change to C and to T, respectively. Constructs were transiently transfected into U2-OS cell and luciferase activities were quantified. Luciferase activity of the reporter constructs 135-c and 172-t was significantly elevated compared to wild-type. We conclude that the Rad51 promoter variant 135G>C is indeed a functionally relevant substitution but its oncogenic impact for breast cancer appears to be low and may require concomitant BRCA2 inactivation.

P 005

A frequent splicing mutation of XRCC4 : implications for breast cancer ?

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Mutations of the canonical acceptor and donor splice dinucleotide signals are a frequent cause of inherited disease and are usually considered to be pathogenic. We report on the identification of a frequent splicing mutation of the DNA repair gene XRCC4 („X-ray cross-complementing gene 4“) that occurs at polymorphic frequencies in several populations. The mutation, IVS7-1G>A, abolishes the conserved acceptor site of the last intron in the XRCC4 gene and presents with an allele frequency of about 1 in 10. Transcript analyses demonstrated the exclusive activation of a cryptic acceptor site within the last exon in carriers of the IVS7-1G>A mutation. This aberrant splicing gives rise to a shorter XRCC4 protein isoform that is expressed at wildtype levels in lymphoid cells from individuals homozygous for the IVS7-1G>A allele. Because the XRCC4 protein has a central function in non-homologous end-joining, and deficiency in DNA double strand break repair is thought to be a hallmark of breast cancer, we examined the allele and genotype frequencies of the splicing mutation in breast cancer patients and in random individuals from three distinct populations of German, Byelorussian and Saudi Arab origin. An excess of homozygotes for the IVS7-1G>A allele was found among 500 German breast cancer cases compared with 400 German random donors (OR= 4.1, $p<0.05$). The same tendency was noted in smaller series of Byelorussian breast cancer patients (n=136) and Saudi Arab breast cancer patients (n=36), yielding a combined crude odds ratio of OR=2.8 (95% CI 1.1-7.5) for all three cohorts. In summary, these results identify IVS7-1G>A as a common XRCC4 splicing mutation in diverse ethnicities and suggest that homozygosity for this allele could play a role in the genetic predisposition towards radiation sensitivity and breast cancer.

P 006

The CHEK2*1100delC mutation: inherited breast cancer susceptibility with low penetrance

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The CHEK2 gene encodes a cell cycle checkpoint kinase that modulates the radiation-induced DNA damage response. A frameshift mutation, 1100delC, abolishes the kinase function and has been proposed in two recent studies to underlie a substantial fraction of familial breast cancer (Meijers-Heijboer et al. 2002, Vahteristo et al. 2002). We have investigated whether this mutation may account for some proportion of cases in a large hospital-based series of unselected Lower Saxonian breast cancer patients. Using a newly established ARMS-based screening assay, we identified the 1100delC allele in 11 out of 985 breast cancer patients (1.1 %) compared with 1 out of 400 random controls (0.3 %; crude OR= 4.5, 95% CI 0.7-29.3). The median age at diagnosis was 67 years in the 1100delC heterozygotes compared with 58 years in the total series. None of the 1100delC carriers had bilateral breast cancer. Two of the eleven carriers reported a first- or second-degree relative with breast cancer. There were seven cancers of other sites in the first-degree relatives of the 1100delC carriers. The relatively low proportion of cancers in close relatives and the late age at diagnosis in the patients are inconsistent with the previous postulate of this mutation as causing Li-Fraumeni syndrome (Bell et al. 1999). In conclusion, our study corroborates the nature of the CHEK2*1100delC mutation as a breast cancer susceptibility allele in the German population but the data also indicate that its penetrance is low.

P 007

Different spectra of somatic mutations in different NF1 patients - implications for the search of genetic modifiers of the disease

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Neurofibromatosis type 1 (NF1), an autosomal dominantly inherited disorder, is mainly characterized by the occurrence of multiple dermal neurofibromas and is caused by mutations in the NF1 gene, a tumor suppressor gene. The variable expressivity of the disease and the lack of a genotype/phenotype correlation prevents any prediction on the patients´ outcome and points to the action of genetic factors modifying the severity of the disease. The analysis of somatic NF1 gene mutations in neurofibromas from NF1 patients revealed that each neurofibroma results from an individual second hit mutation, a fact showing that factors which influence somatic mutation rates can be regarded as potential modifiers of NF1. Here we present the results of a mutational screen of numerous neurofibromas from two NF1 patients, a mother and her daughter, both severely affected with hundreds of neurofibromas. In 13 of 33 tumors analysed the second hit mutation could be detected. Three tumors showed LOH in the NF1 gene region and ten tumors small mutations. The predominance of point mutations, small deletions and insertions as second hit mutation demonstrated in the two patients, together with the results of studies, which showed LOH as predominant second hit in neurofibromas of other patients, provide further evidence that in different patients different factors may influence the somatic mutation rate and thereby the severity of the disease. Our findings have a direct impact on the search strategies for genetic variants modifying tumorigenesis in NF1 patients because they suggest a subdivision of the patients according to their predominant type of second hit mutations prior to further analysis.

P 008

Familial adenomatous polyposis: Phenotypes in patients with APC splice site mutations

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Familial adenomatous polyposis (FAP) is an autosomal-dominant colorectal cancer predisposition syndrome caused by germline mutations in the APC tumour suppressor gene. A consistent correlation between site of mutations in the APC gene and clinical phenotype has been reported in FAP. However, in the case of splice site mutations not only the location within the gene but also the relative position within the splice site has to be considered. Among 448 germline mutations detected in 917 unrelated FAP patients we identified 30 base exchanges in or adjacent to splice site sequences. While mutations in the highly conserved positions of the splice sites (AG/GT) are known to destroy the splice site, the effect of variations at the less conserved positions on splicing and on the phenotype are not well known. Change in splicing efficiency can be evaluated by use of splice site prediction programs (e.g. http://www.fruitfly.org/seq_tools/splice.html) and experimentally examined by mRNA analysis. For example, a mutation at the splice donor site of intron 9 of the APC gene (IVS9+3A>G) detected in a patient with attenuated FAP results in a predicted reduction of splicing efficiency from 0.93 to 0.4; by mRNA analysis a partial deletion of exon 9 was proven. In contrast, the mutation IVS9+5G>A (in a patient with typical FAP) almost completely abolishes the splice donor site (to 0.07) and results in a complete deletion of exon 9. We will present another 22 mutations detected at different positions of the splice sites and correlate them with the splicing efficiency predicted by computer programs and the clinical phenotype. This data will contribute to the knowledge on genotype-phenotype correlations in FAP.Supported by the Deutsche Krebshilfe

P 009

Germline Mutations in the von Hippel-Lindau Tumorsuppressor Gene

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Von Hippel-Lindau disease (VHL) is a tumor susceptibility syndrome based on germline mutations in the VHL tumor suppressor gene. It is characterized by a variety of benign and malignant tumors, including renal cell carcinomas, cerebellar and retinal hemangioblastomas, pheochromocytomas and other tumors and cysts.We report on the germline mutations in two German unrelated VHL families. The whole coding region of the VHL gene was sequenced. This sequencing revealed signals appearing as a homozygous point mutation for one member of family A. Whether this result is an effect of a homozygous point mutation or a heterozygous mutation on one allele accompanied with an intragenic deletion on the other was tested applying FISH analysis. FISH with probes for the chromosomal region 3p25-p26 including the VHL gene showed no loss of allelic signals. Homozygous mutations in the germline have not been identified so far. As homozygous germline deletion in the mouse model have been proven to be lethal, further studies are on the way to verify the homozygous nature of the mutation in the patient. Family B showed the mutation G810A (predicted to cause the aminoacid exchange R200Q) previously described and an additional DNA alteration not reported yet: G707C (V166L). So far, it is not clear whether this is a polymorphism or whether a compound heterozygosity causes the disease.

P 010

Identification of genomic rearrangements in the BRCA1-gene by MLPA- method in cases of familial breast-cancer

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Large genomic rearrangements in one of the known breast cancer genes (BRCA1/2) can cause hereditary breast cancer. So far only time-consuming and expensive methods such as Southern Blotting were available for the identification of such mutations. We now evaluated a new PCR-based method, multiplex ligation dependent probe amplification (MLPA), for their detection.MLPA analyses (MRC Holland, Amsterdam) were carried out on genomic DNA of index patients from 75 high-risk families. Previous analyses of BRCA1/2 using dHPLC and sequencing had failed to detect disease-causing mutations. For the quantitative analysis of the BRCA1 gene, 25 exon-specific probe pairs with integrated universal primer sequences were applied in one single probe mix, specifically ligated and multiplex-amplified with fluorescently labelled universal primers. The results were visualised on a DNA sequencer (ABI 3100). To validate the method, two control samples containing a duplication of exon 13 and a deletion of exon 22 were included.We found previously described deletions of exons 1a-b and 2 in two

families and of exon 17 in another family. Mutations were confirmed in the two control samples. Duplications or deletions detectable by the MLPA method were excluded in the remaining patients. The recently published MLPA- technique is a rapid and cost-efficient method for the detection of genomic rearrangements in hereditary breast cancer. The frequency of such mutations in our high-risk patients studied was lower (3/75= 4%) than reported in other studies (up to 30%) (Montagna et al., Hum Mol Genet 12, 2003, 1055-1061).

P 011

Impact of endoscopic surveillance and prophylactic surgery in German Patients with Familial Adenomatous Polyposis

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Background: Familial adenomatous polyposis (FAP) is an autosomal-dominant condition characterized by the occurrence of hundreds to thousands of colorectal adenomas. If adenomas are left untreated, colorectal cancer (CRC) will develop in virtually all cases. Endoscopic surveillance and prophylactic surgery have been shown to improve the outcome of FAP patients by preventing death due to colorectal cancer. In Germany, the implementation of the recommended surveillance procedure in FAP patients and relatives at-risk as well as the impact of the screening procedures on survival has not been evaluated systematically so far. Methods: The presented retrospective study includes patients with definite or suspected diagnosis of FAP. Clinical data and family history are obtained from a questionnaire answered by the participants, completed by medical records and personal telephone interviews. Results: Until now, 266 patients from 165 unrelated families were investigated (181 probands, 69 call-up cases, 16 patients diagnosed by chance). The mean age at diagnosis in the probands was 34 years (range 4-70) versus 23 years (range 6-51) in the call-up group. 39 % of the probands had CRC at the time of diagnosis versus 8 % of the call-up patients. The stage of CRC was significantly earlier in the call-up group (Dukes A: 61 %) than in the probands (Dukes A: 36 %). The mean age at colectomy was 35 (range, 7-70) years in the probands and 27 (range, 7-53) years in the call-up group. 86 % of call-up cases had undergone preoperative screening colonoscopies at regular intervals, 14 % only sporadically. The frequency of postoperative surveillance (rectoscopy, pouchoscopy) varied: In 77 % it was performed at regular intervals, in 9 % sporadically and in 11 % no surveillance took place. Prophylactic duodenoscopies were performed in regular intervals in 52 %; duodenal polyps were detected in 76 % of these patients. The cumulative colorectal cancer risk and the cumulative survival of the patients without surveillance compared to those who underwent regular screening examinations and to the general population will be presented. Conclusions: Our results indicate that prophylactic screening examinations increase early diagnoses and well timed surgery in FAP patients, leading to an improved prognosis due to prevention of CRC. The screening examinations recommended for FAP have been implemented incompletely until now, although the intensity of pre- and postoperative surveillance has improved during recent years.

P 012

Familial Pancreatic Cancer in Germany - An Update

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Pancreatic cancer (PC) is the fifth leading cause of cancer-related mortality with a very poor prognosis. More than 90% of pancreas cancers are ductal adenocarcinomas. Epidemiologically, cigarette smoking is a well established risk factor. It is estimated that 3-5% of PC-cases are caused by a genetic predisposition. An 18 to 53-fold risk has been reported among first degree relatives of PC patients in pancreatic cancer families (FPC). To investigate the inherited components in PC we started to enroll families with FPC and with pancreatic cancer-melanoma syndrome (PCMS) in the German Familial Pancreatic Cancer Case Collection (FaPaCa). In a total of 42 families the spectrum of cancers and diseases was analysed. Similar to previous studies hereditary PC appears to be exceedingly heterogeneous, as evidenced by its association with other diverse cancers. In our collection, a combination of PC and breast cancer (BC) was found in five (11,9%) families. Up to date, BRCA2 germ-line mutations have been identified in two out of 14 FPC-families. In both families BC in addition to PC was present. PC and malignant melanoma was observed in nine (21,4%) families. A CDKN2A germline mutation was found in 2 of 5 PCMS and in none of 31 FPC families. Both families with CDKN2A mutation did not show the FAMMM phenotype. In accordance with previous studies we confirmed the association of a CDKN2A mutation with PCMS. A fourth family with the joint occurrence of PC and BCC was identified which provided further evidence of a possible basal cell cancer-pancreas cancer syndrome. Molecular investigations in the PTCH gene of these families have been initiated. In an ongoing study additional PC and PCMS families will be collected. High risk family members including those with germline mutations of the CDKN2A or BRCA2 gene will be offered surveillance programs for early detection of PC to reduce the mortality of this cancer in these individuals. Long term observation of high risk individuals will help to assess the PC risk for members of PC and PCMS families. Supported by the Deutsche Krebshilfe, grants 70-2362-Ba2 and 70-2828-Ba3

P 013

Increase of copy number changes with age at operation in unilateral isolated retinoblastomas point to different genetic pathways of tumor progression

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Retinoblastoma (RB) is the most common intraocular tumor in childhood with an incidence of 1in 18000 live births. Mutational inactivation or loss of both RB1 alleles on chromosome 13q14 are a prerequisite for development of all RB, but further genetic alterations are required. Additional chromosome abnormalities have been described in RB in cytogenetic and comparative genomic hybridisation (CGH) studies. However, their role in progression of RB has not yet been clarified in detail. To investigate the genetic pathways of RB progression we studied chromosomal imbalances in unilateral isolated RB with known mutational status at the RB1 gene. A consecutive series of 60 patients with ages at operation (AO) ranging from 2-91.7 months (median 16.6) was analysed. All tumors were of similar sizes. CGH analyses was successful in 57/60 patients. In 41 of 57 tumors chromosomal imbalances were found. The average copy number change (CNC) per abnormal sample was 4.4. Gains at 6p (23/57), 1q (20/57), and losses at 16/16q (16/57) were most frequently observed thus confirming previous reports. However, when CNCs were correlated with age at operation, a steady increase of CNCs with age was observed. In patients with AO <12 months less than 2 CNC/tumor were found while patients with AO >40 months showed more than 2 CNC/tumor. Moreover, gains at 1q and losses at 16/16q were restricted to patients with AO >20 months. Retinal development and retinoblast differentiation progress from central (macula) to peripheral (ora serrata). Thus, in children of younger ages the proportions of retinoblasts and of differentiated retinal cells is different from that in older children. The observed increase of CNCs in unilateral isolated RB with age may, therefore, reflect differences in genetic pathways of tumor progression depending on different differentiation stages and/or location. Funded by the DFG, Ri-1123/1-1

P 014

A severe case of Opitz Trigonocephaly (C) syndrome

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The Opitz trigonocephaly or C syndrome is clinically characterized by trigonocephaly with a prominent metopic crest and lateral frontal depression, unusual oro-facial features like prominent alveolar ridges, limb defects, visceral anomalies, and severe psychomotor retardation.We report on a newborn boy of an unrelated healthy German couple. Pregnancy was complicated by gestational diabetes and polyhydramnion in the third trimester. Amniocentesis revealed a normal karyotype, which was eventually confirmed postnatally. The patient was born at 38 weeks of gestation by spontaneous vaginal delivery. Clinical examination showed trigonocephaly, upslanting palpebral fissures, a broad and flat nasal bridge, anteverted nares, deep set dysplastic ears, a high arched palate and thick anterior alveolar ridges. Furthermore, a short neck,redundant skin, hypoplastic distal phalanges of the fifth fingers, transverse palmar creases, right-sided cryptorchism, marked hypotonia, and a low pitched, hoarse voice were found.Seizures during the first days of life were treated with Phenobarbital. Echocardiography showed atrioventricular septum defect while

brain sonography revealed cerebellar hypoplasia and cavum septi pellucidi cysts. The patient died at the age of nine months inspite of successful surgery of the heart defects at the age of three months.While Opitz trigonocephaly syndrome has probably been overreported, we report here a case of trigonocephaly with additional findings typical of Opitz trigonocephaly syndrome. We discuss the clinical variability of this syndrome by comparing this case with reports in the literature.

P 015

Hyperostosis in two siblings due to a SOST gene mutation

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We report on a 42 year old woman and her 40 year old brother suffering from a rare hyperostotic disease. Both have been misdiagnosed as autosomal dominant Osteopetrosis type II (Albers-Schönberg disease) for a long time. First symptoms occured at the age of approximately four years and consisted in a peripheral facial palsy which at first was unilateral but later involved both sides. Additionally, both sibs developed hearing loss due to a mechanical alteration of the nervus acusticus. Residual hearing capacity is about 50% in the female patient. Her brother is nearly deaf on one side and shows only little residual hearing on the other ear although repeated decompression surgery was performed. Moreover, a progressive thickening of the cranial bones occured with a predominant mandibular overgrowth. Until now, there are no signs of an increased intracranial pressure. Teeth were normal in the female and irregular in shape and position in the male patient. Both patients never experienced a peripheral bone fracture and no impairment of bone marrow function is described. No syndactyly, as frequently observed in sclerostosis, is present in both patients. Concerning family history, no other affected individuals in the family are known. Two further sibs and the daughter of the female patient are unaffected. The parents are not consanguineous and of german origin, although van Buchem disease has mostly been described in Dutch patients. The diagnosis of osteopetrosis type II has been made early in childhood and has never been questioned. Clinical features such as very similar clinical course in two sibs and the lack of any peripheral fractures were in favour of an autosomal- recessive form of a genetic hyperostotic disease. The most probable clinical diagnosis was van Buchem disease or the genetically closely related sclerostosis. Molecular analysis revealed a homozygous splice site mutation /IVS1 + 1 G-C) in the SOST gene in both patients.

P 016

A case of Noonan-like/multiple giant cell lesion syndrome with a new clinical feature and without PTPN11 mutation

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Noonan-like/multiple giant cell lesion syndrome (OMIM 163955) is a very rare disease with less than 20 cases published in the literature. It is characterized by clinical features of Noonan syndrome associated with giant cell lesions of bone and soft tissue. There is obvious clinical overlap between both syndromes, but it is unclear whether they are distinct entities or not.We report on a 19 year old female with Noonan-like/multiple giant cell lesion syndrome with: a hight of 160 cm (10th. perc.), typical craniofacial dysmorphism, prominent jaws, webbed neck, pulmonary valve stenosis, shield chest and pectus excavatum, scoliosis, moderate mental and hearing impairment. X-ray examinations revealed multiple cystic lesions of the jaws and of the phalanges. Histopathologic examination of tissue from a maxilla lesion showed giant cell granuloma. Moreover, she was born with an anal atresia and recto-vaginal fistula – malformations not described so far in patients with Noonan-like/multiple giant cell lesion syndrome.Mutations in the PTPN11 gene are responsible for about half of the individuals with Noonan syndrome. Until today only one family with Noonan-like/multiple giant cell lesion syndrome has been analysed and shown to have a PTPN11 mutation. We could exclude this particular mutation in our patient. DHPLC analysis of the whole coding sequence of the PTPN11 gene detected no mutation. Thus, it remains unclear whether Noonan syndrome and Noonan-like/multiple giant cell lesion syndrome are allelic disorders or not

P 017

In vivo splicing experiments suggest that specific mutations in NF1 exons 4B, 30 and 37 affect exonic splice enhancers

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cDNA based mutation detection analysis reveals that the most common molecular defects in NF1 patients affect mRNA splicing. Among these a high frequency of unusual splicing defects that do not affect the consensus splice sites have been uncovered. Mutations within exonic but also intronic sequences are found to create novel splice sites. Furthermore, nonsense, missense as well as silent mutations have been shown to lead to exon skipping. Several models have been proposed to explain mutation-associated exon skipping. The secondary-structure disruption model applies to pre-mRNA in which local RNA secondary structure is required to promote exon inclusion. In the exonic splicing enhancer (ESE)-disruption model, the mutation disrupts the recognition motif for an RNA-binding protein that enhances splicing, such as a serin/arginerich (SR-) protein and exon inclusion is no longer favoured. In a cohort of minor NF1 lesions identified in the Austrian and the Belgium laboratory one missense and 3 nonsense mutations were associated with obvious exon skipping in the patient samples. Using minigene constructs we now tested inclusion of the wildtype and mutated sequences of the three affected NF1 exons in in vivo splicing assays. Our results confirmed mutation associated loss of the exons also in this hybrid minigene context. To test whether the ESE-disruption model would apply for the NF1 mutations found in our cohort of mutations we analysed the exonic sequences using two ESE prediction programs available on the world wide web, i.e. ESE finder developed by Cartegni L. et al. and RESCUE-ESE by Fairbrother WG et al. These analyses predict that the mutations we analyzed by in vivo splicing assays destroy SR protein binding sites. To further ascertain the splicing enhancer activity of the predicted ESE sites disrupted by the NF1 mutations we are currently assessing their ability to rescue splicing of the splicing enhancer dependent SXN exon 2. Sofar our results strongly suggests that disruption of in cis acting exonic splice enhancer sites are responsible for the nonsense and missense mutation associated exon skipping observed in our NF1 patients.

P 018

Clinical study with valproic acid in SMA carriers

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Recently, our research group discovered valproic acid (VPA) to be able to elevate full-length (FL) SMN2 RNA andprotein levels in fibroblast cell cultures from spinal muscular atrophy (SMA) patients by transcription activation andrestoration of the correct splicing pattern. Since VPA is an FDA approved drug, successfully used in longtimeepilepsy treatment revealing only rare and mild side effects, this observation opens the realistic chance for a firstSMA therapy. Thus, our group started a drug treatment study with VPA in 12 parents of SMA patients. Thoseprobands possess only one SMN1 copy and 1-3 SMN2 copies (2x 1 copy, 7x 2 copies, 3x three copies). During theclinical protocol, blood samples of the probands are taken every 14 days. After isolation of total RNA, real-timePCR analysis is performed to determine FL and truncated SMN RNA using GAPDH as an internal standard. SMNprotein levels are quantified by flow cytometry analysis of freshly isolated peripheral blood mononuclear cells.To achieve base line values of SMN RNA and protein, the first three blood analyses will be carried out while theprobands receive no medication. Then, medication of the probands is started with 300 mg VPA per day. Eachweek, the dose is increased by 300 mg up to the maintenance dose of 1200-1800 mg per day to reach a bloodlevel of 70-100 µg/ml VPA (blood level of epilepsy treatment). Blood levels of FL-SMN / truncated RNA and proteinwill be measured three times under the maintenance dose. Values under VPA treatment will be compared withthe respective base line levels. In case VPA is able to clearly up-regulate FL-SMN RNA and SMN protein levels in the carriers, a clinical trial with SMA patients will be performed afterwards.

P 019

Altered Ratio of WT1 Splice Isoforms in Denys-Drash Syndrome - Stop in Kidney Development?

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WT1 encodes a zinc finger transcription factor that is required for urogenital development. Two alternative splice sites lead to four isoforms with different functions, thought to be developmentally regulated. One of them involves the presence or absence of 17 amino acids encoded by exon 5 and the other three amino acids (KTS) between the third and fourth zinc finger. Germline missense mutations result in Denys-Drash syndrome (DDS) characterized by a glomerulopathy, male-to-female intersex disorders and a predisposition to Wilms tumor. The aim of our study was to investigate whether a WT1 missense mutation leads to an abnormal glomerular differentiation as a first step of the progressive kidney disease. For this we have used the ratio of the four WT1 isoforms and the relative WT1 mRNA amount as a marker for the glomerular differentiation stage. After microdissecting glomeruli from whole kidney sections we have performed real-time RT-PCR to quantify the four isoforms in four normal adult kidneys, two fetal kidneys and four kidneys of DDS patients. We could found a developmentally increase of the isoform lacking exon 5 but containing KTS (-/+) and a decrease of the isoform containing exon 5 and KTS (+/+). The changes were gradually seen between the different stages in fetal kidneys from 16 weeks and 24 weeks of pregnancy and in adult kidneys. The relative quantification showed that WT1 is higher expressed in adult than in fetal kidneys. Interestingly, kidneys of DDS patients showed a ratio of WT1 isoforms and a relative amount of WT1 mRNA similar to fetal kidneys. They corresponded to a stage between the 16 and the 24 week of gestation. In conclusion, this demonstrates that the Denys-Drash syndrome is in part caused by a failure of a normal glomerular differentiation.

P 020

Tissue-specific expression patterns of the RAGE receptor and its soluble forms

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The receptor for advanced glycation end products, RAGE, is known to be causally involved in a variety of pathophysiological processes, e.g. immune/inflammatory disorders, Alzheimer disease, tumors, and abnormalities associated with diabetes as arteriosclerosis or disordered wound healing. So far, two human cDNAs have soluble form lacking the transmembrane domain and the cytosolic domain. Apparently the latter form represents a naturally occurring inhibitor of signalling pathways induced by the membrane-standing RAGE receptor. In order to perform a quantitative expression analysis of both RAGE forms, we designed an RT-PCR experiment allowing the simultaneous amplification of corresponding transcripts. As a first result, we were able to identify and characterise three novel human RAGE transcripts all encoding truncated soluble forms of RAGE designated as sRAGE1, sRAGE2, and sRAGE3. Including these transcripts into the expression analyses, the ratios for the full-length RAGE transcript to the sum of its splice-variants encoding the soluble variants varied from 0.56 to 1.72 among the tissues tested. Therefore, the pre-messenger-RNA of RAGE must be subject to extensively regulated alternative splicing activated by extracellular cues of yet unknown cellular signalling pathways. Thus, as deduced from the occurrence at the RNA level it can be hypothesized that there is a complex RAGE regulation network involving isoforms competing for the binding of ligands.

P 021

In NF1, CFTR, PER3, CARS and SYT7, alternatively included exons show higher conservation of surrounding intron sequences than constitutive exons

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It is still not fully understood to what extent intronic sequences contribute to the regulation of the different forms of alternative splicing. We are interested in the regulation of alternative cassette exon events as exon inclusion and exon skipping. We investigated these events by comparative genomic analysis of human and mouse in five experimentally well characterized genes, NF1, CFTR, PER3, CARS and SYT7. In NF1, high intron identity around the 52 constitutive and four alternatively skipped NF1 exons is restricted to the close vicinity of the exons. In contrast, we found on average high conservation of intron sequences over three hundred base pairs up- and downstream of the five alternatively included NF1 exons. The investigation of alternatively included exons in CFTR, PER3, CARS and SYT7 supported this finding. In contrast, the mean intron identities around the alternatively skipped exons in CTFR and NF1 do not differ considerably from those around the constitutive exons. In these genes, the difference in intron conservation could point to a difference between the regulation of alternative exon inclusion and alternative exon skipping or constitutive exon splicing. Additional genome wide investigations are necessary to elucidate to what extent our finding can be generalized.

P 022

Population specific distribution of mutations in the cystinuria genes SLC3A1 and SLC7A9

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Cystinuria is an inherited disorder of defective reabsorption of cystine and dibasic amino acids in the kidney. Excessive urinary cystine results in the formation of renal cystine stones. The disease is caused by a defective heterodimeric amino acid transporter in the renal tubules, encoded by the genes SLC3A1 and SLC7A9. Mutations in SLC3A1 are responsible for the fully recessive Type I cystinuria while mutations in SLC7A9 are mainly associated with the incomplete dominant non-Type I disease. The biochemical classification of both subtypes is routinely carried out by urinary amino acid excretion patterns of the obligate heterozygous parents.Several studies indicate a population specific distribution of genetic variants in the genes SLC3A1 and SLC7A9. We therefore evaluated the occurrence of specific mutations in a cohort of 59 cystinuria patients in relation to their ethnic origin. The patients were screened for mutations in both genes by SSCP and quantitative real-time PCR. Patients originated from middle, southern and south-eastern Europe. In total, we could identify mutations in 64.7% of chromosomes. In the SLC3A1 gene, T216M was the most frequent mutation in the population of south-eastern Europe (50%) while it could only be detected in 12.1% of the total cohort. M467T was detected in 17.1% of the group of middle Europe, in the total cohort it accounts for 12.1% of mutations. The recently identified duplication from exons 5 to 9 in SLC3A1 could be detected in 14.3% of chromosomes in middle Europe and in 8.6% of the total patient cohort, respectively. In the SLC7A9 gene no population specific distribution of mutations can be observed. Nevertheless, G105R is the most frequent mutation in SLC7A9 with a frequency of 6.9%.To sum up, a restricted mutation analysis considering the patients ethnic origin is useful in routine diagnostics and allows the detection of mutations in almost 80% of patients.

P 023

Genetic variants in Silver-Russell syndrome: up-date and applications for genetic diagnosis

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Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterised by severe intrauterine and postnatal growth retardation (IUGR/PGR), small triangular face, clinodactyly V, relative macrocephaly and other less constant features. The syndrome is heterogeneous and various genetic findings have been associated with SRS. However, only chromosomes 7 and 17 are consistently involved in SRS. A subset of 7-10% of SRS patients shows complete or segmental maternal uniparental disomy of chromosome 7 (upd (7)mat); additionally, five SRS patients have been described carrying rearrangements in 7p. Thus, a central role of chromosome 7 in the etiology of SRS can be delineated, while the contribution of chromosome 17 is uncertain. Disease causing mutations in genes on chromosomes 7 and 17 have not yet been identified. Nevertheless, the following diagnostic procedure should be offered routinely to elucidate causative genetic factors in SRS: After exclusion of chromosomal aberrations patients with the typical clinical signs of SRS (severe IUGR/PGR <P3, triangular face, clinodactyly V, relative macrocephaly, asymmetry), should be tested for upd (7)mat, using microsatellite markers localised in the candidate regions 7p12-p14 and 7q31-qter. To exclude cryptic deletions or duplications in 7p12-p14 escaping the aforementioned techniques, we now established a real-time PCR approach based on TaqMan technology. We thereby quantified the copy number of GRB10 in 50 SRS patients, a gene in 7p which has almost been affected by the chromo-

somal disturbances reported for this region. By testing DNA of a patient with a duplication in 7p we could demonstrate the reliability and robustness of this test.

P 024

Analysis of mutations in the epsilon-sarcoglycan gene in an unselected group of patients with different subtypes of dystonias

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Introduction: Primary dystonias are a clinically and genetically heterogeneous group of movement disorders. At least 13 different gene loci are reported for the different subtypes of dystonia, some of them associated with a characteristic phenotype, however with a widely overlapping spectrum of phenotypic expression. Mutations in the epsilon-sarcoglycan (SGCE) gene have recently been found to cause myoclonus dystonia.This term refers to a specific subtype of dystonia characterized by involuntary jerks and dystonic movements of variable expression. Although myoclonus-dystonia is thought to be a distinct disease entity from idiopathic torsion dystonia a widely overlapping spectrum of phenotypic expression of these dystonia subtypes is reported. Considerable clinical variation of SGCE mutation carriers and the variable expression of myoclonus and dystonia seen in different myoclonus-dystonia families leads to the suggestion that mutations in the SGCE gene might be detected also in patients with other subtypes of dystonias, especially in those involving the neck (torticollis) or the upper limb (writer´s cramp).Objective: To evaluate the contribution of mutations in the SGCE gene in patients with different subtypes of dystonias and to determine the phenotypic variability we analyzed the coding sequence of the SGCE gene in an unselected group of 298 patients of different movement disorder outpatient clinics using dHPLC (WAVE DNA Fragment analysis system). This information might be helpful for the appropriate application of DNA diagnostic testing and for genetic counselling.Results: We could not detect any mutation in the SGCE gene in this cohort of patients. However, we were able to detect one published and three unknown polymorphisms in Intron 1,3,4 and in Exon 2 (silent mutation), indicating that dHPLC is a highly sensitive and rapid way of mutation detection in large cohorts of patients. Our findings suggests that mutations in the SGCE gene are only rarely involved pathogenesis of other subtypes of dystonias and seems to be associated with a very distinct phenotype. Genetic testing for mutations in the SGCE gene of patients with dystonia as main symtom is only recommended in cases of association with myoclonic features or psychiatric symptoms. A positive family history is supportive especially if consistent with autosomal-dominant inheritance pattern with incomplete penetrance indicating a maternal imprinting mechanism. Genes involved in the aetiology of dystonias remain to be characterized.

P 025

Molecular breakpoint mapping of constitutional 5q deletions

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We report on a nine year old female patient with mild dysmorphic signs as bilateral epicanthal folds, low-set dysplastic ears, a short nose with anteverted nostrils, conically shaped fingers, increased subcutaneous fat, multiple fine venous teleangiecatses on the back, mild pectus excavatum, and a general, muscular hypotonia. Cytogenetic analyses and FISH studies including region-specific YAC clones revealed a de novo interstitial deletion of the long arm of chromosome 5 with the following karyotype: 46,XX.ish del (5)(q14.3-q23.1)(957h11-,872d11-,904b1x2,854g6x2). Molecular analyses of 12 polymorphic markers on the long arm of chromosome 5demonstrated that the derivative chromosome 5 is of paternal origin. FISH using region-specific BAC clones helped to narrow down the breakpoint regions. Molecular breakpoint mapping was also performed on an already published case with an overlapping deleted chromosomal segment (Raedle et al., 2001).The deleted interval has already been considered as a candidate region for schizophrenia. Due to several cases of schizophrenia in the family history of the father a more detailed analysis was also performed on him. No obvious structural anomalies could be found.

P 026

Modelling germline mosaicism and heterogeneous mutation rates in Duchenne muscular dystrophy

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For Duchenne muscular dystrophy two biological phenomena have been recognised as important: 1. germline mosaicism and 2. different new mutation rates in male and female depending on mutation type. Both principles have been investigated separately and their influence on risk estimation in families has been exemplified in the literature.The aim of this paper is to present a general model which allows to include germline mosaicism an heterogenous mutation rates. Mosaicism is introduced by defining additional alleles at the disease locus in combination with segregation rules. We derived the conditions which have to be fulfilled for a population in mutation selection equilibrium. As a prerequisite for the use of the presented framework for practical applications in genetic counselling model parameters had to be estimated. We use published empirical data and some simplifying assumptions to find admissable solutions for the parameters. As consequence it can be directy demonstated that the proportion of sporadic cases is lower than 1/3 in contrast to the classical hypothesis of Haldane for lethal X-chromosomal diseases incase of germline mosaicism and if certain relations are true for the different mutation rates.Our approach aims to describe the model on the population level and not in individual subjects. This has the great advantage of resulting in tractable algebra. It allows the use of well known algorithms for calculation of likelihoods in pedigrees.

P 027

Two novel candidate genes for the neurological features of Wolf-Hirschhorn syndrome

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The Wolf-Hirschhorn syndrome (WHS, MIM 194190) is caused by deletions in chromosome region 4p16.3 and is thought to be a true contiguous gene syndrome with a yet unknown number of genes contributing to the phenotype. Recently, several candidate genes have been described (WHSC1, WHSC2, LETM1) which are located in or immediately adjacent to the previously defined 165 kb WHS critical region 1 (WHSCR1). We have now focused our attention on the identification and functional characterisation of genes which are involved in the neurological features (e.g. seizures, learning and motor deficiencies, ADHD) of the syndrome. Besides LETM1, which has become a strong candidate for seizures, we identified two further candidate genes by comparative sequencing and intensive database analysis (WHSC3, WHSC5). WHSC5 encodes a protein of approximately 300 aa in human and mouse. The protein most probably belongs to the superfamily of GCN5 related N-acetyltransferases (GNAT), showing the highest homology to members of the camello family of acetyltransferases. Northern Blot analysis revealed two main transcripts of approximately 3 and 6 kb in brain and testis. Whole mount and RNA in situ hybridisation in early developmental stages showed that expression is restricted to specific regions of the brain (roof of neopallial cortex and roof of midbrain). WHSC3, the second gene, is also expressed in brain and testis. Here, the expression is restricted to specific nerve cells (interneurons) in the brain. WHSC3 encodes two short proteins with no significant homologies to sequences in the databases. We have evidence that the proteins may be prohormone or neuropeptide precursors. We generated and tested peptide antibodies against WHSC3. On a Western-blot the antibodies detected two distinct bands between 16 and 18 kDa in protein lysate of murine whole brain, which is in good agreement with the expected protein sizes. The strong expression of both genes in fetal and adult brain tissues strongly suggests an implication in brain development and/or function. Together with their predicted functions in cell signaling and/or early developmental processes, they represent excellent candidate genes for the neurological features in Wolf-Hirschhorn syndrome.

P 028

WHSC4 - a novel ncRNA gene in the Wolf-Hirschhorn syndrome critical region 1

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The Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation syndrome associated with the deletion of the terminal short arm of one chromosome 4. The WHS critical region has been confined to 165 kb in chromosomal subband 4p16.3. Recently, Zollino et al. (2003) suggested a second critical region (WHSCR2) immediately distal to WHSCR1. Clinical and cytogenetic data indicate that WHS is a contiguous gene syndrome, which means that haploinsufficiency of more than one gene in the affected chromosome region contribute to the phenotype. In order to elucidate the etiology and pathogenesis of this syndrome our studies aimed at identifying all genes and regulatory regions (in WHSCR1) contributing to the phenotype by comparative sequencing, computer assisted analysis, as well as functional studies. Two known and three novel genes (WHSC1, WHSC2, LETM1, WHSC3, WHSC5) were identified in the WHSCR1. In addition, we detected several EST matches as well as predicted exon sequences that did not correspond to any of these transcription units. By extensive RT-PCR analysis and rapid amplification of cDNA ends (RACE) as well as Northern blot analysis we could demonstrate that most of these sequences correspond to a single novel gene (WHSC4) which is expressed in a complex pattern in several tissues. WHSC4 contains repetitive sequences, does not display any significant open reading frame and therefore most probably encodes a noncoding (nc) RNA. WHSC4 undergoes complex alternative splicing, leading to at least 4 different transcript classes (A-D). RACE experiments suggested at least 3 different transcriptional start sites and at least 3 different 3´exons. The 5´-end of one of these transcripts (A) overlaps with exon 1 of WHSC3 on the opposite strand indicating that it might function as an antisense transcript. Other transcripts (B-D) span the entire WHSC3 gene and/or overlap with parts of WHSC2 in sense direction. All proved exons of WHSC4 together span about 60 kb of the WHSCR1. The lack of significant open reading frames as well as it's possible antisense function suggests a putative regulatory function of WHSC4. This is currently under further investigation.

P 029

Melanoma Susceptibility Genes CDKN2A and CDK4: Lack of Germline Mutations in German Melanoma Patients

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Introduction: Germline mutations in the tumour suppressor genes CDKN2A and CDK4 play an important role worldwide in the development of malignant melanoma, the most serious form of skin cancer. The association between genetic variants in CDKN2A and melanoma susceptibility seems to be especially strong in familial melanoma cases, here the gene accounts for about 25% of the cases. Mutations in CDKN2A are also involved in pancreatic cancer. In order to shed light on the role of these genes and their contribution to melanoma disease for the first time in the German population, we analysed a group of German melanoma patients for mutations in these two genes. Patients and Methods: Pedigree analysis of 376 melanoma patients revealed 209 patients who showed to have at least one first degree relative with any kind of cancer. Among these patients, we defined three groups: Familial Melanoma Patients (FM) with at least one first degree relative suffering from either melanoma or pancreatic cancer, patients with multiple primary melanomas (MM) and patients with none of these two features (FDRc). The average age of onset among these patients was 52 years. DNA extraction from blood samples was performed using standard methods, as well as the DNA amplification by PCR and mutational analysis by Denaturing High Performance Liquid Chromatography (DHPLC). Results and Conclusions: No deleterious CDKN2A or CDK4 mutation could be detected among the 209 patients, not even in patients deriving from families with many affected members (FM). DHPLC mutational screening revealed some well known polymorphisms which most likely do not have any functional significance. Although patient selection suggested a strong genetic background, the so far best known melanoma susceptibility gene CDKN2A does not seem to contribute to melanoma disposition in this group of German patients, nor does CDK4. The lack of mutations even in familial melanoma patients questions the definition of familial cases. It might be necessary to define this group on the basis of stricter clinical features. This could mean taking patients having either more affected relatives or relatives with earlier age of onset. However, the search for other genes involved in melanoma susceptibility has to go on, as the genetic influence still seems to be obvious.

P 030

FISH-detection of 15q microdeletions in patients with clinically suspected Prader-Willi syndrome

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Prader-Willi syndrome (PWS) is a genetically determined disorder (occurrence 1:10,000-25,000 live-born infants) in which the absence of expression of one or more maternally imprinted genes in the chromosomal region 15q11-13 results in a range of mental and physical symptoms: short stature, muscular hypotonia, excessive appetite with progressive obesity, hypogonadism, mental retardation, behavioral abnormalities, sleep disturbances, dysmorphic facial appearance and other findings. Paternally derived 15q microdeletion (approximately 4 Mb in size) is in about 70% of cases, maternal disomy 15 is in 25% of patients, imprinting mutation is in 2-5% and translocations are in 1% of cases. Patients: In Republic Genetic Center database there were several patients with clinically diagnosed PWS. The karyotypes were examined using GTG-banding (550-800 bands resolution), in one case the visible deletion 15q 11-13 was detected. We report the results of the first in Belarus molecular cytogenetic detection of deletions in these suspected for PWS patients. Method: Peripheral blood lymphocyte culturing and preparation were performed according to standard technique. FISH analysis was carried out using LSI Prader-Willi/Angelman region D15S10 (LSID15S10 SpectrumOrange/CEP 15 D15Z1 SpectrumGreen/PML SpectrumOrange) and LSI Prader-Willi/Angelman region SNRPN (LSI SNRPN SpectrumOrange/CEP 15 D15Z1 SpectrumGreen/PML SpectrumOrange) probes (Vysis). In situ hybridization was performed in details as described at http://www.vysis.com. Results: FISH analysis has revealed the deletion of chromosome 15 critical region in 2 out of 7 PWS suspected patients; that is 28,6%. Conclusion: FISH diagnostics of PWS microdeletion is the effective method and it is the first step in molecular testing of other mechanisms of PWS molecular defects.Work was supported by CATALOGUE CO LTD, and CHERNOBYL FOUNDATION OF THE MOTHER AND CHILD (Japan)

P 031

Genetic diagnostics: Opinions of counsellees, professionals, and members of lay organizations

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Objective: To obtain the opinions of various groups about problems in connection with the application of modern genetic diagnostics.Background: Because of the recent deciphering of the whole human genome, possibilities for the investigation of gene mutations responsible for monogenetic diseases or for the predisposition to polygenetic diseases are rapidly expanding. However, possible cures for genetic diseases are still lacking. Physicians, insurance companies and employers are interested in using genetic diagnostics and the results thus obtained. To date, there are no guidelines or laws regarding the way in which genetic diagnostics should be handled. In Germany, the National Ethics Council has published statements with respect to preimplantation genetic diagnostics (PGD) and the importing of embryonic stem cells. Recently, the CDU/CSU grouping in the German Federal Parliament has discussed a draft bill covering aspects of genetic diagnostics.Design/Methods: A multiple choice questionnaire consisting of 10 questions was prepared. To every question there were three possible answers. The questionnaire was sent to at least 9 different groups: counsellees and accompanying persons, medical students, technical students, members of lay organizations for genetic diseases, student midwives and student occupational physicians. Because the study will only be completed by the end of this year, preliminary aspects will be presented.Results: There is nearly complete agreement in all groups that every person has to decide for himself/herself whether to take predictive genetic analysis. Opinions regarding to whom the results of genetic analysis should be made available are different in the various groups. About two thirds to three quarters of the medical students, accompanying persons, and student midwives agree that results should be available for insurance companies and employers with the person's consent. Most of the members of the lay organization agree with the opinion that results of genetic analysis should never be available for insurance companies or employers. Only a few persons in all groups think that PGD should be forbidden. There is a equilibrium between the opinions that PGD should only be carried out for couples who request this method and the opinion that PGD should only be

performed for couples with an increased risk for a handicapped child. Most of the persons agree with the opinion that a healthy child should only be diagnosed when a genetic disease can be protected. There is strong agreement in all groups with the opinion that genetic diagnosis should only be carried out within the framework of genetic counselling after informed consent.Conclusions: As expected, there are different opinions in the different groups. Persons with (a risk of) genetic diseases and their partners are more restrictive with respect to the use of the results of genetic diagnosis. However, in all groups there is strong agreement that pre- and postnatal genetic analysis should only be applied after comprehensive information is made available to the respective parties.

P 032

Two siblings with ICF syndrome: clinical and cytogenetical follow-up studies

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ICF syndrome is a rare disorder characterized by growth delay, immunodeficiency, craniofacial anomalies, high predisposition to malignancies and chromosomal instability presenting specific centromeric heterochromatin phenomen of autosomes. Mutations in the DNMT3B are responsible for ICF syndrome. Currently autosomal recessive inheritence is suggested. We report on clinical and cytogenetical data of two sisters, now 7 and 3 years old, affected from birth, that were examined in dynamics.Family history: Unrelated young parents were healthy. G1 - spontaneous abortion, G2 - case 1 (proposita - BW=2200g, BL=45cm, OFC=31.5cm), G3 - case 2 (affected sister - BW=1900g, BL=43cm, OFC=31cm). The course of full-term pregnancies and labors were unremarkable. Half-sister (a child of father`s first marriage) was healthy.Phenotype: Patients showed pre- and postnatal severe growth delay (case1 at the age of 6 years had W=13kg, L=110cm, OFC=46,5cm and case 2 being 1 year old had W=6kg, L=62cm, OFC=42cm), mild mental retardation, recurrent respiratory infections, immunodeficiency, anemia. Craniofacial features displayed microcephaly, „bird`s" appearance, hypertelorism, low-set ears, epicanthal folds, arched palate, microretrognathia, eye`s abnormalities (case 1 - strabismus, myopia; case 2 - hypermetropic astigmatism), chest deformation, arachnodactyly, plano-valgus feet. Renal and brain defects were not found during ultrasound examination. Additionally the proposita had cardiomyopathia developing since 2 years old, anemia, trombocytopenia since 4 years old, leukemia since 6 years old. Karyotypes (G-banding), biochemical screening for metabolic diseases were normal in both cases.Cytogenetics: We studied the cytogenetic status for spontaneous chromosome instability of sibs during two years (2001-2002) using 48, 72 and 96 h blood lymphocyte cultures (GTG banding, about 600 metaphases were scored). The frequency of aberrant metaphases increased with time from 15% to 33%. We observed: chromatide and chromosome deletions, dicentric and ring chromosomes, reciprocal and nonreciprocal translocations, markers, additional chromosomes. The specific for ICF syndrome rearrangements, such as chromatide and chromosome breaks in pericentromeric region, isochromosomes, triradial configurations and destroyed triradial figures, interchanges between pericentromeric chromosomal regions, fragmentation and despiralization of the chromatin have been found only recently. Chromosomes 1 and 2 were most unstable in our patients; 10,3% metaphases had aberrant chromosome 1, and 7,1% cells included aberrant chromosome 2. We have studied the bone marrow of proposita when she showed a first signs of leukemia. Cytogenetical abnormalities of different types were detected. Conclusion: We believe that all patients clinically suspected for ICF syndrome should be investigatedfor chromosomal instability signs in dynamics.

P 033

Maternal Uniparental Disomy 16: New case and survey of published cases

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Uniparental disomy (upd) is the occurrence of both homologue chromosomes from only one parent. Upd (16)mat is the most often reported upd; almost all cases are associated with confined placental mosaicism (CPM). Only few upd (16)mat cases are clinically normal, whereas most presented with intrauterine growth retardation (IUGR) in addition to different congenital malformations. Upd (16)mat has therefore been suspected to have clinical effects; however the lack of specificity of the birth defects observed suggests that the phenotype may be related at least in parts to placental insufficiency. We report on a new case of upd (16)mat associated with low level trisomy 16 mosaicism in placenta and fetus. At 19th gestational week intrauterine growth retardation (IUGR) was noted, the fetus died in utero. Apart from mild cranifacial dysmorphisms, he showed anal atresia. While IUGR is probably associated with trisomy 16 mosaicism, anal atresia is a feature previously described only in upd (16)mat. Considering the data in our patient as well as those in upd (16)mat cases from the literature, the following indications for upd (16) testing can be defined: they include trisomy 16 mosaicism, IUGR in addition to congenital anomalies (anal atresia, congenital heart defects). However, there is an overlap of clinical signs in mosaic trisomy 16 cases with upd (16)mat and in those with biparental disomy 16. Therefore, the management of trisomy 16 pregnancies should not differ from those in which upd (16)mat is confirmed. A prenatal testing for upd (16)mat is not useful, but it should be offered postnatally.

P 034

Prenatal diagnosis of a duplication in 22q11.21 led to the identification of a trisomy 22q11.21 in three generations without clinical findings

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We report on a family with a duplication in chromosome 22p11.2-22q11.21 without apparent phenotype. The duplication was initially detected in the fetus of a 20 year old woman referred for amniocentesis. Karyotypic analysis of the father and the grandmother showed a morphologically altered chromosome 22, as well. According to GTG-banding no clear conclusion could be drawn about the type of rearrangement. Using the recently described subcenM-FISH probe set [Starke et al., Med Genetik 2002, 14:262] the derivative chromosome 22 showed two specific signals for the centromeric probe D22Z4, for a centromere-near BAC bK115F6 in 22q11.21 and for the probe midi54, which is specific for all acrocentric p-arms. To exclude an involvement of the CATCH22-critical region a corresponding probe was applied – only one specific signal on each chromosome 22 was detected. Thus, the dicentric chromosome 22 can be described as dic (22)dup (p11.2q11.21). The here described partial trisomy of the centromere-near region 22q11.21 is the first reported case without any clinical signs (for review of genomic disorders on 22q11 see McDermid and Morrow, Am J Hum Genet 2002, 70:1077-1088). Further molecular and molecular cytogenetic characterization of this case may help to further narrow down CATCH22- and/or cat eye syndrome- critical regions. Supported by the Dr. Robert Pfleger-Stiftung and the EU (ICA2-CT-2000-10012).

P 035

Small supernumerary marker chromosomes (SMC): genotype-phenotype correlation and classification

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Small supernumerary marker chromosomes (SMC) are present in about 0.05% of the human population. In about 30% of SMC carriers (excluding the ~60% SMC derived from one of the acrocentric chromosomes) an abnormal phenotype is observed. Clinical outcome of an SMC is difficult to predict as they can have different phenotypic consequences due to (i) differences in its euchromatic DNA-content, (ii) different degrees of mosaicism, and/or (iii) uniparental disomy (UPD) of the SMC's homologous chromosomes. Here we present data from 35 SMC, which are derived from all human chromosomes, apart from chromosome 6, as demonstrated by the appropriate molecular cytogenetic approaches, such as centromere specific multicolor FISH (cenM-FISH), multicolor banding (MCB) and subcentromere specific multicolor FISH (subcenM-FISH). In nine cases without an aberrant phenotype neither partial proximal trisomies nor UPD could be detected. Abnormal clinical findings like psychomotoric retardation and/or craniofascial dysmorphisms were associated with seven of the cases where subcentromeric single copy probes were proven to be present in three copies. Conversely, in eight cases with normal phenotype proximal euchromatic material was detected as partial trisomy. UPD was studied in twelve and subsequently detected in two of the cases with SMC (partial UPD 4p and maternal UPD 22 in a der (22)-syndrome patient), indicating SMC carriers to have an enhanced risk for UPD. At present small proximal

trisomies of 1p, 1q, 2p, 6p, 6q, 7q, 9p and 12q lead to clinical manifestations, while partial proximal trisomies of 2q, 3p, 3q, 5q, 7p, 8p, 17p and 18p may not be associated with significant clinical symptoms. With respect to the clinical outcome a classification of SMC is proposed which considers the present state of moleculargenetic and molecular cytogenetic characteristics. Supported by the Dr. Robert Pfleger-Stiftung.

P 036

Enlarged chromosome 13 p-arm hiding a cryptic partial trisomy 6p22.2-pter

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Here we report on a 2 years old male child with developmental delay, short stature, blepharophimosis, clinodactyly, hypolastic philtrum, abnormal ears and microcephaly. Banding-analysis revealed a normal karyotype apart from an enlarged short arm of one chromosome 13. Rather than NOR- and CBG-staining, we applied the so-called acro-cenM-FISH probeset containing a probe specific for the acrocentric human p-arms, a NOR-specific probe, a probe for Yq12, and all available centromere specific probes for the human acrocentrics. The results allowed to exclude that the enlargement on the suspect chromosome was due to a centromere or p-arm-polymorphism, an enhanced NOR-region or Yq12-derived additional material. As M-FISH was unable to resolve the nature of the rearrangement, the case was studied by glass needle based microdissection and reverse painting. As a result, the region p22.2-pter on both homologous chromosomes 6 was painted with the microdissection-derived probe. The presence of a partial trisomy 6p was proven by multicolor banding, too. In the present case a parental origin of the rearrangement could not be excluded, as the child was adopted and parents remained unknown. The literature revealed that the symptoms of the present patient are in high concordance with those reported for three similar cases with trisomy 6p22or23-pter. In summary, every abnormally large acrocentric short arm marker should be studied in detail by different molecular cytogenetics methods. It must be kept in mind, that M-FISH alone may not always be reliable a method to detect translocations at the end of acrocentric p-arms. Supported in parts by the DFG-436RUS17/40/00-RUS17/49/02

P 037

Highly complex rearrangement between chromosomes 3, 4, 7, 9 and 17 in a healthy female characterized by M-FISH and multicolor banding (MCB)

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Complex chromosomal rearrangements are – according to the literature - constitutional structural rearrangements involving three or more chromosomes or having more than two breakpoints We report on a case of a de novo complex chromosomal rearrangement between 5 chromosomes found in a clinically healthy woman. The only indication for chromosome analysis was a planned ICSI; internal and external genitals, ovaries and hormone status were normal. Conventional cytogenetic analysis showed a rearrangement between chromosomes #3, #4, #7, #9, and #17 whereas karyotype analysis of the parents showed no cytogenetic abnormalities. Application of M-FISH indicated the following aberrations: del (3), der (4)t (17;4;7), der (7)t (3;7), der (9)t (4;9), der (17)t (9;17). To concretize from which chromosomal arm the translocated material was derived, arm-specific probes were used where necessary and revealed that the derivative chromosome 3 had no simple deletion but contained a subtle translocation of material of chromosome 4. In the next step, multicolor banding (MCB) was applied to further characterize the exact breakpoints and to define the orientation of the involved chromosomal material. By this detailed analysis, the karyotype could be established as follows: .ish 46,XX,der (3)t (3;4)(3pter—>3q22::4q34or35—>4qter),der (4)t (17;4;7)(17pter—>17p12or13::4p14—>4q34or 35::7p12or13—>7pter),der (7)t (3;7)(3qter—>3q22::7p12or13—>7qter),der (9)t (4;9)(4pter—>4p14::9q13—>9pter),der (17)t (9;17)(9qter—>9q13::17p12or13—>17qter). The rearrangement consisted of altogether 6 breaks and seemed to be balanced on molecular-cytogenetic level. Although the MCB-technique allows to determine exactly the breakpoints, submicroscopic deletions or duplications close to the breakpoints cannot be excluded. There are only very few cases reported with such a complex chromosomal rearrangement involving 5 chromosomes without clinical signs. As such cases are detected only by chance during course of ICSI the frequency of them in the population still remains unknown. Supported in parts by the DFG (436 RUS 17/49/02).

P 038

Molecular genetic proven Andermann syndrome in Turkish brothers – a clinical report

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We report on Turkish brothers with molecular genetic proven agenesis of the corpus callosum (ACCPN), also called Andermann syndrome. This disorder is characterized by complete or partial agenesis of the corpus callosum, severe and progressive sensorimotor neuropathy, mental retardation, muscular hypotonia, delay of motor development and mild dysmorphic features. Loss of physical and mental abilities is typical. Most of the patients have seizures, many develop psychosis. The Andermann syndrome is predominantly observed in two regions of the province of Quebec. The boys were born after uneventful pregnancies and spontaneously delivered at term with normal body measurements. The parents are second degree cousins. The elder brother showed a developmental delay and muscular hypotonia and hyporeflexia at the age of three months. Furtheron a nystagmus and a scoliosis were observed. Sonographically a corpus callosum was not seen at the age of 4 months and this was confirmed at the age of 22 months by computertomography and magnetic resonance imaging. The EEG examinations revealed disposition to epilepsy with sharp-wave foci, but up to now seizures did not occur. Sensoric as well as motor nerve conduction velocities could be measured as too slow and with too low amplitude (33 months of age). The myography showed complex and polyphase fasciculation potentials. He learned to speak single words and to walk with 3-4 years. The younger boy showed a similar but slightly worse course.Although rare outside Quebec Andermann syndrome should be kept in mind in cases of slightly dysmorphic children with neuromuscular symptoms.

P 039

Frequency of Nijmegen breakage syndrome (NBS) and prevalence of the founder mutation 657del5 in Slovakia

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Introduction: Nijmegen breakage syndrome, an autosomal recessive chromosomal instability disorder, is clinically characterised by congenital microcephaly, facial dysmorphy, growth retardation with prenatal onset, immunodeficicency, and a highly increased risk for lymphoreticular malignancy (1). Cells from NBS patients are hypersensitive to ionising radiation. The gene product, nibrin, is a member of the hMRE11/RAD50 protein complex involved in DNA double-strand break repair and recombination (2). The majority of NBS patients ate of Slav origin and carry the same founder mutation, 657del5, in exon 6 of NBS1 gene. Varon et al.(3) studied the carrier frequency of the 657del5 mutation in newborn of polish, czech and Ukrainian extraction and found a high prevalence of heterozygotes (1/177). The prevalenece in the Czech Republic was 1/154. Based on this, the calculated number of NBS homozygotes was 1/95000 live-births. The observed number, however, was significantly lower (1/271000). The most likely explanation for this discrepancy was underdiagnosis. To our surprise the absolute number of homozygotes diagnosed in Slovakia was highee than in the Czech Republic though the size of the population is only half of it. This prompted us to perform a systematic screen for NBS homozygotes in Slovakia and to estimatethe population frequency of the 657del5 founder mutation. Quite unexpectedly, we found a low frequency of gene carriers and a much higher than expected number of NBS homozygotes.Materi-

al and Methods: We analysed a total of 2499 randomly selected anonymous Guthrie cards drawn from newborn screening tests. The newborns were born in 2001 and 2002 in 15 maternity houses of Slovakia. All samples were analysed for the 657del5 mutation by PCR-single strand conformation polymorphism (PCR-SSCP) analysis as describe before.The close collaboration with pediatricians and clinical geneticists of Slovakia allowed us to investigate DNA samples of 19 children and adolescents born in the period between 1983-2002 with congenital microcephaly of unknown aetiology. All samples were analysed for 657del5 mutation by direct sequencing.Results: The diagnosis of NBS was confirmed by DNA analysis in 13 patients from 10 families. Three families had two affected children. During the period between 1983 and 2002 11 patients from 8 families were born among 1,200.000 recorded live births, the prevalence of NBS among newborn can be rougly calculated as 1/110000. Based on the Hardy-Weinberg law and the above cited incidence of NBS patients the carrier frequency can be formally estimated to 1/150. To our surprise, we found only 2 NBS heterozygotes of the 657del5 mutation in 2499 Guthri cards, giving a carriers prevalence of 1/1250 for the Slovak newborn population. This frequency is much lower than expected.

P 040

A new proximal interstitial deletion of chromosome 4q,del (4)(13.2q21.22): Case report and characterisation of the phenotype

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Deletions of the proximal portion of chromosome 4q are rare cytogenetic findings. While distal interstitial and terminal deletions associated with the loss of bands (4)(q31q33) result in a characteristic and well-defined phenotype, in cases with more proximal 4q deletions a distinct phenotype remains difficult to define. Even in patients sharing similar breakpoints phenotypes may vary significantly. We report a four-year-old girl with a proximal interstitial deletion of chromosome 4 resulting in a karyotype 46,XX,del (4)(pter-q13.2::q21.22-qter). To the best of our knowledge, a deletion with exactly the same breakpoints has not been described previously. The girl presented with severe psychomotor retardation, moderate growth retardation (3rd centile), relative macrocephaly, mild frontal bossing, low-set ears, a left-sided pre-auricular tag, thin upper lip, high arched palate, short fingers and toes, and low-set thumbs. MRI showed mild hypoplasia of corpus callosum and several periventricular lacunar lesions. In the neonatal period, feeding problems were present. Interestingly, at the age of 3.5 years, she had a single episode of lactic acidosis and hypo- and hyperglycemia with glycosuria. Diabetes mellitus was excluded. Comparing the phenotype with that of previously reported patients with overlapping deletions shows some common features but there is no recognisable pattern of dysmorphism. In particular, in contrast to our case no similar disturbances of carbohydrate metabolism have been observed so far. In addition to our cytogenetic and clinical findings, we present results of molecular and molecular cytogenetic investigations in the patient.

P 041

Robinow Syndrome and renal abnormalities - a familial case

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Robinow syndrome is a rare disorder characterized by mesomelic shortening, typical facial appearance („fetal face") and hypoplastic genitalia. The original report of Robinow et al. (1969) documented an autosomal dominant mode of inheritance, but heterogeneity was recognized. The recessive form of Robinow syndrome (RRS; MIM 286310) has a high incidence of spinal segmentation defects such and ribs fusion, which are not seen in the dominant form. The gene of recessive form was identified as the ROR2 gene on chromosome 9q22. The same gene is mutated in autosomal dominant brachydactyly type B. We report a 12 years old girl with classical RRS. She is a first child of healthy consanguineous parents. The limb shortening was detected by ultrasonography at 28 weeks of gestation. At birth severe mesomelic shortening, macrocephaly, characteristic face with hypertelorism, prominent eyes, frontal bossing and alveolar hyperplasia were noted. An X-ray detected thoracic hemivertebrae and ribs fusion. Reexamination at the age of 12 year showed mild mental retardation, short stature, scoliosis, mesomelia, brachydactyly with absent interphalangeal creases and hypoplastic nails, macrocephaly, midface hypoplasia with depressed nasal bridge, high palate, irregular teeth. Ultrasonography detected left incomplete renal duplication. Karyotype was 46,XX. The second pregnancy was medically terminated because of ultrasonographic detection of polycystic kidneys. The female fetus aged 22 weeks had enlarged kidneys with medullary cysts and some features typical for RRS: marked hypertelorism, short nose with flat nasal bridge, short neck, hypoplastic labia minora and clitor. But the length of limbs was normal for gestational age. Karyotype was 46, XX. The renal examination of the father revealed mild enlarged polycystic kidneys without functional abnormality. His two younger sisters also have polycystic kidneys. It is not clear whether renal abnormalities in both cases are RRS symptoms or accidental combination of RRS with ADPK inherited from father. The other question is time of limb shortening detection during pregnancy as an ultrasonographic marker of RRS. The clinical findings in our patients are compared to the data mentioned in the literature.

P 042

First Non-Mosaic Case of Isopseudodicentric Chromosome 18 (psu idic (18)(pter->q22.1::q22.1->pter) is associated with Multiple Congenital Anomalies Reminiscent of Trisomy 18 and 18q- Syndrome

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Cytogenetic abnormalities affecting chromosome 18 are relatively common, and characteristic diagnostic patterns have been described for both trisomy 18 and for the terminal deletion syndromes of 18p and 18q. In contrast, isodicentric or isopseudodicentric chromosome 18 is a rare cytogenetic aberration, which results in a combination of partial trisomy and partial monosomy of chromosome 18. Only few cases of an isopseudodicentric chromosome 18 with partial deletion of 18q have been published, and all these cases were mosaic. We describe here a hypotrophic newborn with a lateral cleft lip and palate and multiple craniofacial dysmorphisms, a combined heart defect, unilateral hypoplasia of the kidney, bilateral aplasia of thumbs, and generalized contractures. Cytogenetic analysis revealed an isopseudodicentric chromosome 18 with breakpoint in 18q (46,XX,psu idic (18)(pter->q22.1::q22.1->pter)). The isopseudodicentric chromosome 18 was observed in 100% of blood lymphocytes and umbilical cord fibroblasts, thus indicating a non-mosaic finding of the isopseudodicentric chromosome in the child. An elongated derivative chromosome 18 had also been found prenatally in amniotic cells. In contrast, a terminal deletion (18q-) was detected in placental cell cultures. The breakpoint was mapped to a 0.9 Mb region on 18q22.1 (located 64.8 to 65.7 Mb from the telomere of the p-arm) by a novel quantitative PCR approach with SYBR green detection. The results indicate an identical breakpoint of the isopseudodicentric chromosome 18 in the child and the 18q- chromosome in the placenta. To our knowledge this is the first case of a fetus carrying an isopseudodicentric chromosome 18 with breakpoint in 18q (46,XX,psu idic (18)(pter->q22.1::q22.1->pter)) in non-mosaic form that has been viable, but was associated with severe congenital malformations of the child.

P 043

Fibromuscular dysplasia-an important differential diagnosis of Marfan syndrome-case report of an 52 year old patient

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Fibromuscular dysplasia is an arterial occlusive disease of children or young adults that can lead to stroke, hypertension, claudication or myocardial infarction. Additionally, aneurysms and arterial dissections are frequently observed, as in Marfan syndrome. Fibromuscular dysplasia of arteries is occasionally associated in Recklinghausen neurofibromatosis and Noonan syndrome. Most nonsyndromic cases are sporadic. However, case reports in sibs raise the possibility of recessive inheritance in some families, and vertical transmission has been demonstrated repeatedly, suggesting autosomal dominant inheritance. Gene loci have not been identified.We report a 52 year old German patient with dolichostenomelia and tall stature. His body length of 186 cm is above the 90th centile. He has had spontaneous renal arterial dissection, hypertension and extreme aneurysms of both arteriae iliacae requiring aortobifemoral bypass operations. Fibromuscular dysplasia of the intimal type was verified pathohistologically. CCT demonstrated older cerebral infarctions. It remains unclear if the patient`s only adult daugh-

ter is affected too. She has high diastolic blood pressure of with unknown cause, but no other manifestations. The family history is otherwise unremarkable.We wish to call attention to fibromuscular dysplasia as a differential diagnosis of Marfan syndrome.

P 044

Minor anomalies in a patient with a karyotype 46,XY,der (3)t (3;4)(p26;p16)

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Terminal deletion of the short arm of chromosome 3 has been proposed as a distinctive syndrome. In all patients band 3p26 and a variable extent of band 3p25 have been involved in the mostly de novo deletions. The phenotype of these patients is characterized by microcephaly, growth retardation, mental retardation, and facial dysmorphism. Duplications of the short arm of chromosome 4, however, are less common, in most cases half of the p-arm is duplicated and the phenotype is rather variable.We report on a 7 years old Turkish boy who presented with mild mental retardation and moderate speech delay, mild facial dysmorphisms as flat mid face, large protruding ears, upslanting palpebral fissures, mild progeny, prominent lower lip, and funnel chest. No internal malformations have been found, vision and hearing were normal as were MRT, EEG, and extensive laboratory investigations.Cytogenetic analysis performed on peripheral blood lymphocytes using GTG banding techniques revealed an abnormal karyotype, 46, XY,del (3)(pter->p26). Fish analysis with specific whole chromosome painting probes (wcp1-22, X and Y), MCB mixed probes for chromosome 3, and subtelomere probes for chromosome 3p confirmed the cytogenetic result. Conventional cytogenetic and molecular cytogenetic analysis of the boy's parents with MCB mixed probes for chromosome 3 and subtelomere probes for chromosome 4 showed a balanced translocation between chromosome 3 and 4 in the mother, karyotype 46,XX,t (3;4)(p26;p16). Re-evaluation with the subtelomeric probe of chromosome 4p and subtelomere screening of 42 telomere ends with subtelomeric localized primers confirmed a partial monosomy of 3p26.1-pter and in addition a partial trisomy of chromosome 4p16.1-pter. The deletion was narrowed down to 3.7 Mb (± 0.1Mb). By using four different primer pairs the duplication of chromosome 4p was estimated to extend minimum 5.4 Mb and maximum 11Mb.Many patients with either terminal deletions of chromosome 3 or 4 and either terminal duplications of chromosome 3 or 4 have been published. Only one report on a terminal 3p deletion combined with a terminal 4p duplication (Grammatico et al., 1998) exists. We will discuss the genotype/ phenotype correlation of our patient with that published in the literature and with that of patients presenting the phenotype of 3p- deletion syndrome or 4p- duplication.

P 045

Identification of the origin of a marker chromosome 7 in a child with dysmorphic signs and congenital heart defect

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We report on a case of additional marker chromosome mosaicism. Cytogenetic analysis was performed postnatally and revealead mos 47, XX + mar [26] / 46, XX [14]. The karyotypes of the parents were normal. The marker chromosome was identified by fluorescence in situ hybridization (FISH) and derived from chromosome 7. A marker chromosome derived from chromosome 7 has not been reported until now. A uniparental disomy 7 was excluded. The child revealed congenital heart defect and dysmorphic signs.The psychomotoric development of the little girl was retarded and followed up for two years.

P 046

PHENOTYPICAL VARIATION IN COUSINS WITH THE IDENTICAL PARTIAL TRISOMY 9 (pter-q22.2) AND 7 (q35-qter) AT 16 AND 23 WEEKS GESTATION

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From the study of numerical and structural chromosomal abnormalities there is convincing evidence and accumulating information of a direct karyotype to phenotype correlation. Knowledge of phenotypical consequences of a specific chromosomal imbalance is important for genetic counseling and prenatal diagnosis. However, for unbalanced non-Robertsonian translocations a precise karyotype to phenotype correlation is difficult to predict since varying breakpoints result in different lengths of the monosomic and trisomic segments and the combination of the same trisomy with different monosomies, or vice versa, can result in diverging phenotypes. Therefore, the study of the karyotype to phenotype correlation in affected relatives of the same age and the identical unbalanced translocation provides a good model to investigate phenotypical consequences of a specific genetic imbalance. We report of two second trimester fetuses with the identical major partial trisomy 9 (9pter-9q22.2) and minor partial trisomy 7 (q35-qter) resulting from a familial translocation (7;9)(q35;q22.2)mat. One fetus presented with a Dandy-Walker malformation, micropolygyria and mild dysmorphic features, whereas the other fetus showed unilateral cleft lip and palate without cerebral anomalies or dysmorphic features. Both fetuses had no features in common. Potential mechanisms for this different phenotypical expression of the same unbalanced translocation resulting in partial trisomy 9 and 7 in the two cousins and possible consequences for genetic counseling and prenatal diagnosis are discussed.

P 047

Mild ornithine transcarbamylase deficiency in a boy caused by somatic mosaic for a novel mutation

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Ornithine transcarbamylase deficiency, inherited as an X-chromosomal trait, is the most common urea cycle disorder. Hemizygous boys typically present with severe hyperammonaemic encephalopathy in the neonatal period but may have a milder phenotype usually depending on the nature of the underlying mutation. We present the case of a boy with developmental retardation and recurrent episodes of restlessness, vomiting and mild jaundice, in whom OTC deficiency was diagnosed at the age of 2 years. Biochemical findings at diagnosis included elevated transaminases and bilirubin, reduced Quick, massive excretion of orotic acid, and plasma ammonia concentrations of 221-273 µmol/L. Molecular studies with denaturing gradient gel electrophoresis and direct sequencing consistently showed a pattern resembling heterozygosity for a novel mutation at the stop codon (TGA->TTA), denoted X355L. Chromosomal studies showed a normal male karyotype. Somatic mosaicism for X355L was confirmed by finding different proportions of normal and mutant alleles, respectively, in lymphocytes and fibroblast cells. X355L causes the addition of 14 C-terminal amino acids to the OTC protein and may be expected to severely affect enzyme function. Somatic mosaicism as cause of OTC deficiency has only been reported once but may be difficult to recognise with standard methods when the wild type predominates in genomic DNA obtained from blood. It could explain some cases of OTC deficiency with normal molecular findings. The results in our patient explain the relatively mild phenotype and also excludes a germ cell mosaic in one of the parents. There is thus no increased risk for subsequent children with OTC deficiency in this family.

P 048

Clinical findings in a patient with proximal chromosome 6-duplication

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We report on the first child of healthy, non-consanguineous parents. The boy was born after 39 weeks of gestation. Birth measurements were below average. He presented with facial dysmorphisms (ptosis of the eyelids, small and overlapping helices, anteverted nares, retrognathia), microcephaly, and mental and motoric retardation. Short fingers and toes, hypermobility of the joints, and hip dysplasia were also noted. After the age of two weeks he developed epilepsy as well as feeding problems.Sonography of the brain showed agenesis of the corpus callosum and a Dandy-Walker malformation. In addition, an ASD II with L-R shunt was demonstrated using echocardiography.Cytogenetic analysis revealed a proximal duplication of chromosome 6q11-q21. The parents had normal karyotypes, 46,XX and 46,XY.While duplications

of the distal region of chromosome 6q have been reported in the literature in approximately 23 cases, only one case with a proximal duplication (46,XY,dup (6)q11-q15) was published by Giordano et al. (Clin Genet 1994: 46:377-379), but the clinical findings in that case do not show much overlap with those of the patient reported here.

P 049

Townes-Brocks syndrome: clinical variability in two patients of one family

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The autosomal dominantly inherited Townes-Brocks syndrome (TBS) is a malformation syndrome including characteristic anal, thumb and ear anomalies. Many other organ system abnormalities may be present. The expression is extremely variable. The disease-causing gene for TBS was identified as SALL1, a zinc finger transcription factor. We describe a new family with TBS and demonstrate the variability of the disorder in an affected woman and her son. The boy shows the full spectrum of anomalies including anteposition of the anus, sacral dimple, hypospadia penis, triphalangeal thumbs with ulnar deviation, postaxial hexadactyly of fingers, clubfoot on the left and flatfoot on the right, microcephaly, cleft palate, retrogenia, deep set ears and suspicion of hearing loss. He was born in the 39th gestational week with a birth weight of 3200 g and a length of 49 cm. There were feeding problems. A gastrooesophageal reflux was diagnosed. In contrast the mother has hypoplastic thumbs as the only feature of TBS. There are bilateral short thumbs with shortening of the distal phalanges. Cytogenetic analysis was performed on peripheral blood lymphocytes. The analysis of G-banded chromosomes revealed a normal karyotype 46,XY in the boy. Molecular analysis is planned. TBS was described first in 1972 by Townes and Brocks (J. Pediat. 81: 321-326). Surka et al. stated that hypoplastic thumbs should be considered a feature of TBS (Am. J. Med. Genet. 102: 250-257, 2001). This case report illustrates the intrafamilial variability of TBS. Careful examinations of relatives of patients are necessary for the differentiation of sporadic and familial cases of TBS and for the differential diagnosis to other disorders like the VA (C)TER (L) association.

P 050

THE DYNAMIC OF LIPID CHANGES DEPENDS ON APOLIPOPROTEIN E GENOTYPE AND LIFESTYLE

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Introduction: Carrier of the apolipoprotein E4 allele in industrialized countries show significant higher serum cholesterol concentrations, and a higher prevalence of cardiovascular diseases compared to natives e.g. in Northern Mexico following a traditional lifestyle. Purpose: To determine the influence of a cardiovascular intervention program on the lipid profile, stratified by four different homozygous and heterozygous manifestations of the apolipoprotein E (apo E).Methods: This 3-week lifestyle intervention study was composed of a special diet: low in saturated fatty acids and sugar, and high in complex carbohydrates, in combination with high physical activity. At the beginning and during the course of the study blood was drawn weekly for plasma lipid profile and additionally the participants' apo E allele were determined after the study. Results: Apolipoprotein E3/E3 (61%), was pointed out as the most common apo E genotype in this sample followed by E3/E4 (34%). Both heterozygous E2/E4 and homozygous E4/E4 polymorphism was represented each by one men. All participants decreased their cholesterol levels: The serum cholesterol in men dropped in average by 90,7±23,9 mg/dl, and the triglyceride levels by 81,6±90,8 mg/dl. The changes were slightly less in women: 62,5±16,7 mg/dl for cholesterol and 34,2±58,6 mg/dl for triglycerides. However, women with geno-type apo E3/E4 improved their cholesterol levels by 70,5±14,5 mg/dl compared to 58,6±16,6 mg/dl for those with geno-type E3/E3. The dynamic of the lipid changes showed a geno-type dependent structure. Conclusions: A lifestyle intervention on the basis of a diet low in fat and high in complex carbohydrates and physical activity can outweigh the hypercholesterolemic predisposition with apo E4 allele carrier in women.

P 051

CORRELATION OF NUTRIENTS AND SERUM CHOLESTEROL CONSENTRATION DEPENDING ON APOLIPOPROTEIN E GENOTYPE

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Introduction: The interaction of some nutrients (saturated fatty acids, cholesterol, polyunsaturated fatty acids, soluable dietaty fibre) with serum cholesterol is well known by megastudies. Carrier of the apolipoprotein E4 allele in industrialized countries show significant higher serum cholesterol concentrations, and a higher prevalence of cardiovascular diseases compared to the wildtype ApoE E3/E3. To determine the influence of the geno-type of ApoE on the correlation between nutrients and lipid profile, four different homozygous and heterozygous manifestations of the apolipoprotein E were stratified. Methods: From a 7-day food record the nutrients for 100 participants were calculated, the serum cholesterol levels were measured and the ApoE genotypes were determined. Results: Apolipoprotein E3/E3, was pointed out as the most common apo E geno-type in this sample followed by E3/E4. Both heterozygous E2/E4 and homozygous E4/E4 polymorphism was present. The correlation between nutrients and lipid values showed a genotype dependent structure. Conclusions: A lifestyle intervention on the basis of a diet can outweigh the hypercholesterolemic predisposition with apo E4 allele.

P 052

Complex cardiac malformation with pulmonary atresia in a male infant with a 7q22 interstitial deletion

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We describe a male infant with an interstitial deletion on 7q22. The child was born at 36 weeks of gestation via cesarean section because of placental insufficiency. Manifestations include a complex cardiac malformation with pulmonary atresia, extremely hypoplastic pulmonary arteries, atrial - and ventricular septum defects (ASD,VSD) and atypical Ductus arteriosus Botalli. Main extracardiac manifestation comprised severe hypotrophy (birthweight 1260g), low set ears, under riding toes and digits and hypoplasia of the corpus callosum. Molecular cytogenetic analysis defined a deletion of 26 Mb at most. Reports on deletions of 7q22 in patients with cardiac malformations are rare. While some cases with ASD and VSD in patients with similar deletions have been described, no reports on conotruncal malformations and/or pulmonary atresia exist.

P 053

Pseudoxanthoma elasticum (PXE): A case report

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder of connective tissue, involving elastic fibers in the skin, the arteries and the retina. This results in laxity and loss of elasticity in the affected dermal areas, arterial calcifications with a predisposition to early onset arteriosclerosis and retinal haemorrhages leading to the characteristic angoid streaks and macula degeneration. Recently mutations in an ABC transporter gene, ABCC6, have been identified in PXE patients. Here we describe the clinical findings and the identification of two mutations in the ABCC6 gene in a 60 year old man with PXE, who presented with severe atherosclerosis of the peripheral and coronary arteries. The molecular diagnosis of PXE was complicated due to a neighbouring pseudogene. The implications for the molecular diagnosis of PXE are discussed.

P 054

Subtelomere FISH using a checklist detected rearrangements in 20% of patients, including a de novo balanced translocation with breakpoint near the CTNS gene at 17p13.3

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Segmental aneusomy is a common cause of malformation and mental retardation (MR). Recently cryptic rearrangements diagnosed by the FISH subtelomere test of the 41 unique chromosome ends (subtelomere FISH) or by alternative approaches have been recognized as a major cause of MR. Presently some 20 subtelomere screen studies including more than 2500 patients have been reported. These studies are not directly comparable because different techniques and patient ascertainment criteria were used, but a recent overall metaanalysis of 14 studies indicated that causal aberrations were found in 5.8% of patients (97 out of 1718; ranging 2-29%; 95% confidence interval 4.60-6.84). We report 20% (10 out of 50; confidence interval 10.03-33.72) cryptic rearrangements at the chromosome ends, all bona fide causal, found in a prospective subtelomere FISH study of 50 unrelated children ascertained using a clinical checklist. The study group comprised 30% children with mild (DQ 50-69) or yet unclassifiable MR due to young age and this subgroup also made up for 30% of detected chromosome aberrations. Familial unbalanced inversion or translocation was found in 5 patients, de novo deletion in two patients and de novo unbalanced translocation in two patients. Patient 5, who had a large head, vision defect, increased body hair, omphalocele, heart defect, enlarged kidneys, MR, speech defect and transient homocyst (e)inemia, showed a de novo balanced translocation of chromosomes 17p13.3 and 20q13.33. There has been no previous report of a balanced disease-associated rearrangement diagnosed by the subtelomere screen. We located his 17p breakpoint near CTNS, the gene for cystinosis. Dysfunction of CTNS could have caused or contributed to his homocyst (e)inemia. - Analysis of clinical data indicated that premature birth at 36 weeks of gestation occurred slightly more frequently in patients with vs without a rearrangement (p=0.097). Possibly adding this criterion would further improve the checklist. In conclusion, the checklist-assisted patient ascertainment for subtelomere FISH enabled finding the probable cause of the MR in 20% of patients. The approach was also helpful in the very young children with yet unclassifiable degree of MR.

P 055

Screening unclassified Fanconi anemia cell lines for mutations in BRCA2/FANCD1

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Biallelic mutations in the BRCA2 gene were recently recognized as a rare cause of Fanconi anemia (FA), explaining the underlying gene defect in patients of complementation group FAD1. Using BRCA2 immunoblotting we screened 37 lymphoblastoid cell lines from FA patients who could not be assigned to complementation groups FAA, FAC, FAD2, FAE, FAF and FAG via retroviral gene transfer. 3 of the 37 cell lines repeatedly failed to show a BRCA2 signal, whereas all other lines displayed the expected 380 kD band. Exon scanning sequencing of BRCA2/FANCD1 gene to date yielded two nonsense mutations, one 2-bp-deletion and one splicing mutation, all compound, in two of the three BRCA2-negative cell lines. The deletion and one of the stop mutations are listed in the Breast Cancer Information Core data base (BIC). Since most homozygous BRCA2 -/- knockout mice are embryonic lethals, it is likely that only certain types and combinations of biallelic mutations are compatible with survival (albeit affected by FA) in humans. Assuming a carrier frequency for BRCA2/FANCD1 mutations in the order of 1/250 in Germany, one would expect several hundred FAD1 patients in our population. However, close to 90% of the 200 plus known FA patients in Germany belong to complementation groups FAA, FAC and FAG, such that less than 20 of such patients could be due to biallelic mutations in BRCA2. The discrepancy between the expected and observed number of patients implies that biallelic mutations in BRCA2 contribute to spontaneous pregnancy loss in humans.

P 056

Analysis of unclassified FA cell lines for mutations in BRCA2

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Recently it was found, that biallelic mutations in BRCA2 are responsible for Fanconi Anemia (FA) complementation group FA-D1 and FA-B. Therefore we screened 42 unclassified lymphoblastoid FA-cell lines on western blot for expression of BRCA2/FANCD1. Three of the analyzed cell lines showed no detectable BRCA2/FANCD1-protein, why we started to sequence the BRCA2/FANCD1-gene of these patients. By now we found two stop-mutations, one deletion and one splicing mutation in two of the three examined cell lines. The deletion and one of the stop mutations are registered in the Breast Cancer Information Core (BIC) as breast cancer causing mutations, while the other mutations are unrecorded. Our data combined with the previous published FANCD1-mutations show no hot spot regions or specific mutations in the BRCA2/FANCD1-gene leading to Fanconi anemia.

P 057

Biomarker discovery and identification in laser microdissected head and neck squamous cell carcinoma with ProteinChip technology, two-dimensional gel electrophoresis, tandem mass spectrometry and immunohistochemistry

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Head and neck cancer is a frequent malignancy with a complex, and up to now not clear etiology. Therefore, despite of improvements in diagnosis and therapy, the survival rate with head and neck squamous-cell carcinomas (HNSCC) is poor. For a better understanding of the molecular mechanisms behind the process of tumorigenesis and tumor progression we have analyzed changes of protein expression between microdissected normal and tumor tissue by ProteinChip technology. For this cryostat sections from head and neck tumors (n = 57) and adjacent mucosa (n = 44) were laser-microdissected and analyzed on ProteinChip arrays. The derived mass spectrometry profiles (n = 47/41) exhibited numerous statistical differences. One peak significantly higher expressed in the tumor (p = 0.000029) was isolated by two-dimensional gel electrophoresis and identified as annexin V by in-gel proteolytic digestion, peptide mapping and Tandem MS/MS analysis. The relevance of this single marker protein was further evaluated by immunohistochemistry. In this study we could show that biomarker in head and neck cancer can be found, identified and assessed by combination of ProteinChip technology, 2DE, and immunohistochemistry. Such studies, however, make only sense if a relatively pure microdissected tumor tissue is used. Only therewith minute changes of protein expression between normal and tumor tissue can be detected and it will become possible to educe tumor associated protein pattern which might be used as a marker for tumorigenesis and progression.This work was supported by the BMBF.

P 058

Analysis of proteomic differences between normal colon mucosa and colon adenomas as well as colon carcinomas by tissue microdissection and protein chip array technology

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To establish proteomic patterns of the molecular cancerogenesis in the colon deep frozen (-80°C) tissue samples from normal colon mucosa (n = 18), colon adenomas (n = 21), and colon carcinomas (n = 19) were microdissected from unstained histological sections with Laser microdissection and pressure catapulting (LMPC; PALM). The crude protein extracts were analyzed on a strong anionic exchanger (SAX2) Pro-

teinChip Array in a ProteinChip Reader (SELDI PBS II; Ciphergen Biosystems). In colon adenomas prominent new peaks – not detectable in normal mucosa – appear at 15.1 kDa, 15.9 kDa, 24.6 kDa and 41.7 kDa. Expression of proteins with mw of 4.7 kDa, 7.6 kDa, 9.1 kDa, 10.1 kDa, 10.3 kDa and 24.6 kDa is up regulated in comparison with the normal mucosa. No down regulated peaks are recognizable. In carcinomas proteins with mw of 5.6 kDa, 10.5 kDa, 11.3 kDa, 12.0 kDa and 14.0 kDa are up regulated, whereas peaks at 5.0 kDa, 8.4 kDa, and 9.0 kDa are down regulated. The results indicate that protein patterns of the normal colon mucosa, colon adenomas and carcinomas are obviously different and correlate with benign and malignant epithelial growth in the colon. This allows the identification of proteins and the estimation of their biological significance associated with development and progression of colon neoplasias. Bioinformatics and further analyses to identify these proteins are in progress. Furthermore the results demonstrate the high importance of the combination of tissue microdissection and protein chip array technology in cancer research.This work was supported by the BMBF.

P 059

Molecular cytogenetic characterization of a de novo translocation (7;10) in a patient with some features of MATCHS syndrome

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We report a 4 year old girl who was investigated for developmental delay, hypotonia and macrocephaly. She had a bossed forehead, downslanting palpebral fissures, a broad flat nasal tip, thoracic kyphosis and slight pectus. Her hands and feet were small and slender. She had significant speech delay and oromotor dysfunction with swallowing difficulties. Chromosome banding analysis revealed a de novo translocation 46,XX,t (7;10)(q34;q23.3). Following FISH with region-specific BAC clones from chromosome 7q33-q36.1 and 10q22.1-24.1, we identified breakpoint-spanning clones on both derivative chromosomes. In addition to the reciprocal translocation, deletion of a 7 Mb segment from 7q34 had occurred. The deleted region lies about 4 Mb distal to the breakpoint on the derivative 10. It is known that 10-30% of apparently balanced de novo translocation in patients with a human genetic disease are associated with microdeletions up to several megabases in length, however these deletions are usually directly in a breakpoint region (s). The patient´s phenotype may be caused by haploinsufficiency of a dosage-sensitive gene (s) in the deleted region. Alternatively, it could be due to disruption of the PTEN gene at the chromosome 10 breakpoint. Mutations in PTEN are associated with MATCHS (macrocephaly, autosomal dominant, thyroid disease, cancer, harmartomata and skin abnormalites) and this patient has some features compatible with a diagnosis of MATCHS. Studies are underway to confirm inactivation of PTEN. To our knowledge this is the first reported case of a de novo translocation with a deletion at some distance to the translocation breakpoint.

P 060

Evidence for a novel connexin 26 compound heterozygous mutation resulting in deafness

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Mutations of the gap junction beta 2 (GJB2) gene coding for the protein connexin 26 are known to be a major cause of nonsyndromic sensorineural hearing loss (NSHL). A family consisting of normal hearing parents and two children with NSHL was analyzed for Cx26 mutations. Peripheral blood lymphocyte DNA was used to amplify the Cx26 coding region by polymerase chain reaction, followed by complete sequencing. Phenotypic characterization including audiometric testing was completed for both children and their parents. The two affected children were found to be compound heterozygous for the very frequent mutation 35delG and a previously unreported nucleotide substitution 40A->G leading to substitution of an evolutionary conserved amino acid (N14D). The parents were each heterozygous, specifically the father a 35delG heterozygote and the mother a 40A->G heterozygote. Currently, 100 normal hearing control individuals are screened for the presence of the novel nucleotide substitution. The information based on conservation of amino acid residues and coexistence with a second GJB2 mutation suggests that this missense change should be responsible for the deafness phenotype.

P 061

Molecular cytogenetic analysis of a patient with de novo translocation 46,XY,t (1;6)

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We present a 8-year-old boy with macrocephaly, development problems, a cardiac anomaly (atrial septal defect) and recurrent infections. He has very distinct physical features: hypertelorism with blue sclerae and small palpebral fissures, prominent nose, thin tented upper lip, small and low set ears. His hands showed fetal finger pads more marked on the 3rd and 5rh finger and he also had overlapping 3rd and 5rh toes. Cytogenetic analysis demonstrated an apparently balanced de novo translocation (1;6)(q32;q22). FISH with region-specific large-insert clones identified a breakpoint-spanning BAC, RP11-338c15 on chromosome 1q32.3 and a breakpoint-spanning PAC, RP1-157N22 on chromosome 6q22.31. The breakpoint region on chromosome 1 contains the Activating Transcription Factor 1 (ATF1) gene, a cyclic-AMP-dependent transcription factor, and two genes with unknown function: The breakpoint PAC on chromosome 6 contains only one putative gene predicted by genescans. Molecular analyses are underway to show whether the patient´s phenotype is caused by inactivation of a gene (s) in the breakpoint region.

P 062

A rare form of partial trisomy 20p - accompanied by jumping translocations

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Cases of partial trisomy 20p are not uncommon and mostly caused by duplications of (20)(pter->p11 to q11) due to familial translocations.We report on a newborn girl who was referred for karyotype analysis because of VSD, ASDII with aneurysma of the cardiac septum and a dystopic kidney. The child was born after an uneventful pregnancy at term by caesarean section. Birth weight was 2650 g (3-10 %), lenght 53 cm (75-90 %) and head circumference 35 cm (50-75 %). Examination at 5 weeks shows a length of 56 cm (50 %), a reduced weight of 3400 g (3-10 %) and OFC of 37,5 cm (75-90 %).Several dysmorphic features were found: brachycephaly, a round and flat face, depressed nasal bridge, anteverted nares, flat philtrum, mild retrognathia, posterior rotated ears, wide-spaced nipples, ventral placed anus and single palmar crease.The parents are healthy and nonconsanguineous (the mother was 33 years, the father 49 years, respectively). The mother had had 3 abortions.The karyotype analysis of the child showed a de novo partial trisomy 20p caused by an isochromosome i (20p) and “jumping translocations” of the long arm of chromosome 20 to the telomers of 5p, 6q,11p and 17p. The breakpoint is located in 20q11.1 verified by FISH analysis with probe CEP20 (D20Z1) which could not detect any chromosome 20 centromer-alphoid sequences at the translocation chromosomes.This is the first report of an isochromosome for the short arm and jumping translocations involving the whole long arm of chromosome 20.Similar rearrangements have been reported rarely in the literature. Reddy et al. (2000) described a patient with multiple distinctive craniofacial features caused by partial trisomy 9 with an isochromosome i (9p) and jumping translocations of the whole long arm and Marques-de-Faria and Hackel for chromosome 12 (1989). Possible mechanismens for these postzygotic rearrangements are discussed.

P 063

The Hypermobility Syndrome

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Hypermobility of multiple joints is a feature common to many Heritable Disorders of Connective Tissue (HDCT). The vast majority of hyperlax individuals, particularly children, are free of further symptoms. However, there is also a small group of patients, who experience frequent and severe pain since early life. This combination of joint hyperlaxity and symptoms including arthralgia, back pain, dislocations and sprains has been called „Hypermobility syndrome“ since the first publication of Kirk in 1967. It is most often seen in the Benign Joint Hypermobility Syndrome (BJHS), which some authors consider to be identical to the hypermobility type of Ehlers-Danlos Syndrome (formerly EDS III). It is rarely noticed in the genetic and pediatric literature. We therefore report on a 16 years old female patient with recurrent pain be-

ginning at the age of ten years in the knees and spreading to the ankles, wrists, fingers, shoulders and back during the next years. The pain increased with use of the joints and after intensive physiotherapy. Rheumatoid arthritis was ruled out by X-rays and blood tests. She received psychological therapy for several months and was unjustifiably accused of malingering and hypochondria. We diagnosed hypermobility according to the Beighton criteria with a score of 7/9. In addition she has striae on the tighs and two thin, but not papyraceous scars. We urge to consider hypermobility syndrome in the differential diagnosis of any patient with unexplained chronic musculoskeletal pain. ReferencesBeighton PH, Grahame R, Bird HG. Hypermobility of joints. 3rd ed. London, Heidelberg, Springer Verlag 1999.Grahame R. Pain, distress and joint hyperlaxity. Joint Bone Spine 2000,67:157. Kirk JH, Ansell BM, Bywaters EGL. The hypermobility syndrome. Ann Rheum Dis 1967,26:425.

P 064

Detection of a partial trisomy of the telomere-associated region of 11p and a partial monosomy of the telomere-associated region of 11q as a cause for Jacobsen Syndrome

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A neonatal thrombocytopenia (<150,000 thrombocytes/µl) occurs in 1% of all newborns. The differential diagnosis includes infections, disseminated intravascular coagulation, immune-mediated thrombocytopenia, iatrogenic factors (drugs) and genetic bone marrow failure syndromes. Here we report on a premature infant born in the 32nd week of pregnancy with complex dysmorphic abnormalities including facial dysmorphia, partial agenesis of the corpus callosum, pulmonary hypertension, ureteral stenosis and thrombocytopenia associated with distinct dysmegakaryopoiesis. Cytogenetic analysis revealed a structural aberration of the long arm of one chromosome 11. On this chromosome, FISH (fluorescence in situ-hybridisation) analyses showed the loss of the telomere-associated region of the long arm being replaced by additional material from the telomere-associated region of the short arm. The resulting partial monosomy 11q and the partial trisomy 11p could be confirmed by CGH analysis (comparative genomic hybridisation). Jacobsen syndrome is a contiguous gene syndrome caused by deletions of 11q of variable size. In the majority of patients breakpoints occur within the fragile site in 11q23.3. The largest deletions described so far extend from 11q23 to 11qter. The clinical appearance depends on the size of the deleted region: craniosynostosis and cardiac defects are present when the deletion involves the region distal of 11q23. In the case of smaller deletions, only thrombocytopenia and facial dysmorphisms are present. This indicates that candidate genes for differentiation and maturation of thrombocytes may be located distal of the marker D11S933. Our findings support the assumption that defects of these genes cause thrombocytopenia. Thus, we show that a new genetic variant may be responsible for the phenotype of Jacobsen syndrome. Moreover, we discuss the differential diagnosis of congenital aberrations leading to defects of thrombopoiesis and finally to thrombocytopenia.

P 065

Genetic Diagnostic of the GJB2-Gene: Retrospection of one year experience

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Mutations in GJB2 gene encoding the connexin 26 protein are the most common cause of familial, non-syndromal sensorineural severe hearing impaiment (HI). Moreover, in sporadic non-familial cases of HI high frequencies (11-37%) of GJB2 mutation carriers are observed.Due to its small size of the coding sequence, the GJB2 gene can conveniently be screened for alterations at relatively low expenses. Therefore, the university clinic of Tübingen offers a diagnostic testing of this gene.In 2002, 40 patients with severe sensorineural HI underwent a molecular genetic testing for GJB2. In all cases the coding region of GJB2 was analyzed by sequencing.Mutations in GJB2 were detected in 35% (14/40) of patients. While 12.5% (5/40) displayed two mutations, in 22.5% (5/40) only one mutation was observed. Mutations in a further connexin gene, GJB6 encoding connexin 30, were previously shown to occur in a compound heterozygous manner with GJB2 mutations. Furthermore, regulatory defects, e.g. mutations in the GJB2 promotor could be involved in manifestation of hearing impairment in these patients. Because of the high frequency of single mutation carriers, these two regions should also be considered for diagnostic testing.

P 066

Low incidence of PDS mutations in patients displaying non syndromic hearing impairment and German controls

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Pendred syndrome is the most common form of syndromic deafness characterized by congenital sensorineural hearing loss, thyroid goiter, and positive perchlorate discharge test. Recently, this autosomal recessive disorder was shown to be caused by mutations in the PDS gene, which encodes an anion transporter called pendrin. In addition, patients demonstrating non syndromic hearing impairment also displayed mutations within this gene. Therefore, we analyzed 200 German patients with non syndromic, severe to profound hearing impairment as well as 100 control individuals for three frequently observed mutations in PDS. The DNA changes E384G, T416P, L236P, and donor splice site mutation 1001+1G®A were analyzed using Readit, SSCP and restriction digests.None of these mutations was detected in control individuals indicating a rare (0/100) occurence in the German normal population. Although described as frequently involved in other populations, the three mutations analyzed also demonstrated a low frequency (1/200) in the German patients.

P 067

Additional partial trisomy 17q in a de novo translocation (3;11) in a newborn with severe cardiac anomalies

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We report on a male newborn with partial trisomy 17q in a de novo translocation (3;11), recognized by a late prenatal diagnosis. He is the second child of unrelated parents. No genetic disorders in the family are known. Sonographic examinations at 12th week of gestation revealed a fetal nuchal translucency of 5,1 mm. Recommanded investigations like CVS or amniocentesis were refused by the parents until 31th week of gestation when further ultrasound anomalies like a complex heart defect, brain malformations and a general growth retardation of the fetus were found. Cytogenetic investigation of amniotic cells showed a de novo translocation t (3;11)(p14.3;qter) which was confirmed by FISH analysis. Due to the severe pathological findings a chromosomal unbalance was assumed. Parents accepted further cytogenetic investigations only after birth. The patient was born at term, birth weight was 2250g, length 46cm, head circumference 29,5cm. He shows a complex heart defect as a single ventricle with double outlet, dysmorphic facial features, cleft palate, bilateral low grade kidney tissue differentiation as judged sonographically, kryptorchism and club feet. High resolution banding and multicolor FISH revealed an additional segment of chromosome 17q on the derivative chromosome 3 leading to the following karyotype with no apparent unbalance of chromosome 3 and 11 material.46,XY.ish der (3)t (3;17) (3qter-3p14.3:: 17q23.2-17qter)(wcp3+,wcp11-, wcp17+, D3 S4559-, D34560+),der (11)(11pter-11qter:: 3p21.2- 3pter)(wcp3+,wcp11+,wcp17-, 3S4560+, D3S6571+,ViJ2yRM2072+) Most of the phenotype features of partial trisomy of comparable chromosome 17q segments according to the literature are present in the patient.

P 068

The 342-kb deletion in GJB6 is not present in patients with non-syndromic hearing loss from Austria

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Hearing loss (HL) is the most prevalent inherited sensory disorder. About 1 in 1000 children are affected by moderate to profound hearing impairment. The incidence of HL in children is much higher if mild to moderate HL is included. An unparalleled heterogeneity with more than

100 genes is estimated to be involved in deafness. However, mutations in one single gene, the connexin 26 gene (GJB2), account for up to 69% of autosomal-recessive non-syndromic hearing loss (NSHL) and up to 37% of cases with sporadic NSHL in various populations. Mutations in other genes are rare causes of HL with the exception of a 342-kb deletion involving GJB6 (connexin 30 gene). This deletion has been reported to be the second most common mutation causing prelingual NSHL in Spain, and was frequently observed in patients from France and Israel. It results in NSHL in homozygous status and, furthermore, in heterozygousity with a GJB2 mutation. We screened 331 patients with NSHL being negative or heterozygous for GJB2 mutations for this GJB6 deletion using a multiplex PCR. All probands were of Austrian origin. None of them was carrying the deletion in GJB6 indicating that the occurrence of this deletion is restricted to certain populations.

P 069

Branchio-Oto-Renal (BOR) syndrome in a patient with mental retardation, visual impairment and complete bilateral absence of nipples

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BOR syndrome is a rare autosomal dominant disorder that is caused by mutations of the EYA1 gene. Clinical expression is highly variable however typical manifestations include anomalies of structures derived from the branchial arch, like preauricular pits, pinnae abnormalities, hearing loss and renal hypoplasia. The prevalence is about 1:40.000 in the general population and about 2% of deaf children. Some of the patients with BOR syndrome show a contiguous gene deletion syndrome involving the EYA1 gene locus at 8q13.3 and also resemble features of Duane syndrome, which was mapped to the same chromosomal region. A second BOR locus at 1q31 was recently identified by linkage analysis of a large family with BOR syndrome where renal anomalies are absent. This variant form is therefore also called branchio-otic (BO) syndrome. Here we report on a severely impaired 8 year old male patient, who shows all characteristic features of BOR syndrome fully expressed except a serious renal anomaly. He rather has bilateral slightly smaller kidneys still within the lower normal size range. On the other hand he shows several additional anomalies, some of them were either not or extremely rarely reported in BOR syndrome patients including some distinct dysmorphic features, severe mental retardation, visual impairment, choanal atresia and absence of nipples. No further family members show any of these anomalies or symptoms of BOR or BO syndrome. Because of this plethora of abnormalities in the patient an unbalanced chromosomal aberration was suspected. An obvious anomaly like a larger deletion could be ruled out by routine cytogenetic and FISH analysis using a BAC clone from the EYA1 gene locus however the presence of a cryptic aberration as a microdeletion at 8q13.3 or 1q31 is still not unlikely. Since bilateral absence of breast and nipples is a very rare anomaly but at least one patient with a combination of this and BOR syndrome has already been described it seems reasonable to assume that a gene locus for an autosomal dominant form of athelia might be located close to one of the BOR or BO syndrome gene loci

P 070

Clinical features of 6 patients with LEOPARD-Syndrome and mutation analysis of the PTPN11-gene

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LEOPARD-Syndrome (LS) is a rare autosomal dominant disorder characterized by multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia in males, retardation of growth, and sensorineural deafness. Many of these features including facial dysmorphism are shared with Noonan syndrome (NS) and mutations in the PTPN11-gene have been found in both conditions. We report clinical features of 4 sporadic patients (patient 1-4) and one family with at least 2 affected family members (patients 5 and 6) with a diagnosis of LS. Patient 1 had been evaluated by neuropediatricians because of developmental delay prior to development of lentigines. Patient 3 was only diagnosed after cardiac arrest at age 26. The other patients were seen for evaluation of café-au-lait spots and/or multiple lentigines. Analysis of the PTPN11-gene revealed a Tyr279Cys mutation in patients 1, 2 , 5 and 6 and a Thr468Met mutation in patients 3 and 4. Both mutations are known to be associated with LS. All patients had facial features of NS. Echocardiography revealed a hypertrophic cardiomyopathy in patient 3. Patient 1 and 4 had a mild aortic valve insufficiency. However, no patient had a pulmonary stenosis. Profound sensorineural deafness was present in patient 2 and a very mild sensorineural deafness could be diagnosed in patients 3 and 5.

P 071

MR/MCA-Syndrome due to a familial translocation of chromosome 18p and 20q

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We present a dysmorphic and retardet child at age 13 months. The boy was born after uneventfull pregnancy. In retrospect, upslanting palpebral fissures, a broad nose and broad nasal tip could noted. During the second pregnancy the mother introduced to us because of unclear retardation of her first child. At age 13 month the boy could not sit, walk and did not speak any words. Chromosome analysis of periphere lymphocyte cultures revealed a suspected short arm of chromosome 18. FISH analysis using telomere probes (ToTelVysion Probes,Fa.Vysis) presented a trisomy of 20q tel and a monosomy of 18p tel. Further investigations demonstrated a familial reciprocal balanced translocation of 18p and 20q. Using telomere probes,a rapid and save prenatal diagnosis could carried out. Other members of the family were diagnosed to carry the balanced translocation. The clinical and cytogenetical results are discussed.

P 072

A SALL4 missense mutation causes preaxial polydactly, hypophyseal hypoplasia, and Type II Duane anomaly.

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Okihiro syndrome consists of radial ray anomalies in combination with Duane anomaly which is caused by agenesis of the VI. cranial nerve and/ or its nucleus in the hindbrain and misinnervation of the lateral rectus muscle of the eye by fibres of the oculomotor nerve (N. III). Okihiro syndrome results from truncating mutations in the putative zinc finger transcription factor gene SALL4 on chromosome 20q13.13-13.2. Similar to SALL1 mutations in Townes-Brocks syndrome, missense mutations within the SALL4 gene have not been observed in Okihiro syndrome, raising the question if such mutations would be pathogenic and if so, which phenotypic features would be associated with them. Here we describe a missense mutation in the SALL4 gene which is the first pathogenic missense mutation found in a SALL gene. The mutation results in an exchange of a zinc-coordinating Histidine within a C2H2 double zinc finger domain which is crucial for zinc finger structure. The mutation was found in a patient who shows unilateral incomplete preaxial polydactyly, hypophyseal hypoplasia and a single central incisor. Most notably, however, the patient has no Type I Duane anomaly as typical for Okihiro syndrome patients with limited abduction but displays limited adduction with only slight limitation of abduction (Type II Duane anomaly). Based on this observation, the phenotype of SALL4 gene missense mutations seems very difficult to predict, and such mutations might not be identified in typical Okihiro syndrome patients.

P 073

High incidence of the R276X SALL1 mutation in sporadic but not familial Townes-Brocks syndrome and report of the first familial case

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Townes-Brocks syndrome is a rare autosomal-dominant malformation syndrome caused by mutations in the SALL1 gene on chromosome 16q12.1. All mutations identified so far are truncating, i.e. nonsense mutations, short deletions, short insertions and one splice site mutation. Previously, one mutation (826C>T; R276X) has been detected in seven independent families with sporadic TBS but not in families with inherited TBS. We have detected the R276X mutation in eight additional families with sporadic TBS, and found that it causes TBS in 15 out of 29 cases of sporadic TBS with detectable SALL1 mutations. The absence of the mutation in familial cases of TBS with SALL1 mutations had previously raised the question if the R276X mutation results in infertility, which proved to be a problem for genetic counseling of carriers of this mutation. Here we present the first two generation family with the R276X mutation. Both the mother and her newborn daughter have typical TBS but the daughter is more severely affected. Comparison of all known cases with the R276X mutation suggests a higher incidence of congenital heart defects (including two cases with Tetralogy of Fallot previously not observed with SALL1 mutations) as compared to other SALL1 mutations. Therefore, the low frequency of the R276X mutation in familial cases is not due to absence of fertility at least in females but might result from reduced genetic fitness of mutation carriers.

P 074

X-linked inheritance of corneal endothelial dystrophy in a large family reveals further genetic heterogeneity of this condition

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The endothelial (posterior) corneal dystrophies, which result from primary endothelial dysfunction, include autosomal dominant Fuchs´ endothelial dystrophy (FECD; MIM 136800), posterior polymorphous dystrophy (PPCD; MIM 122000) and congenital hereditary endothelial dystrophy in which an autosomal dominant (CHED1; MIM 121700) and an autosomal recessive form (CHED2; MIM 217700) have been described, and which are all thought to represent defects of neural crest terminal differentiation. We studied 26 individuals from a large Austrian family who were variably affected with features of a corneal endothelial dystrophy. The phenotype ranged from scattered vesicles that went unnoticed throughout life to a congenital endothelial dystrophy requiring a graft in early childhood. In this family, women were less severely affected from corneal alterations than men. In addition, no instance of male-to-male transmission of the disease was encountered establishing X-linked inheritance of the corneal dystrophy in this family. While mutations in COL8A2 and VSX1 account for a percentage of FECD and PPCD cases, respectively, the mapping of the disease gene in the family reported here can provide additional insight in the function and dysfunction of the corneal endothelium.

P 075

A new case of ring chromosome 12

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Constitutional ring chromosome 12 is a rare structural aberration. We report on a 10 month old girl with a ring chromosome 12. After an uneventful pregnancy the girl was delivered at 40 weeks of gestation. The birth weight (3090 g), height (51 cm) and head circumference (34,5 cm) were within the normal range. The girl is the third child of a 34 year old woman. At the age of 5 months, she presented with respiratory problems. The only clinical findings were a developmental delay (4370g/62 cm/39,5 cm) and a cardiac myopathy with dilatation. Cytogenetic analysis of peripheral blood lymphocytes revealed a mosaic karyotype. Most of the metaphases showed a ring chromosome 12 (75%) while 22% of the metaphases presented a normal female karyotype, and the remaining 3% of the cells showed monosomy 12. In a small number of cells two ring chromosomes (1.5%) and double rings (0.5%) were seen. FISH analysis with subtelomeric probes of chromosome 12 revealed the loss of subtelomeric regions on both arms. A locus specific probe assigned to chromosomal region q24.2 to 24.3 hybridised to the ring chromosome, thus suggesting the breakpoint on the long arm is in q24.3. Based on these studies the breakpoints were interpreted as r (12)(p13.3q24.3). Ring chromosome 12 is a rare abnormality with only seven patients reported in the literature. The clinical findings observed are microcephaly, mild mental retardation and some minor dysmorphisms (e.g. low-set ears, clinodactyly, unusual or single palmar creases, café-au-lait spots). In all these cases the breakpoints were assigned to the most telomeric regions which results in minimal deletion of chromosomal material. This is similar to our case with only few clinical manifestations.

P 076

Mosaicism of partial Tetrasomy 14q: case report and review of the literature

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The third boy of a healthy turkish consanguin couple was born after an uneventful pregnancy with weight 2490g (3. centile), length 48 cm (10. centile) and head circumference 34 cm (10. centile). The boy presented with the following facial dysmorphisms: High arched eye-brows, epicanthal folds, broad and high nasal bridge, bulbous nasal tip, flared nostrils, thin lips and retrognathia. On his left hand a simian crease was present. At age 6 weeks he developed inspiratory stridor and vomiting. Further investigations showed an omphalocele and abnormally wide placed nipples. An eye examination proved bilateral coloboma of the iris. Echocardiography showed a minimal insufficiency of the mitral valve. MRI-scan of the brain demonstrated a thinning of the corpus callosum. Karyotype analysis from lymphocytes revealed a marker chromosome in part of the mitosis which originated of an isochromosome 14q proximal. Lymphocyte studies of the parents showed a normal female and male karyotype, respectively. FISH with the ?-satellite probe for the centromeric region of chromosome 14 and 22 showed two signals in the marker chromosome with sufficient discrimination between the active and inactive centromere. Thus, the boy´s karyotype raised from lymphocyte cultures was 47,XY,+i.ish i (14)(pter-q13 :: q13-pter)[16]/46,XY[47], QFQ, GTG. A partial tetrasomy 14q was verified. Re-examination of our patient at age 10 months showed a marked psychomotor delay, he was unable to sit idependently and showed no speech development. His body measurements were below the third centile. Currently, interphase analysis is performed on buccal smear to further investigate the distribution of the 2 cell lines.An identical tetrasomy of the region 14pter-14q13 has only been reported once in the literature. In this case ultrasound abnormalities and the abnormal karyotype led to a termination of the pregnancy in the second trimester. The fetus showed ventriculogemaly, talipes, camptodactyly and clinodactyly. Mild facial dysmorhisms included a prominent nasal bridge, a protuberant forehead and low-set ears with posterior angulation. There are some common features with our patient.

P 077

Bleomycin vs. ionizing radiation in diagnostic testing for Ataxia telangiectasia

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Increased cellular radiosensitivity is a hallmark of ataxia telangiectasia (AT), AT-like disorders and the Nijmegen breakage syndrome. Large size and a detection rate below 100% render routine mutation analysis of the ATM gene difficult. Functional tests such as measuring the cellular response to ionizing radiation (IR) employ g-rays or so-called radiomimetic agents, of which bleomycin (BM) is most widely used. BM is a mixture of closely related glycopeptide antibiotics isolated from Strept. verticillus and has been shown to induce a type of DNA damage similar to that of IR. We used two-parametric flow cytometry to compare radiation- vs. BM-induced G2 phase arrest relative to the growth fraction (GF) in 72 h in vitro lymphocyte cultures of 7 AT and 39 control blood samples. IR dosage of 1.5 Gy ensured optimal discrimination between AT resp. AT-like disorders (G2/GF: 0,41+0,13) and control samples (G2/GF: 0,09+0,03). BM at 1 mg/ml was also capable of discriminating AT from control cells (sensitivity 100% in our study). However, discrimination was less pronounced and inter-sample variation was greater using BM compared to IR (G2/GF[AT]: 0,36+0,07; G2/GF[control samples]: 0,15+0,06). The aminothiols WR-1065 (amifostine) and cysteamine are potentiators of BM and showed ID50 values of 7 µM and 100 µM, respectively, in our study. Used at concentrations well below these values, preliminary results show that these compounds may allow to improve the discrimination of AT in the BM diagnostic assay. Differences in the composition of various BM preparations, different oxidative activation of DNA-

bound BM depending of the redox status of cultured cells, variations in the intracellular activity of BM hydroxylase and greater non-specific toxicity of BM than IR may all contribute to the superior performance of ionizing radiation in diagnostic testing for A-T.

P 078

Spectrum of Mutations in the Connexin 26 Gene among 142 Czech Patients with Congenital or Prelingual Deafness

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Background: Mutations in Connexin 26 gene (Cx26) are the most common known cause of congenital and prelingual deafness. The mutation 35delG is by far the most common in Cx26 in the Caucasian population. No data about Cx26 mutation-spectrum from a larger group of Czech patients with congenital deafness were available before our study. In 2002 we published the high frequency of 35delG mutation among 29 Czech cochlear implant users. We further observed the 35delG heterozygous frequency in Czech hearing population to be 1: 29,6, similar to central Europe. Now we report the spectrum of mutations in the Cx26 gene in a serie of 142 Czech congenital deafness patients, including the previously reported cochlear implant users.Methods: Allele specific PCR together with the fluorescent fragment analysis were used for the detection of 35delG mutation. Direct sequencing of the entire coding region of the Cx26 was performed in the patients, which were not homozygous for the 35delG mutation.Results: 178 individuals including 142 patients with congenital or prelingual deafness and their 36 healthy relatives were tested. No causal mutation in Cx26 gene was detected in 70 out of 142 deafness patients (49,3 %), including 6 patients with a harmless polymorphisms only (Val153Ile and Arg127His). Both disease-causing mutations were detected in 58 out patients (40, 8 %). Only one pathogenic mutation was detected in further 14 patients (9, 9 %), so at least one causal mutation in Cx26 gene was found in 72 out of 142 patients (50, 7 %).Among the 58 patients in whom both disease-causing mutations were detected, the allele frequencies for the mutations were as follows: 35delG on 95 alleles (82%), T24X on 12 alleles (8,4 %), 313del12 on 5 alleles (3,5 %). Mutations: 358delGAG, Lys15Thr, Glu147Lys, 340kb deletion involving the Cx30 gene were found once each in patients with both causal mutations.Besides the 35delG, following mutations were further detected in patients carriing one mutation only and in the patients relatives: Met34Thr, Leu90Pro, Val37Ile, Lys221Asn.The mutation W24X was in all cases detected in the patients of the Gipsy origin. A digenic origin of the congenital deafness was detected in one patient with a 313del12 mutation in the Cx26 gene together with the 340kb deletion involving the Cx30 gene. Both normaly hearing parents of this patients are heterozygous for each of the mutation. Neverthless, the Cx30 deletion seems to be very rare in the Czech population, since it was not found in any of the 600 control chromosomes.Conclusions: Spectrum of mutations in Cx26 gene in the Czech population was evaluated. Mutations in Cx26 gene are a very frequent cause of autosomal recessive prelingual hearing loss in the Czech population, similarly to other European countries. 35delG is by far the most common mutation in Cx26 in Czech population and was found in homozygous state in 30,3 % (43 out of the 142) of Czech patients with prelingual deafness. Surprisingly, the W24X mutation found in Gipsy patients is the second most common mutation.Supported by IGA NM 7417-3 and by VZ 11100003

P 079

Supernumerary der (1) marker chromosome containing the original centromere and euchromatin from 1q21.1->q21.3 characterized by reverse painting and FISH

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The female patient described here showed dystropy and marked dysmorphic stigmata at birth; and at the age of 9 months developmental delay of at least 2 - 3 months without growth retardation was noticed. Cytogenetic analysis detected a very small supernumerary marker chromosome in almost 100% of PHA stimulated cultured lymphocytes. The marker presented as uncharacteristically pale after G-banding and seemed telocentric by C-banding in more than 95% of metaphases examined. Only in few metaphases the marker had the appearance of a ring chromosome. To identify the chromosomal origin, 15 copies of the marker were collected by microdissection. Reverse painting of this microdissection library gave strong FISH signals flanking the 1q12 heterochromatic region. CISS hybridization with a whole chromosome paint 1 resulted in strong signals on the whole marker. Localized FISH probes from proximal 1p and 1q helped to more precisely identify the contents of the marker: The marker consists of the centromere and euchromatin from q21.1-q21.3 representing a segment between 5 and 7 Mb, the q12 heterochromatin was reduced to a still detectable minimum. No euchromatin from 1p could be detected on the marker. BACs RP11-337-c18, RP11-458i7, RP11-363i22, and RP11-674d19 presented each one single FISH signal both on the normal chromosomes 1 as well as on the marker suggesting that the marker contains only a single dose of the corresponding genomic region. In contrast, BACs RP11-30i17, RP11-300l20, and RP11-21j4, and YAC 966c10 hybridized each to a single locus on the normal chromosomes 1, whereas on the marker they presented two distinctly separated FISH signals suggestive of a duplication. These findings imply a multi-step process of consecutive structural changes during formation of this marker. Even regarding a „ring-history" of the marker, the observed single versus double FISH signals were a consistent property of each clone tested and did not vary from cell to cell. Therefore, our data disclose the structure of a relatively stable marker chromosome derived from a ring chromosome and do not support a variable behaviour of an unstable ring chromosome itself.

P 080

An unusual reciprocal translocation detected by subtelomeric FISH:Interstitial and not terminal.

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An 11 month-old boy with a pattern of dysmorphic signs, an ASD, right inguinal hernia, bilateral undescended testes, bilateral urinary reflux and right renal dysplasia, and developmental delay revealed an abnormal chromosome 11 with enlarged terminal long arm in his karyotype. The paternal karyotype was normal 46,XY, while the mother showed the same 11q+ and in addition a 2q- chromosome. Chromosome paiting with chromosome 2 and 11 libraries (Vysis, Inc., Downers Grove, IL, U.S.A.) confirmed a reciprocal translocation with a tiny 11 signal on 2q and a larger 2 signal on 11q. Thus, a reciprocal terminal exchange was assumed resulting in duplication of distal 2q material and a small subterminal 11q deletion. The karyotype of the mother would thus be 46,XX,t (2;11)(q35;q24.3). Fluorescence in situ hybridisation with 2q and 11q subtelomeric probes (Vysis, Inc., Downers Grove, IL, U.S.A.) was performed in order to confirm the reciprocity of the translocation. However, to our great surprise we did not find the expected pattern, namely an 11q signal on the rearranged 2 and a 2q signal on the rearranged 11. Instead, there was no signal on the 2q- while the 11q+ contained both the 11q signal and, more proximally, the 2q signal. Thus, it turned out that the translocation was not an terminal reciprocal exchange, but was interstitial on both chromosomes which is highly unusual since most interstitial translocations are non-reciprocal. Thus, the results "moved" the aneuploid segments to a more proximal position, especially the deleted 11q segments since it does not contain the subtelomeric FISH region. Microsatellite marker analysis confirmed a subterminal 11q deletion in 2 informative constellations and was suspective for a 2q duplication in one informative marker. The molecular cytogenetic results in this family show that reciprocal translocations should be investigated not only with whole chromosome paiting, probes but also with further probes for the subtelomeric region since, for genetic counseling, karyotype-phenotype correlation and gene mapping it is of increasing importance to determine the breakpoints and aneuploid segments as precise as possible.

P 081

Premature ovarian failure in an otherwise normal woman with de novo unbalanced X/autosome translocation characterised by subtelomere screening

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We describe a thirty-one year old woman with a de novo unbalanced t (X;1) that resulted in a deletion of Xq26.3-qter and a trisomy of 1q42.1-qter. To our knowledge, only a few cases of pure trisomy 1q42-qter have been reported. The delineation of a distinct phenotype of these cases remains doubtful and difficult because of coexisting deletions of other chromosomes. Nevertheless, some clinical findings like macro-

cephaly, and more unspecific minor craniofacial dysmorphisms, intrauterine and postnatal growth retardation, developmental delay, and cardiac defects have been reported constantly. Although being trisomic for the region 1q42.1-qter, our patient lacked any of the above mentioned features. She was referred for chromosome analysis because of the diagnosis of premature ovarian failure. Conventional chromosome analysis was done on the patient and her parents and revealed an altered X chromosome in the majority of the metaphases. By the application of FISH with X-specific library, with a subtelomeric probe set and with a probe generated by microdissection of the aberrant X chromosome the patient`s chromosomal imbalance could be interpreted as de novo partial trisomy 1q, present in all analysed mitoses. This discrepancy between karyotype and phenotype suggested the spreading of X inactivation throughout the translocated segment of 1q. The premature ovarian failure of our patient can most likely be explained by the distal deletion of the long arm of the X chromosome. The maintenance of genes and gene order within the „critical regions" on Xq have been proposed for a normal ovarian function. The exact location of the breakpoint on Xq and the parental origin of the translocation, for which exclusively paternal derivation has been demonstrated, are being determined.

P 082

A child with supernumerary small marker chromosome and uniparental disomy 22 due to trisomy rescue

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Trisomy rescue is one of various proposed mechanisms in formation of supernumerary marker chromosomes (SMC). However, postzygotic reduction of a chromosome to a SMC has never been observed in clinical cases. Indirect evidence is given when a small SMC is associated with uniparental disomy (UPD). Here, we report a child with SMC, UPD and confined placental mosaicism for trisomy 22. A small de novo marker chromosome derived from chromosome 14 or 22 was diagnosed in amniocytic fluid during prenatal diagnosis due to maternal age. Follow up investigations at birth revealed mosaicism 47,XX,+min (14 or 22)46,XX. Using FISH the SMC was positive for the probe D14/22Z1, but negative for the probes MIDI54 (i.e. a probe for the acrocentric`s p-arms) and D22Z4. According to microsatellite analysis, both chromosomes 22 were inherited from the mother (UPDmat). Trisomy of the whole chromosome 22 was present in short term culture of placenta, where cytotrophoblast cells are investigated, but not in cultured placental cells which represent esenchymal mesoderm. The newborn girl was phenotypical normal. Motoric and mental development is according or ahead of developmental milestones (free walking with 10 month, first words with 12 months). Cardiac and abdominal formations were excluded by sonografic examinations. The present case confirms that maternal UPD 22 most likely is clinically silent. According to FISH results, UPD 22 and 47,XX,+22 in the placenta we conclude that the SMC was derived from heterochromatic centromeric material of chromosome 22. This case gives evidence for the first time that early somatic formation of a marker chromosome is a real existing mechanism to rescue a conceptus with trisomy. Supported in parts by the Dr. Robert Pfleger-Stiftung.

P 083

Real Time PCR in diagnosis of 21-hydroxylase deficiency

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Congenital adrenal hyperplasia (CAH) is one of the most common autosomal recessive metabolic diseases and 90-95% of cases are due to 21-hydroxylase deficiency. Identification of defects in the corresponding gene, CYP21, is the basis for prenatal diagnosis and therapy of female fetuses in attempts to reduce virilization of external genitalia. Molecular diagnosis is hampered by the complicated and variable structure of the CYP21 gene locus on chromosome 6p, which results from duplication events. Prevalent is a bimodular arrangement comprising the active gene CYP21 and a highly homologous inactive pseudogene, CYP21P, in near proximity. The molecular defects result predominantly from recombinations between CYP21 und the CYP21P pseudogene leading to deletion/duplication of CYP21 or to conversions with transfer of deleterious sequences from the pseudogene. Since deletions and large gene conversions account for one third of CYP21 defect alleles determination of gene copy number is an important element of mutation detection. To improve and enhance diagnostics of CAH we established a real-time PCR approach for quantification of gene copy number. The setup involves co-amplification of CYP21 together with a known bi-allelic reference gene (HSA). CYP21-specific primers and probe were located within exon 3, a region with clear differences between CYP21 and CYP21P; the FAM-labelled TaqMan probe carries a TAMRA quencher. HSA specific primers and probe were designed according to Thiel et al., 2002, using a VIC labelled probe with a non-fluorescent quencher. Optimization of primer and probes concentrations allowed synchronization of CYP21 and HSA reactions resulting in nearly identical amplification rates. Simulation of deletion and duplication alleles was done by bisection and doubling of template DNA and resulted in significant and reproducible shifts of the Ct value. (Thiel et al., 2002, Eur J Hum Genet 11: 170-178)

P 084

Identification and characterisation of a small supernumerary euchromatic marker chromosome derived from chromosome 10 by microdissection and

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We report the identification and characterisation of a supernumerary marker chromosome by microdissection followed by forward and reverse painting in a female patient presenting with short stature. The fluorescence in situ hybridisation showed that the marker chromosome originates from chromosome 10 and includes the euchromatic bands p11.2 and q11.2, which was verified by hybridising a whole chromosome painting probe (wcp10) specific for chromosome 10. The marker was found in 17 % of the analysed peripheral blood lymphocytes and this finding could be confirmed in a second cell system (oral mucosa cells) by interphase FISH using an alphoid centromeric probe for chromosome 10. Parental karyotypes were normal. The karyotype of the patient can be described as mos47, XX,+mar.rev ish der (10)(p11.2q11.2) (wcp10+) /46,XX. Cytogenetic and clinical data on additional cases with marker chromosomes 10 are extremely rare. To our knowledge there are only two cytogenetically comparable cases with a supernumerary marker chromosome and small partial trisomy for the pericentromeric regions of chromosome 10. Only one of these cases has been described clinically after birth.

P 085

Heart malformation in a boy with a 6 Mb interstitial deletion in 8p23.1

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We report on a patient with a complete artrioventricular (AV) canal defect and pulmonary stenosis. The boy was born after 37 weeks of gestation with a birth weight of 2150g (<3.centile). Length was 41 cm (<3. centile) and head circumference was 31 cm (<3. centile). Because of heart malformation and dystrophy chromosome analysis was undertaken. Following GTG-banding, a numerical normal male karyotype with a deletion in the short arm of one chromosome 8 was supposed on a 450 band level. Fluorescence in situ hybridization (FISH) with 8 different YACs (RZPD, Berlin) from the 8p22-8p23 region confirmed that suspicion. The YACs 871a08, 966b05 and 770e09 showed hybridization signals on only one chromosome 8 per metaphase. FISH with a 8p subtelomeric probe gave normal results. In conclusion, the patient shows an interstitial deletion in 8p23.1 with an estimated size of not more than 6 Mb. Additionally performed multicolor banding (MCB) with a chromosome 8 specific probe set gave normal results, indicating for a resolution limit of MCB similar to CGH - i.e. about 10 Mb. The location of the deleted region coincides with the 6 Mb critical deletion region for congenital heart defects (Devriendt et al. 1999), which contains the transcription factor GATA-4. Supported in parts by the Wilhelm Sander-Stiftung (99.105.1-2).

P 086

Molecular cytogenetic characterisation of human papillomavirus-transfected cell lines by spectral karyotyping

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High-risk human papillomavirus (HPV) types 16 and 18 are involved in the multistep process of cervical carcinogenesis. For HPV-induced immortalisation - a prerequisite for tumour growth - genomic changes such as HPV integration into the host genome and distinct structural and numerical aberrations are necessary. In the present study cell lines of HPV-transfected keratinocytes were analysed by spectral karyotyping (SKY). SKY-Fluorescence- in situ - hybridisation (FISH) is a method which has revealed a number of previously undetected or incompletely identified chromosome aberrations.We could confirm previously described chromosomal imbalances which had been identified by comparative genomic hybridisation (CGH) such as loss of 10p and gain of chromosome 11 (Solinas-Toldo et al. 1997). These aberrations are stable and were detected in all cell passages. Additional new aberrations detected by SKY-FISH are a t (3;5) - which we also found in HeLa-cells- and the t (10,14), or i (10q) which might be a cause for loss of 10p. Further frequently occuring new aberrations were t (1;19), t (3;15) and i (8q).In conclusion, in this study we detected some novel structural rearrangements in HPV 16 or 18-transformed cell lines, whose relevance as prognostic markers in cervical cancer will be investigated further.

P 087

Definition of new BAC probes that enable clinical FISH tests, including prenatal diagnosis, of the CES marker chromosome

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Supernumerary bisatellited marker chromosomes (SBMCs), especially if found at prenatal diagnosis, may pose serious diagnostic problems. SBMCs appear very similar by karyotyping, but there are various types and subtypes, which are associated with very different clinical prognoses, ranging from the fully normal phenotype at the mild end of the spectrum to the severe syndromal mental retardation with the dicentric 15q12-q13. The dicentric 22q11.2 (the typical CES marker chromosome) represents a major diagnostic problem. No specific diagnostic test and no specific DNA probe for the CES chromosome can be commercially purchased, and the established FISH probe, cos121 (Liehr et al. Clin Genet 1992, 42, 91-96), is not freely available and genetically not well-defined by modern standards. This study aimed at establishing new well-defined DNA probes to be used in clinical FISH tests of SBMCs.We report metaphase FISH studies on 8 new unrelated cases of a typical or atypical CES marker chromosome. Probes included alphoid probes, cos121 and 10+ clones from the CTA library (Kim et al. PNA, S 1996 , 93, 6297-6301). Some map positions (Kim et al. 1996) were not supported by our results and current data (Genbank, Ensembl, UCSC Genome Browser). Three BACs yielding clear signals on typical CES chromosomes were identified and may be used in clinical testing. Other BACs enabled the breakpoint mapping on the CES chromosomes. - This study included five prenatal cases of a CES chromosome. Complex ethical issues were discussed. Following fetal ultrasonography and in-depth genetic counselling, three children were born, one pregnancy was terminated, and one pregnancy is in progress. All cases, clinical and FISH findings correlated favourably, i.e. inconsistencies were not observed.We thank the referring colleagues Drs. C. Behrend, C. Gillitzer, K. Sandig, M.-L. Mazauric, P. Kozlowski and G. K. Hinkel.

P 088

Terminal deletion 14q32 in a boy with severe developmental delay and dysmorphisms detected by FISH subtelomere screening

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We report on a mentally and motorically retarded boy with dysmorphisms whom we investigated at the age of 2 3/4 and 3 3/4 years. He is the first child of a healthy, non-consanguineous couple of Turkish descent. After an uneventful pregnancy and delivery by caesarean section due to breech position, birth measurements were normal (2810g, 49cm, 34cm; apgar scores 10/10). At the age of 5 months, the psychomotoric delay was first noticed. His further development was severely retarded (sitting with 22 months, crawling with 28 months, no standing in upright position and a speech ability of 2-3 words with 2 3/4 years). His craniofacial dysmorphisms included epicanthal folds, down-slanting palpebral fissures, small nose with broad nasal bridge and anteverted nares, thin upper lip, large philtrum, dysmorphic ears and a low hair line. He had bilateral simian creases and hypospadia. At the age of 2 1/2 years, his body weight was above average (15,5kg; 90-97th centile). At the age of 2 3/4, his length was significantly above average as well (103cm; >97th centile), his head circumference was normal (50cm). Screening for metabolic diseases and conventional cytogenetic analyses of peripheral blood lymphocytes (400 bands per haploid genome) and skin fibroblasts gave normal results. Brain MRI-scan showed a disturbance of myelinisation. Re-examination at the age of 3 3/4 years additionally showed generalised hyperlaxity of the joints. A screening for subtelomeric chromosome aberrations (Vysis, ToTelVysion) detected a terminal deletion 14q. Parental chromosome analyses showed a normal karyotype. FISH with the subtelomeric probes deleted in the patient did not reveal a subtelomeric aberration in the parents demonstrating that the aberration arose de novo. In order to delineate the breakpoint, hybridisations of a panel of BAC probes on patient´s metaphase spreads are in progress. A genotype-phenotype correlation based on the exact size of the 14q deletion as well as on a review of the literature will be presented.

P 089

Molecular cytogenetic characterisation of Howell-Jolly Bodies

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Howell-Jolly-Bodies (HJB) are known as nuclear fragments in erythrocytes which often appear in patients with pernicious anaemia and after splenectomy. Its DNA content, however, has never been defined unequivocally. Therefore, we selected formaldehyde-fixed erythrocytes of a splenectomised male patient with the help of an extended Pasteur pipette and characterised the DNA of the HJBs with molecular cytogenetic techniques. For that, we amplified and labelled the DNA by DOP PCR and performed reverse painting on normal metaphase spreads. Preliminary results of four HJBs led to different but specific centromeric signals on chromosomes 1/5/19, X, 7, 17, respectively. Signals on euchromatic or other heterochromatic chromosome regions could not be detected. Consequently, we suppose that HJBs preferentially consists of centromeric heterochromatin of different chromosomal origin. Furthermore, we assume that HJBs result from a normal DNA degradation process which eliminates euchromatic material first. This degradation process may have some similarities to the formation of marker chromosomes which often consists of confined centromeric DNA as well.

P 090

Molecular cytogenetic analysis of a de novo 5q23.2q31.2 deletion in a dystrophic boy with minor congenital anomalies

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Only few patients with constitutional interstitial deletions of 5q have been reported, and no common phenotype could be established. Most cases were investigated by conventional cytogenetic techniques only, and the size of the deletions varies considerably. We report on a 1-year-old boy with a de novo interstitial deletion 5q23.4q31.2 who presented with dystrophy, statomotor retardation and multiple minor congenital anomalies, among which long slender fingers and toes, bilateral clinodactyly of the toes IV and V, and large low-set ears were the most prominent. Additional features were a broad nasal bridge, hypertelorism, long philtrum, retrognathia, and a high palate.Conventional chromosome analysis revealed a de novo interstitial deletion 5q23.4q31.2 which was confirmed by CGH. Further molecular cytogenetic investigations using YAC and BAC clones were undertaken to determine the precise extent of the deletion which is about 24 Mb (proximal breakpoint between markers D5S622 and D5S2120, distal breakpoint between markers D5S463 and

D5S638).Dystrophy, psychomotor retardation, and minor facial anomalies are the only common features in the few known cases with smaller interstitial deletions of 5q (reviewed by Courtens et al. Am J Med Genet 1998). Patients with larger deletions comprising the FAP locus suffer from Gardner syndrome.Interestingly, the Fibrillin 2 (FBN2) gene is located near the centromeric breakpoint and might be deleted in our patient. Mutations in FBN2 cause Beals-Hecht syndrome (congenital contractural arachnodactyly). The long toes and fingers which have also been described in at least one other patient with a similar deletion of 5q might be due to haploinsufficiency of FBN2.

P 091

Molecular analysis of the 17q breakpoint of a patient with a translocation t (17;20)(q25;q13) associated with SRS

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A severe Silver-Russell syndrome (SRS), a heterogenous disorder mainly characterized by pre- and postnatal growth retardation, lateral asymmetry and other morphological abnormalities, ascertained in a girl with a translocation t (17;20)(q25;q13) inherited from her phenotypically normal father, was reported by Ramirez-Dueñas et al. (1992). Here we report the molecular analysis of the breakpoint region on chromosome 17q25 based on a BAC / PAC contig established for this region. Efforts to map the molecular breakpoint in 17q25 determined several BAC and PAC clones giving signals on both chromosomes 17 and der17 in FISH analysis using proband metaphase chromosomes so establishing a refined clone contig for the region of interest. According to the mutation analysis of a 3,5 cM interval on chromosome 17q25 for hereditary neuralgic amyotrophy (HNA; Meuleman et al., 2001) we started mapping several candidate genes onto the contig. One of these genes (SEC14L1) could be localized nearby the region of interest by PCR approach. Further refining the existing clone contig by STS content mapping and sequencing the BAC / PAC insert ends resulted in defining the breakpoint within 81,5 kb in the 5`- region of SEC14L1. However, there is evidence to localize the breakpoint to an even smaller region of about 5 kb upstream SEC14L1 considering the sequence data of a single PAC clone showing conflicting results in hybridization experiments. Corresponding analysis of the breakpoint region on chromosome 20 resulted in the identification of a PAC clone RP1-232n11 spanning the breakpoint including one exon of the gene PTPRT, so far another candidate gene located nearby the region of interest. FISH experiments using PAC and BAC subfragments and RFLP analysis in order to identify aberrant fragments specific for the translocation will now be used to further localize the molecular breakpoint hence giving novel information on the genetic background of the development of Silver-Russell syndrome.

P 092

Molecular characterization of a pericentric inversion of chromosome 3

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Chromosomal rearrangements provide an important resource for the analysis of mutations alterating the phenotype of the affected patients in consequence of shortened, deleted or differently expressed gene products. Here we report the case of two adult and one infant proband in a family with reduced body height (below percentile 3) otherwise lacking dysmorphic features. Karyotyping revealed a pericentric inversion 46XX inv (3)(p25;q26), being heterozygous in all tested probands. Assuming an association between reduced body height and observed inversion we established a BAC contig for each breakpoint region 3p25 and 3q26 consisting of eight clones in total by in silico analysis confirmed by FISH experiments. Searching for candidate genes in the region of interest we focused on the gene SHOT (SHOX homologous gene on chromosome three, SHOX2) as this is described sharing 83% homology at amino acid level with the homeobox gene SHOX (short stature homeobox) (Blaschke et al., 1998) whose mutations and haploinsufficiency are thought to be responsible for several syndromes associated with short stature like Ullrich-Turner syndrome (45,X0) (Rao et al., 1997). FISH analysis so far results in physical mapping of two BAC clones possibly spanning the breakpoint covering SHOX2 confirmed by PCR approach and Southern hybidization experiments. Further on RFLP analysis as well as detailed FISH experiments using isolated subfragments of these BAC clones as probes will result in narrowing of the breakpoint region, so giving evidence for a relation between the inversion and the altered phenotyp of the probands.

P 093

Chromosomal evolution of the PKD1 gene family in primates

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The autosomal dominant polycystic kidney disease (ADPKD) is mostly caused by mutations in the PKD1 (polycystic kidney disease 1)-gene located in 16p13.3. Moreover, there are several genes homologous to PKD1 that are located proximal to the master gene in 16p13.1, and there is evidence that these homologues form a family of pseudogenes located in 16p13.1. In contrast, there is a single copy gene on mouse chromosome 17 and no pseudogenes could be detected in the mouse genome. The question arises how the human situation originated phylogenetically. To solve this question we applied comparative FISH-mapping of a human PKD1-containing genomic BAC clone and a PKD1-cDNA clone to chromosomes of a variety of primate species and the dog as a non-primate outgroup species. Summarizing our comparative mapping data, it may be concluded that PKD1 must have undergone amplification very early in primate evolution while it is present as a single copy gene in the dog. Interestingly, only in the gorilla, the bonobo, the chimpanzee and the human further chromosomal rearrangements, i.e. transpositions, in addition to the amplification have clearly separated the pseudogenes from the master PKD1 gene in a species-specific pattern.

P 094

Molecular Cytogenetic Analysis of Breakpoints in Two Cases with Deletion 7q36

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Two female patients with de novo deletions 7q36 are reported. From the cytogenetic aspect both deletion breakpoints seem to be identical. Molecular cytogenetic analysis by fluorescence in situ hybridization (FISH) using a panel of 4 YAC clones mapped the breakpoint in both cases within a 1 Mbp region in 7q36.2 . Subsequent FISH-analysis by means of 8 BAC/PAC clones derived from the breakpoint-overlapping YAC clone narrowed down the positions of the breakpoints within an interval as small as 300 - 400 kbp. However, the clinical symptoms in both patients significantly differ. The differing phenotypes are discussed in view of hemizygosity for SHH and HLXB9, both genes being located within the deleted segment.

P 095

Purification and biological activity of recombinant hGH, TNFalpha and FeldI proteins overexpressed in bacterial and eucaryotic systems

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In the last years proteomics is rapidly developing as a new field connected with protein structure and function. Now each protein predicted by genome analysis can be obtained as recombinant protein. Biologically active recombinant proteins can be expressed in bacterial or eucaryotic systems. We used for expression of recombinant proteins E.coli cells and mammary glands of transgenic animals. Human tumor necrosis factor TNFalpha and two chains of major cat allergen FeldI were purified from bacteria. Human growth hormone hGH was overexpressed in homozygotic female rabbit. All recombinant proteins have 6xHis tag for purification on metal affinity chromatography column (Talon). Histidine tags were removed from hGH and FeldI chain 1 and 2 by cleavage of sequence recognized by thrombin or enterokinase introduced between tag and protein sequence. Biological and cytotoxic activity of recombinant proteins was estimated. Additionally, for both FeldI chains immunological analysis was accomplished by surface plasmon resonance technology. Human growth hormone activity was analyzed towards growth promoting activity using growth hormone dependent cells Nb2-11. For all recombinant proteins amino acid sequencing was performed.

P 096

Evolution of the Y-chromosomal AZFa region in higher primates

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Compared to other regions on the human and great ape Y chromosomes, the genomic segment encompassing the functionally defined AZFa locus has undergone higher X-Y sequence divergence, which is detectable by fluorescence in-situ hybridisation (FISH). We have comparatively mapped a human Y chromosomal PAC contig that includes AZFa by FISH analysis in human and great apes. The hybridisation results for the PAC clones of the classical AZFa region differ from the surrounding clones due to a lower conservation of the X/Y homology in higher primates. This allows an evolutionary definition of an interval enclosing AZFa with a size of about 1.1 Mb. This region includes the genes USP9Y, DBY and UTY and is limited by evolutionary breakpoints within the PAC clones 41L06 and 46M11. These breakpoints restrict an area of possible male specific evolution that may have resulted in the acquisition of male specific functions, including a role in spermatogenesis.

P 097

The coherence between isochore pattern and replication timing in the NF1 gene region is conserved between human and mouse

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A feature of the mammalian genome is the local differentiation of its GC content: Long stretches of alternate DNA sequence composition are separated by sharp boundaries. Beside the visible banding pattern of chromosomes, this so-called isochore structure is correlated with a number of functional features like gene density, recombination frequency and replication timing. Within the human NF1/FHN3 gene region a GC-poor isochore (39% GC) of several hundred kb in length is immediately followed by a long sequence stretch with an average of 51% GC. The orthologous region of the mouse genome mirrors this GC distribution and the sharp isochore boundary exactly. The isochores of both species are therefore evolutionary conserved, although after the separation of human and mouse the DNA sequence diverged. To study the correlation between sequence composition and replication timing for both species in detail interphase FISH was performed. The results clearly demonstrate that in both species GC-rich isochores are replicated early during S phase whereas neighbouring GC-poor sequences are replicated late. The boundary between early- and late-replicating sequences is sharp and precisely coincides with the boundary between the GC-rich and GC-poor isochores. Moreover, fiber-FISH experiments with a probe spanning the isochore boundary revealed, apart from linear structures, a number of Y-shaped structures representing replication forks. This demonstrates directly that in the human and in the mouse NF1/FHN3 gene region the replication fork is arrested for a longer time period within the isochore transition region. The isochore transition proves to be a conserved landmark of mammalian chromosomes.

P 098

A repetitive sequence present in human chromosome 1q21, 1p12 and 1p36.1 lead to new insights into the evolution of chromosome 1 and its homologues in human and 4 ape species

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The phylogeny of human and ape chromosomes is not yet fully established as proven as well by the present study. Here, a locus-specific probe (BAC b35B4) together with probes of the MCB1-probeset have been used. b35B4 derives from 1q21 and contains 143 kb of non-repetitive DNA, however, it produces three specific FISH-signals in 1q21, 1p12 and 1p36.1 of Homo sapiens (HSA). According to database search (NCBI) 123kb of b35B4 are present in at least 5 copies in 1q21, leading to a strong FISH-signal. In 1p36.1 59kb of b35B4 with ~95% sequence homology are present twice in tandem order. Additionally, 21.5 kb with ~90 homology to b35B4 are located once in 1p12. Human chromosome 1 was studied in comparison to its homologues in Hylobates lar (HLA), Gorilla gorilla (GGO), Pan troglodytes (PTR) and Pongo pygmaeus (PPY). The results clearly indicated, that there was an up to present unrecognized pericentric inversion in the evolution between apes and HSA #1. Moreover, a duplication of the sequences homologous to human 1p36.1 could be detected in PTR. Finally, in HLA there is also a homologous region to HSA 1p36.1 and the region homologous to HSA 1q21/1p12 is split onto two different chromosomes. The present ZOO-FISH study using human BAC-probe led to new insight into the evolution of chromosome 1; details not recognized by M-FISH, chromosome bar-code or MCB could be detected. Supported by DFG (PO284/6-1) and INTAS (2143). Ape cell lines were kindly provided by Dr. Rocchi (Italy) and Dr. Hameister (Germany).

P 099

SubcenM-FISH defines up to present hardly characterizable centromere-near rearrangements

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Recently, we described an approach closing a gap in molecular cytogenetic techniques: the subcenM-FISH. We demonstrated, that subcenM-FISH probe sets, consisting of a centromere specific satellite probe, one centromere-near BAC in q and p (excluding the acrocentric chromosomes) and arm-specific pcp probes, characterizes the pericentric region in small supernumerary marker chromosomes [Starke et al., Med Genetik 2002, 14:262]. In the present study the suitability of subcenM-FISH for the characterization of centromere-near breakpoints is established for 14 cases with derivative or rearranged chromosomes derived from chromosome X, 1, 4, 5, 6, 2, 10, 16, 19, 20, 21 or 22. The rearrangements have been defined as dicentrics [idic (X)(p11.21 or 11.22), dic (16;22)(16pter-> 16q24.3::22q11.22->22pter), dic (22)(p11.3->q11.22 or q11.23::p11.1->q11.22 or q11.23), dic (22)(pter ->q11.21::p11.2->qter)], inversions [inv (1)(p12;21.1), inv (2)(p11.2q11.2), inv (10)(p11.1q21.1), inv (19)(p13.11q13.12-q13.13)], duplications [inv dup (4)(p11p16), dup (5)(pter->q11.1::p12->qter), dup (16)(q11.2q13)], translocations [t (5;6)(p11-12;q11.2)] or derivative chromosomes [der (21)(:q21-q22.1->p11.2::q21-q22.1->qter), der (21)(p13->p12-13::q13.33->q13.32::q11.21->p12-13::q11.21-11.22->(?)q13.32::p13->p13)]. In 2 additional cases, chromosomes after GTG-banding suspected to have pericentric rearrangements, could be shown to have satellite sequence heteromorphisms; i.e. in one case the alpha-satellite region of one chromosome 6 was extremely small, in another case the corresponding region on a chromosome 20 was extremely enlarged. In summary, we conclude that subcenM-FISH is the method of choice for the characterization of SMC as well as of centromere-near rearrangements in larger derivative chromosomes. Supported in parts by the Dr. Robert Pfleger-Stiftung, the Herbert Quandt Stiftung der VARTA AG and the EU (QLRT-1999-31590).

P 100

Towards generating a catalogue of genes specifically expressed in the human retinal pigment epithelium

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Our project is focused on the identification of genes specifically or abundantly expressed in the human retinal pigment epithelium (RPE), a mononuclear layer of cells in the posterior pole of the eye intimately involved in many hereditary and complex eye disorders. To achieve this goal, we generated an RPE cDNA library using a suppression subtractive hybridisation (SSH) technique to enrich for differentially expressed genes and rare sequences. Thus far, 2379 randomly selected cDNA clones were sequenced and bioinformatically analysed. Overall, the sequences correspond to 340 annotated or hypothetical genes which were subjected to a number of selection criteria to determine potential tissue-specific and ubiquitiously expressed genes. By querying GeneAtlas and HuGeIndex, 122 housekeeping genes and 35 tissue-restricted genes (e.g. liver- or kidney-specific) were identified. By Northern blot analyses, the expression profiles of an additional 38 genes were determined identifying 21 housekeeping, 2 tissue-restricted and 15 RPE/neuronal-specific genes. An extensive search of expression data from the literature identified another 38 genes with ubiquitious expression. The remaining 122 genes were then assessed by their hypothetical function and all genes involved in ubiquitous cellular pathways were determined bringing the number of housekeeping genes or genes expressed in a tissue-restricted fashion to a total of 243. This renders 97 genes with an unknown and potentially RPE-specific expression. Their expression is currently being analyzed in ten human tissues by quantitative real-time (qRT)-PCR. This should provide a number of genes with important functions in

the RPE which then can be assessed for their potential role in human retinopathies.

P 101

Malignant melanoma of the skin displayed two deleted regions in 1p36.3

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Deletions in 1p36 in malignant melanoma have been found in high percentages in nodular melanomas and melanoma metastases. Despite many efforts, no candidate tumour suppressor gene in this region could be assigned to malignant melanoma so far. To further determine a possible tumour suppressor gene locus, we carried out a deletion mapping of chromosome 1p36 at nine microsatellite loci in 74 malignant melanomas. Loss of heterozygosity (LOH) in this region was found in 77% of nodular melanomas (NM), 86% of metastatic melanomas, but only 20% of superficial spreading melanomas (SSM). Regarding the allelic losses, the nodular and metastatic melanoma samples could be divided into three groups: one showing LOH at the more telomeric loci D1S243 and D1S468 (1p36.33), one displaying allelic loss at the more centromeric loci D1S214 and D1S253 (1p36.32-31), and one with LOH over all informative loci between D1S243 and D1S160. We did not found any significant correlation between a deletion in any of the in vestigated loci and survival data of the patients. But our data confine the deleted region in malignant melanoma to a very small area around 1p36.32 thus facilitating the search for the tumour suppressor gene with importance in malignant melanoma.

P 102

Cloning and characterization of three putative non-coding RNA genes exclusively expressed in retina

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The human retina is a multi-layered neuronal tissue specialized for the reception and processing of visual information. To identify novel genes expressed in this highly complex system, we have generated a human retinal cDNA library by a suppression subtractive hybridisation (SSH) technique. We have sequenced 1113 clones and on the basis of BLASTN algorithm analysis classified them into four categories including those with i) significant homology to known human genes (766/1113), ii) significant homology to partial transcripts and hypothetical gene predictions (162/1113), iii) no homology to known mRNAs (149/1113), and iv) vector sequences and clones derived from mitochondrial genes (36/1113). Clones from category 2, representing a total of 92 unknown transcripts, were selected for expression analysis by RT-PCR in 20 human tissues. This resulted in the identification of 29 genes specifically or abundantly transcribed in the human retina. Of these, three were further characterized. The putative full-length cDNA sequences were established for L33, L35 and L38 with a length of 1.6 Kb, 1.1 Kb and 3.0 Kb respectively. These sizes correspond well with the data obtained from Northern blot analysis. The longest open reading frame was found for L35 with 44 amino acids. This led us to suggest that these genes may belong to the growing number of non-coding RNA genes, producing functional RNA molecules rather than encoding proteins. Such transcripts act directly as structural, catalytic or regulatory RNAs, and are synthesized under defined conditions (e.g. during specific developmental stages or in a cell specific manner). Our current efforts are directed towards establishing the exact nature of those three transcripts. We expect this work to contribute to our further understanding of retinal physiology.

P 103

Methylation analysis of several tumor related genes shows low frequent methylation of CDKN2A and RARB in uveal melanomas

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Uveal melanoma is the most common form of primary eye cancer. Loss of an entire chromosome 3 (monosomy 3) is present in about 50% of uveal melanomas and is significantly correlated with metastatic disease. Long-term studies have shown that 4 years after diagnosis approximately 70% of patients showing monosomy 3 in the primary tumor have died of metastases, whereas tumors with disomy 3 rarely give rise to metastatic disease. In many tumors, recurrent loss of genetic material is part of a two step inactivation of tumor suppressor genes. Inactivation of second alleles might be caused by local mutations such as point mutations, gene deletions or epigenetic silencing. The observation of isodisomy of chromosome 3 in some uveal melanomas supports the hypothesis that epigenetic factors might play a role in tumor development and metastatic disease. Therfore, we have performed a comprehensive methylation study of the promoter regions of genes reported to be involved in tumorigenesis, with a special emphasis on genes located on chromosome 3 (fragile histidine triad (FHIT), von Hippel-Lindau (VHL), beta-catenin (CTNNB1), activated leukocyte cell adhesion molecule (ALCAM) and retinoic acid receptor-beta 2 (RARB)). In addition, the methylation patterns of the CpG rich regions 5´of the E-cadherin (CDH1), p16/cyclin-dependent kinase inhibitor 2 A (CDKN2A) and retinoblastoma (RB1) genes were analyzed by bisulfite genomic sequencing or methylation specific PCR (MSP). Furthermore, the SNRPN and D15S63 loci, which are located in the imprinted region on chromosome 15, were included in the study. The methylation patterns of these genes were analyzed in 20 uveal melanomas with monosomy 3 and in 20 uveal melanomas with disomy 3. Aberrant methylation was detected in 9 of 40 tumors analyzed: three tumors showed hypermethylation of RARB, and in 3 other tumors, the CDKN2A gene promoter region was methylated. For both genes no correlation of chromosome 3 status and methylation was found. Interestingly, the imprinted SNRPN and D15S63 loci showed an altered methylation pattern in 3 tumors with disomy 3. We conclude that methylation in the analyzed genes occurs infrequently in uveal melanoma and appears not to be associated with the chromosome 3 status.

P 104

Mutation analysis in autosomal dominant hereditary spastic paraplegia in Germany

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Hereditary spastic paraplegias (HSP) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterised by progressive spasticity and hyperreflexia of the lower limbs. Autosomal dominant hereditary spastic paraplegia 4 linked to chromosome 2p (SPG4) is the most common form of autosomal dominant hereditary spastic paraplegia (40 %). It is caused by mutations in the SPG4 gene encoding spastin, a member of the AAA protein family of ATPases. SPG3 accounts for approximately 10 % of autosomal dominant HSP. It is caused by mutations in the SPG3A gene encoding the protein atlastin. To date, only five disease causing mutations in the SPG3A gene have been described. In this study the spastin gene of HSP patients from 130 apparently unrelated families in Germany was analysed. We identified mutations in 31 out of the 130 HSP families; only two mutations were found in more than one family. Among the detected 27 different mutations were 14 missense mutations, 8 deletions, 3 nonsense mutations as well as 2 mutations that affect splicing. Most of the mutations are located in the conserved AAA cassette-encoding region of the spastin gene. Missense mutations account for the majority of SPG4 mutations in this population. Out of the SPG4 negative families 13 families with early onset of symptoms were selected for SPG3A gene analysis. In five out of these families a mutation could be identified. Thus SPG4 and SPG3A analysis should allow molecular genetic diagnosis in about 50 % of autosomal dominant HSP.

P 105

Genetic characterization of Wilms' tumors (nephroblastoma)

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Occurring with a frequency of ~1 in 10.000, Wilms' tumor is one of the most common solid tumors of childhood. It arises from embryonic kidney cells and most frequently presents as an unilateral (95 %) and sporadic (98 %) tumor. We have established the German Wilms' tumor bank containing more than 300 Wilms' tumor samples. All new tumor samples undergo screening for mutations of WT1 and ß-catenin as well as screening for loss of heterozygosity (LOH).Mutations of WT1 and ß-catenin which occur in approximately 10 % of Wilms' tumors, respectively, are frequently associated with each other. A high percentage of ß-catenin mutations including both in-frame deletions (del ser 45) and base pair substitutions is detected in Wilms' tumors with stromal histological subtype. These tumors are also prone to WT1 mutations which are analysed by DHPLC and SSCP. Sequencing tumor samples deviating from the exon specific DNA melting pattern, we identified new mutations in WT1, e.g. insertions and nonsense mu-

tations. LOH at different chromosomal regions (e.g. 11q, 16q, 22q) could be correlated with a weaker response to chemotherapy and poor outcome. In Wilms' tumors with predominantly blastemal histology we found an almost complete absence of ß-catenin mutations, but an increased allele loss for chromosome 11q that appears independent of frequently found 11p13 and 11p15 alterations. Therefore, chromosome 11 seems to harbor three loci involved in Wilms' tumor development, namely 11p13 (WT1), 11p15 and a locus at 11q which has to be narrowed down in future. Aiming at a further characterization of genetic alterations leading to the development of Wilms' tumors we recently started real time RT-PCR and cDNA microarray analysis (12.000 clones). Expression profiles in different Wilms' tumors are combined with the stratified clinical data in order to search for new molecular prognostic factors.

P 106

Disruption of the PDGFB gene in a patient with a chromosome 1;22 translocation and presumed Costello syndrome: accumulation of aberrant PDGFB fusion proteins in the extracellular matrix

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Costello syndrome is a rare congenital disorder characterized by postnatal growth deficiency, mental retardation, coarse face, loose skin of the neck, hands, and feet, cardiomyopathy, and nasal papillomata. Autosomal dominant inheritance of the trait with de novo mutations has been suggested whereas the genetic basis of the syndrome is yet unknown. We are studying a female patient initially diagnosed with Costello syndrome carrying an apparently balanced translocation t (1;22)(q24.2;q13.1). Molecular characterization of the 1;22 translocation revealed that the patient carries a mosaic of two derivative chromosomes 1, including one in which the PDGFB gene is disrupted. In 18 patients with Costello syndrome, mutation analysis of six genes belonging to the PDGF (R) family, PDGFA, PDGFB, PDGFC, PDGFD, PDGFRA, and PDGFRB, revealed no pathogenic mutation in any of them. These findings prompted us to reevaluate the clinical symptoms of the translocation patient, the results of which challenge the diagnosis of Costello syndrome. In total RNA isolated from lymphocytes of the translocation patient, we identified four different fusion transcripts by RT-PCR consisting of PDGFB exons and of parts of chromosome 1q24.2. In two of the mRNAs, exon 6 of PDGFB, encoding the 41 C-terminal amino acid residues, was absent. Immunofluorescence analysis showed that these two aberrant PDGFB-EGFP proteins encoded by the fusion RNAs are localized in aggregates in the extracellular matrix (ECM), whereas the normal protein was dispersed and formed a network-like structure in the ECM. Deposition of PDGFB into the subcellular matrix provides the potential for retention of growth factors in preformed storage pools. Although the aberrant PDGFB proteins lacking the basic C-terminus remain in the extracellular matrix, it is questionable whether they are available for subsequent mobilization, possibly by the release of appropriate enzymes, and are able to bind to high affinity PDGF receptors. Thus, we propose that aberrant localization of the PDGFB mutant proteins in the ECM may contribute to the disease phenotype of the translocation patient.

P 107

Loss of heterozygosity of the entire short arm of chromosome 1 is a recurrent alteration in uveal melanoma with monosomy 3.

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Uveal melanoma is the most common primary ocular tumor with an annual incidence of about six cases per million people. Approximately 50% of the patients die of metastases predominatly in the liver. The metastases mainly arise from primary tumors which show loss of an entire chromosome 3 (monosomy 3), whereas tumors with disomy 3 rarely give rise to metastatic disease. The results of global gene expression analysis of 20 primary tumors showed that uveal melanoma with monosomy 3 and disomy 3 represent two distinct entities. The same set of expression data revealed genes differentially expressed between both entities. Three genes showing reduced or loss of expression in tumors with monosomy 3 map within a region of 4 Mb on the short arm of chromosome 1 (1p31). Previously, this region was found to be deleted in all primary uveal melanomas and metastases showing loss of heterozygosity (LOH) at 1p by cytogenetic analyses. This indicates a potential role of genes located in this region in the initiation or progression of the tumor. To correlate chromosome 1p alterations with disease outcome, we performed microsatellite analysis of 50 uveal melanomas (27 tumors with monosomy 3, 23 tumors with disomy 3) which were derived from patients with a follow up between 6 to 12 years. We used 9 markers on the short arm, with 3 markers located in 1p31, and one marker on 1q. LOH on 1p was found in both uveal melanoma entities but was more frequent in tumors with monosomy 3. Interestingly, loss of the entire short arm of chromosome 1 was observed in 8/27 tumors with monosomy 3 but in no tumor with disomy 3 ($p = 0.0035$). By comparing partial 1p deletions in tumors with monosomy 3 we were able to define a smallest region of deletion overlap (SRO) at 1p35 flanked by the markers D1S233 and D1S507. In tumors with disomy 3 an SRO was defined by the marker D1S507 at 1p36. Kaplan-Meier analyses revealed that patients with monosomy 3 in the tumor have a reduced tumor related survival when 1p LOH is also present in the tumor. However, due to small number of uveal melanoma patients included in this study this result did not reach significance.

P 108

Mitochodrial DNA variability in the Kazakh population of Middle Asia

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Information about mtDNA variation in Middle Asia is very crucial for understanding of Asian mtDNA phytogeny as well as for reconstruction of Asian population history. The sequence of the first hypervariable segment of mtDNA was determined in 246 individuals from three district of Kazakhstan. 120 polymorphic positions and 192 different HVS 1 haplotypes were revealed. For correct haplogroup affiliation the ambiguous HVS 1 sequences were additionally screened for RFLP markers. 38 gaplogroups of mtDNA was determined. HVS 1 haplotypes occuring once (unicum haplotypes) were determined 64,6% in Kazakhs. Unicum haplotypes is different values in the populations of the Volga-Ural region: for Chuvaschs -–64%, for Bashkirs–88%, for Tatars –80%.Index variability of gaplotypes is 0,99 in Kazakhs. Index variability of gaplotypes are analogical values in the populations of the Volga-Ural region: for Chuvaschs and for Bashkirs–0,98, for Tatars –0,99.Index of gene diversity (calculated about of the frequencies gaplogroups) is 0,93 in Kazakhs. From literature it is known that the same values index of gene diversity in population for Kirgizs - 0,87, for Uzbeks – 0,92, Tajiks - 0,85 [Golubenko et.al., 2002].The populations of kazakhs have generally higher values of gene diversity of mtDNA by complicated ethnic history of the Kazakh population. The comparison of Kazakhs and other ethnic origin reveals the complicated structure of mtDNA gene pool in Middle Asia.

P 109

Alu insertion polymorphisms (ACE, TRA25 and PV92) in Kazakhs

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Alu insertion polymorphisms are objective markers for checking linguictic and archaeological theories about the origin and migration of ancient human populations, because they have played an important role in evolution by creating new mutations and gene combinations. The vast majority of Alu insertions occur in non-coding regions and are thought to be evolutionarily neutral. However, an Alu insertions in introns of genes for tissue plasminogen activator (TPA) and angiotensin converter enzyme (ACE) are associated with heart disease. The most a number of human-specific Alus are dimorphic - an insertion may be present or absent on each of the paired chromosomes of different people and were inserted within the last million years, during the evolution and dispersion of modern humans. Three Alu insertion polymorphisms were examined in Kazakh population of Middle Asia, represent to Turkic-speaking ethnic groups. A total of

224 unrelated healthy Kazakh people were studied. A portion of ACE, TPA25, PV92 genes from genomic DNA was amplified by PCR and analyzed on a 6% polyacrylamide gel. Each fragment was classified according to genotypes. Genotypes and allelic frequencies were revealed: for ACE gene - 26,3% (II), 22,3% (DD), 51,4% (ID), 52,0% (I), 48,0% (D), for TRA25 gene - 21,9% (II), 26,3% (DD), 51,8% (ID), 47,8% (I), 52,2% (D), for PV92 - 28,1% (II), 23,2 (DD), 48,7% (ID), 52,5% (I), 47,5% (D). The results were compared with available data on other modern Caucasian and Asian populations. The area of Kazakhstan has been a place of interaction of many ethnic layers during a historically long period. Thus, these revealed differences in allele and genotype frequencies of Alu dimorphisms are possible to explain by a complicated ethnic history of Kazakh population. It was confirmed, that the analysis of Alu insertion polymorphisms are tools for reconstructing human prehistory.

P 110

Antiphospholipid antibody syndrome in a large kindred is associated with at least two genetic defects one of which maps to chromosome 10p12

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The antiphospholipid antibody syndrome (APS) is an acquired thrombophilic and/or thrombocytopenic condition of unknown origin associated with the presence of antiphospholipid antibodies. This general definition conflicts with evidence for multifold clinical courses and the existence of familial APS. A large, four generation family (24f/20m) was evaluated both clinically and genetically. 11 family members presented with thrombocytopenia (<150/nl), 10 with migraine and 5 with arterial and/or venous thrombosis. 10 persons had more than 2 different antiphospholipid antibodies and 4 carried a factor V Leiden mutation. According to standard diagnostic criteria 10 patients had APS characterized by autoimmune age-dependent thrombocytopenia (9), migraine-like headaches (8) and thrombosis (3). Severe thromboembolism did only occur in the presence of a factor V Leiden mutation (2). Under a genetic model of dominance with incomplete penetrance of APS in this family, a genome wide linkage analysis with 170 highly polymorphic short tandem repeat (STR) markers was performed. Although no evidence for linkage of APS was identified, thrombocytopenia maps to chromosome 10p12, a genomic locus previously identified for non-syndromic thrombocytopenia by Savoia et al., 1999. Maximum 2-point LOD scores for thrombocytopenia in family L1 at chromosome 10p12 ranged between 2.4 and 5.0 (at 95% penetrance). Detailed haplotype analyses mapped the thrombocytopenia gene between STR-markers D10S2325 and D10S1426, a genomic locus of 26 cM. Only patients with thrombocytopenia share the affected haplotype. No linkage of antiphospholipid antibody titers, migraine or thrombotic events could be identified. Our data suggest a complex genetic model for an APS-like phenotype in this family: thrombocytopenia but no other features of APS are linked to chromosome 10p12. Two family members with APS carry the factor V Leiden mutation. The nature of at least one additional genetic factor remains enigmatic.

P 111

Mild citrullinemia caused by mutations in the argininosuccinate synthetase (ASS) gene

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Citrullinemia can be caused by deficiency of the argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), or defects of the mitochondrial transporter citrin (encoded by the SLC25A13 gene). Patients with classical citrullinemia present in the neonatal period with severe encephalopathy due to hyperammonemia. Mainly from Japan, there are also reports of patients with a mild or late-onset citrullinemia induced by citrin deficiency. Since the introduction of extended newborn screening programs using tandem mass spectrometry, some asymptomatic children with elevated plasma citrulline levels have been detected in Germany. The need for treatment and follow-up programs of those hitherto asymptomatic patients are uncertain. In this study, we provide evidence for the genetic defect in 19 individuals from 16 unrelated families with a mild phenotype (11 homozygotes and 8 heterozygotes). Molecular analysis showed mutations in both alleles of the ASS gene in all patients investigated. Most of the found mutations (35/38 alleles) are private missense mutations. The mutation Ivs11+49 C>T generating a cryptic splice site and creating a premature stop codon was found on 3/38 alleles. The mutation W179R was detected on 15/38 alleles, mostly in patients with turkish origin. In summary, genotyping of individuals with mild citrullinemia demonstrated that mild citrullinemia in european and turkish patients is allelic to classical citrullinemia rather than to citrin deficiency as found in Japan. The knowledge about certain mutations which are associated with mild, late-onset, or asymptomatic citrullinemia will be important for prenatal diagnostics and may guide the medical care of affected individuals.

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Mutation analysis and in vitro expression of human N-acetylglutamate synthase

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The mitochondrial enzyme N-acetylglutamate synthase (NAGS) produces N-acetylglutamate serving as an allosteric activator of CPS1, the first enzyme of the urea cycle. Activity of mammalian NAGS is enhanced by L-arginine. Autosomal recessively inherited NAGS-deficiency (NAGSD) leads to severe neonatal or late-onset hyperammonemia.Until now only few patients affected by NAGSD have been reported and the gene involved was described very recently. In this study, 10 families were analyzed for NAGS gene mutations (8 neonatal onset, 2 late-onset). 9/10 patients revealed homozygous for the individual mutations. In total we identified 10 private mutations, 7 missense, 1 nonsense, 1 splice-site mutation, and 1 insertion. In order to functionally analyze the deficient NAGS activity, we developed an in vitro expression system using the NAGS deficient E. coli strain NK5992. We expressed the NAGS pre-protein including the mitochondrial target sequence, the mature protein as well as a highly conserved core protein. NAGS activity was detected in all three expressed proteins but varied regarding activity level and response to stimulation by L-arginine. Expression of mutations showed a decrease in enzyme activity as well as in response to stimulation by L-arginine. In conclusion, we provide the genotypes of a series of NAGS deficient patients and a NAGS in vitro expression system for the characterization of mutations which will help to understand the highly variable clinical course of patients.

P 113

An efficiency-compensating mathematical model for real-time quantitative PCR

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The newly developed real-time quantitative PCR (polymerase chain reaction) technology is becoming a commonly accepted quantification method for DNA and mRNA within a wide range of concentration. It provides real-time analysis of product generation by detecting fluorescent signal given by double strand DNA binding dye SYBR Green I or hybridization probe. There are two types of quantification methods: relative and absolute. In most quantification assays of both methods, a standard curve based on a serial of known DNA/mRNA input is used. The assumption of these methods is that all standard and unknown samples have the same value of PCR efficiency. In this study, we present a mathematical model by which users can achieve the actual curve of target samples to calculate the starting concentration of DNA/mRNA based on a standard curve, even when PCR efficiencies of standard and unknown samples are different. Suppose the standard curve is expressed as: CP=Ss×lgC0+Is, then the actual curve is: CP=Su×lgC0+ Is× Su / Ss (CP crossing point, S slope, C0 initial concentration, I intercept). The standard curve can be obtained from LightCycler software by making a serial dilution of standard samples. The amplification efficiency of unknown samples can be calculated by different methods. The CP value is given by LightCycler software. Thus, the initial concentration of target sample (C0) can be calculated. This mathematical model provides a more accurate method of nucleotide quantification.

P 114

Spectrum of mutations in the factor VIII gene among patients with severe and mild to moderate haemophilia A

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Haemophilia A is a common X-linked bleeding disorder caused by various types of mutations in the factor VIII gene. The most common intron 22-inversion is responsible for about 40% of the severe hemophilia A cases while large deletions, point mutations and small rearrangements are responsible for the disease in the rest of patients. We report on the spectrum of mutations in the factor VIII gene, found in 100 German haemophilia A patients (50 severely affected and 50 with mild to moderate bleeding disorder), negative for the intron 22-inversion. The whole coding region of the gene has been sequenced in all patients. Southern blot analysis for detection of gross rearrangements was performed in addition in the patients with an obvious normal sequence. Disease causing mutations (30 novel and 62 recurrent) were identified in 92 of the patients. Thirty of the mutations, identified in the severely affected group (about 55%)are leading to a premature termination of the protein biosynthesis. More than the halve of these changes are located in exon 14 of the factor VIII gene. We also identified 18 missense and 2 splice site mutations. The majority of the missense mutations (12/18) affect the A1 and C1 domains, whereas no such mutation was detected in the factor VIII B-domain.The prevalence of missense mutations among the moderately/mildly affected patients was 72% (36/50). About one third of these mutations lie in the A2 domain, one affects the B/A3 junction and the rest are spread over the A1, A3, C1 and C2 domains. Three splice mutations and four small deletions/insertions in exon 14 of the factor VIII gene were also detected in this group. Our analyses failed to identify disease causing mutations in the remaining seven individuals. Thus, the detection rate of the methods used in this study was 100% for the group of the severely affected haemophiliacs, negative for the common intron 22 inversion, whereas only 86% of the mild/moderate affected patients show disease causing mutations in the coding region of the factor VIII gene. Genotype-phenotype correlations and possible explanations for the lack of factor VIII mutations in a certain proportion of mildly affected haemophiliacs will be discussed.

P 115

Identification of whole-exon RB1 gene deletions in patients with retinoblastoma

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Hereditary predisposition to retinoblastoma is caused by mutations in the RB1 gene. Oncogenic point mutations, base substitutions and small length mutations, have been detected in almost all of its 27 exons and the promoter region. With the progress in point mutation-screening-technologies, detection of these kinds of mutation has become available as a routine service. However, identification of large deletions, which probably add up to 20% of all oncogenic RB1 gene mutations, is still difficult if only single or few exons are lost. Medium sized deletions of various size and location are notoriously difficult to detect and, therefore, different methods have been developed to address this problem. In order to screen the RB1 gene for mutations of this kind, we have used an approach based on quantitative multiplex PCR amplification. In 3 sets, 25 regions that contain exons of the RB1 gene are simultaneously amplified (QM-PCR). Each reaction set also contains primers for amplification of a region located on chromosome 15 (internal control). One primer of each pair carries a fluorescent label (FAM) at its 5'-end. QM-reactions are run for 19 cycles only in order to stop the reaction while in the exponential phase. QM-products are evaluated using a capillary DNA analyzer (ABI). Relative amounts of individual products are determined by comparing peak integrals. We have analyzed DNA from tumors (36) and peripheral blood (34) of 70 patients with retinoblastoma. Most of these samples had previously been screened for point mutations with negative result.Samples from 18 patients, including five tumors with loss of heterozygosity, showed a marked reduction or complete loss of products for single or several neighbouring exons. Peripheral blood DNA from one patient showed significantly more product corresponding to the exon 10 region thus indicating the presence of an insertion including the PCR target region. Samples from 15 patients showed less clear-cut changes in relative product amounts: we found signal reductions for single exons and neighboring exons in 10 and 5 samples, respectively. In order to confirm deletions involving exon 7 we performed quantitative real-time PCR using Taqman technology. Results of real-time PCR were concordant with that of QM-PCR in the two samples with marked reduction of exon 7 products. Of the 6 samples with less clear-cut changes 3 showed real-time PCR results compatible with hemizygous deletions. To confirm deletions in other regions of the RB1 gene we are now establishing appropriate Taqman and long-range PCR assays. Our results confirm that quantitative multiplex PCR complements existing methods for RB1 gene mutation screening.

P 116

Update on the spectrum of *TRPS1* mutations in the tricho-rhino-phalangeal syndrome types I and III

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Mutations in the TRPS1 gene, which codes for a zinc finger transcription factor, lead to the development of the tricho-rhino-phalangeal syndrome (TRPS) types I and III. They are characterized by craniofacial dysmorphism, brachydactyly and short stature. The latter signs are more severe in TRPS type III. In a previous study (Lüdecke et al. , 2001, Am J Hum Genet 68:81-91), we described 35 different mutations in the TRPS1 gene. Nonsense mutations were associated with TRPS I and missense mutations, exclusively found in the GATA-type zinc finger (GATA-zf), caused TRPS III.Here we report the identification of 22 novel TRPS1 mutations. Together with seven mutations reported by others, a total of 64 different TRPS1 mutations have been found in 88 unrelated patients, up to now. Twelve mutations are recurrent mutations. Worth noting is that 24 (27 %) of the 88 patients have mutations in the 123 bp exon 6 or the adjacent intron 6 splice donor site. Nineteen of the 33 base substitutions as well as the twelve out-of-frame insertions and the nineteen out-of-frame deletions lead to premature translation stop codons. The Y915X mutation in the GATA-zf is the first nonsense mutation that is associated with a TRPS III phenotype as determined by metacarpophalangeal pattern profile analysis. Three base substitutions affect splice signals. The remaining eleven base substitutions cause missense mutations. Seven of them affect the GATA-zf, and five of them cause TRPS III. Interestingly, the A919T mutation causes TRPS III, whereas exchange of the same amino acid to valine (A919V) results in a TRPS I phenotype. Two missense mutations (R952C and R952H) affect the nuclear localization signal and prevent nuclear entrance of TRPS1. Two missense mutations (C1217R and C1217Y) alter the first cysteine residue of the first IKAROS-like zinc finger. The six patients in whom we identified the last four missense mutations present with a TRPS I phenotype.Our current results extend our knowledge of TRPS1 mutation and confirm, in general, the previously described genotype-phenotype correlation in TRPS.

P 117

Analysis of gene expression by cDNA arrays to identify candidate markers in renal cell carcinomas (RCC)

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Renal Cell carcinomas can be classified in conventional (both granular and clear cell variants), papillary, and chromophobe carcinomas. An optimal clinical management of renal cell carcinomas requires an accurate pathological characterization of these tumor types. But also the molecular mechanisms for the transition from non-metastatic tumor to metastatic forms are of interest. To find and analyse these complex mechanisms high parallel techniques like cDNA microarrays has to be used. For this study we considered histologically well characterized samples of the conventional clear cell variant. To characterize candidate markers in metastatic (n=5) and non-metastatic tumor (n=5) samples versus normal tissue (n=10) we monitored the expression levels of 648 genes by using cDNA microarrays. Total RNA was isolated from snap frozen tissue with commonly used protocols. After purification of mRNA a T7 amplification was performed and the cDNA was labelled with Cy3 or Cy5, respectively. We hybridized tumor mRNA against a reference of mRNA from normal renal tissue of the same patient as well as tumor mRNA from metastatic samples against tumor mRNA from non-metastatic samples. Patterns of gene expression revealed several genes that were up or down regulated in the tumor samples. These genes were for instance VWF, VEGF and VCAM1 which were previously described as markers for renal cell carcinomas by other groups. Furthermore we found specifically over expressed genes in the metastatic tumor samples with the potential to characterize a renal cell carcinoma as metastatic or non-metastatic. These results show that cDNA arrays are an appropriate tool to find markers responsible for classification of renal cell carcinomas. For further evaluation of these differentially expressed genes a specific real-time RT-PCR assay has to be performed.This project was supported by the BMBF.

P 118

Consequences of reversible ERBB2-expression in breast carcinoma cells: Molecular analyses of intracellular signaling cascades and regulated target genes

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The ERBB2 protooncogene is a member of the EGFR gene family consisting of ERBB1 (synonymous: EGFR), ERBB2 (synonymous: HER2), ERBB3 and ERBB4. The genes encode type I receptor tyrosine kinases involved in the transmission of proliferative as well as differentiating signals. The level of ERBB2 expression is directly correlated with the prognosis of several human malignant neoplasias, like breast and ovarian carcinoma. Upregulation of ERBB2-expression in tumor tissue can frequently be attributed to genomic amplification of the ERBB2-locus on chromosome 17q21. A different mechanism known to result in an inadequate ERBB2-activation is shedding of large parts of the aminoterminal extracellular portion of the receptor protein. Both processes lead to ligand-independent activation of ERBB2 signaling cascades that ultimately lead to the cellular changes associated with malignant transformation. We used the Tet-regulatory system to reversibly overexpress a mutant version of the ERBB2-protein (NeuNT; the homologous rat-protein constitutively activated by a point-mutation) in human MCF7 breast carcinoma cells. Tet-induced NeuNT-expression in several independent MCF7/Tet-NeuT clones resulted in prominent phenotypic alterations of the cells 16-48h after NeuNT-induction. Morphologically the cellular changes that could be observed resemble the epithel-to-mesenchyme transition that has been described for the process of carcinogenesis. In addition we observed complete reversibility of these phenotypic alterations in experiments with transient oncogene-expression. In order to correlate the phenotypic response with intracellular signaling pathways, inhibitors for different signal transduction cascades known to be engaged in ERBB2-signaling (PI3K/AKT, P42/P44 MAPK, JNK, P38 MAPK) were applied. Using this approach we could attribute the NeuNT-mediated phenotypic epithelial-mesenchymal transition of MCF7-cells to the activation of the p38 MAPK-pathway. Interestingly, NeuNT-expression was not accompanied by a proliferative response of the MCF7/Tet-NeuNT cells, as judged by FACS- and thymidine-incorporation analyses. Comparative expression-analyses of NeuNT-induced vs. non-induced cells yielded several transcripts displaying a differential expression profile compatible with a block in cellular proliferation.

P 119

Pitfalls in the molecular diagnosis of Ehlers-Danlos syndrome (EDS) Type IV, the vascular type

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Among the heterogeneous group of Ehlers Danlos syndrome, EDS type IV is the most severe one often resulting in life-threatening complications as a consequence of the extreme arterial fragility and spontaneous rupture of the colon or the gravid uterus. Major diagnostic features are thin translucent skin with a visible venous pattern over the chest, abdomen, and extremities; extensive bruising and delayed wound healing; a characteristic facial appearance with tight skin, a thin nose, thin lips, hollow cheeks and prominent eyes. EDS type IV is an autosomal dominant trait and is most often caused by structural defects in the pro-alpha1 (III) chain of collagen III encoded by the COL3A1 gene. Despite the above mentioned characteristic clinical symptoms there is a broad phenotypic spectrum making the diagnosis difficult unless a complication has occurred. When suspected, the diagnosis of EDS type IV can be supported by ultrastructural and biochemical findings and is confirmed by the identification of a pathogenic mutation in the COL3A1 gene. Since EDS type IV results from abnormalities in structure, synthesis, or secretion of type III procollagen, these may be detectable in dermal fibroblasts. Electron microscopy can detect a thinned dermis with small collagen fibre bundles and small fibril diameters. Decreased immunhistochemical staining of collagen III in dermal fibroblasts or organ tissue indicates reduced synthesis, reduced incorporation or increased degradation of collagen fibrils. Biochemical demonstration of procollagen or collagen III with abnormal stability or electrophoretic mobility in skin fibroblasts hints to a structural COL3A1 defect.We report on the molecular diagnosis in four cases with clinically suspected EDS type IV, two sporadic individuals and two families. Only in one case the dermatological diagnosis which was made after the occurrence of a carotid-cavernous fistula lead directly to the request of a molecular genetic analysis of the COL3A1 gene. In the two families preceding ultrastructural investigation of a skin biopsy of the index patients was in agreement with a collagen type III defect. Additional immunhistochemical examination in one of the familial cases indicated decreased collagen type III staining and supported the clinical diagnosis. Surprisingly, biochemical analysis of skin fibroblasts detected no aberrant electrophoretic mobility of collagen III alpha chains. Biochemical analysis in the fourth case argued against a structural COL3A1 defect in the same way. Nevertheless, subsequent mutation screening of all 52 coding exons of the COL3A1 gene by direct sequencing identified characteristic glycine substitutions in all four cases, located at different positions in the triple-helical domain and suspected to be pathogenic. In one of the sporadic cases clinical and biochemical investigations would have been misleading and the patient would not have been diagnosed for EDS type IV without the identification of a COL3A1 mutation. This study encourages the combination of diagnostic approaches for the confirmation of the clinical diagnosis EDS type IV and supports the application of mutation screening especially if ultrastructural and biochemical findings are contradictory.

P 120

A novel missense mutation Gly1579Ser in the GAP related domain of the TSC2 gene is associated with minimal clinical manifestations in a three generation TSC family

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Tuberous sclerosis (TSC) is an autosomal dominant disorder with a broad phenotypic spectrum. The clinical manifestations of the disease can vary considerably, both between and within families, making genotype-phenotype correlations in general not possible. Here we describe a family segregating a novel missense mutation, 4753G>A (G1579S), in exon 36 of the TSC2 gene. Only the index patient fulfilled the present diagnostic criteria for TSC: prenatal sonography revealed two cardiac rhabdomyomas, at the age of eight weeks he developed first hypomelanotic macules, subsequent development is normal up to now. Molecular genetic analysis at the age of two years identified the missense mutation G1579S in exon 36 of the TSC2 gene by direct sequence analysis of all TSC1 and TSC2 exons. In an attempt to classify the variation, three additional mutation carriers could be identified in the family. However, they all showed clinical signs associated with TSC but insufficient for a definitive diagnosis. The 4753G>A mutation leads to a change of a glycine residue in the GAP related domain of tuberin highly conserved between human, mouse, rat, fugu, drosophila and even caenorhabditis. To obtain additional evidence that the TSC2 4753G>A mutation was the pathogenic mutation in this family, the effect of the G1579S substitution on tuberin function was investigated. Compared to wild-type tuberin, the G1579S variant was hypophosphorylated, showed a reduced ability to form a complex with hamartin and was unable to inhibit the phosphorylation of ribosomal protein S6, supporting the conclusion that the TSC2 4753G>A substitution was the pathogenic mutation in this family. For the understanding of the molecular pathogenesis of tuberous sclerosis findings in families like this strengthen the importance of careful clinical examination in patients who lack classical signs of TSC and of functional investigation of missense changes in potentially functional domains of tuberin.

P 121

Translationally silent mutations can cause epidermolysis bullosa

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Translationally silent mutations are generally considered to be not pathogenetically relevant in human disease. In rare instances the change of a nucleotide cause interference with the spliceosome leading to aberrant splicing. The genotypical and morphological consequences of

a silent mutation leading to preferred usage of a weak splice site have not been analysed yet. We report on a patient suffering from junctional epidermolysis bullosa, an inherited blistering skin disease. Blistering was noticed since birth. Later severe teeth decay and nail dystrophy did accompany the skin symptoms. Immunofluorescence microscopy showed profound reduction of linker protein laminin 5, which is located in the dermo-epidermal junction zone. Transcriptomic analysis revealed homozygosity for the mutations 3009C->T in the exon 20 of the LAMB3 gene, which is coding for the beta3 chain of the laminin-5 protein. The nucleotide transition 3009C->T does not alter codon translation. Rather it introduces an exonic splice site, which is preferentially recognized by the spliceosome over the unaltered consensus splice site farther downstream, although the newly introduced consensus sequence shows only a splice site score of 68.6 in contrast to 92.2 of the original one. The aberrantly used 5´splice site did not result in correct 3´splicing. Rather, two cryptic splice sites in the exon 21 leading to at least two new transcript which are 44 and 170 bp shorter, respectively. These result in premature stop codons. The results show that translationally silent mutations can be pathogenetically relevant.

P 122

Novel PCR-Reagent systems for Molecular analysis in Human Genetics

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Recently Eppendorf AG introduced a set of novel PCR-Reagents, which represent versatile tools for the molecular analysis of mutations and function of genes in the post HUGO period:A totally new Hot Start technology that raises questions about virtually every feature of established technologies and an RT-PCR system covering all common RT applications with a simple modular system which enables maximum compatibility and flexibility. Both systems use a novel buffer technology which is able to buffer the magnesium concentration and in this way provides the correct magnesium quantity at all times without an optimization step. Here we present Data for:**Hotstart and quantitaive Real-Time PCR using Hotmaster Taq Polymerase**Sensitive single cell Multiplex RT-PCR using cMasterRTplusPCR **Longe Range and High Fidelity PCR using Triplemaster PCR SystemThese newly introduced reagents, in combination with existing products such as the TripleMaster PCR System, demonstrate the consistent conversion of the researcher's desire for simplification of tools through innovative technologies.

P 123

X-linked spondyloepiphyseal dysplasia tarda – A new missense mutation

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Spondyloepiphyseal dysplasia tarda is a genetically heterogeneous disorder of the skeletal system characterized by short stature and progenient osteoarthritis with an early onset. This X-linked disorder is caused by deletions or point mutations in the SEDL gene, coding for a 140 amino-acid protein with a putative role in endoplasmatic reticulum-to-Golgi vesicle transport as part of the transport protein particle (TRAPP) and mapping to Xp22.2. Herein we report a family with two male cousins showing symptoms of Spondyloepiphyseal dysplasia tarda as disproportionate short stature, barrel chest, lower back pain and early need for hip arthroplasty. After amplification by PCR followed by sequencing protein coding exons of the SEDL gene we demonstrated a substitution of a single nucleotide in exon 5 at nucleotide position 254 resulting in an exchange of a T for a G (ATG to AGG) causing a missense mutation M85R in the translated gene product. Genotype analysis of the mothers was carried out also confirming the X-linked type of inheritance as both were found to be female carrier of this recessive disease.Compared to the already described 4 missense mutations (F83S, S73L, V130D, D47Y), this new M85R transition causes more severe symptoms comparable to mild courses of nonsense mutations assuming the alteration of a significant protein domain.These data may contribute to figure out the complex function of this evolutionarily highly conserved protein in eukaryotic organism.

P 124

Identification of candidate genes for Emery-Dreifuss muscular dystrophy using GeneSeeker

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Until now two genes, STA and LMNA, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Scanning 93 patients suffering EDMD or associated phenotypes at the Institute of Human Genetics Greifswald revealed that mutations in STA and LMNA together account only for 36 % of the cases. Obviously, further genes are likely to be involved in EDMD. Forced by the lack of families suitable for a classic positional cloning approach we started a candidate gene approach.A priori functionally related proteins to emerin and lamin A/C such as lamin B1 (LMNB1) and B2 (LMNB2), lamin B receptor (LBR), lamina-associated polypeptides (LAP1 and LAP2), nurim (NRM) and the integral inner nuclear membrane protein (MAN1) are promising functional candidates. Moreover, factors interacting with emerin and/or lamin A/C (Narf: nuclear prelamin A recognition factor, Zmpste24: zinc metalloproteinase) were considered as candidates. An additional group of candidates were those, which are expressed specifically in heart and skeletal muscle - the preferentially affected tissues in EDMD (FLNC encoding filamin C and SMPX encoding a small muscular protein). But until now we have not been able to identify any certain disease causing mutation for EDMD in the genes examined.So we used a new web-based tool for the identification of candidate genes for human genetic disorders called GeneSeeker (Van Driel et al., 2003). Using GeneSeeker with the assumption that EDMD and limb-girdle muscular dystrophy (LGMD) are clinically overlapping, we identified a new candidate gene designated Popeye 1 (POP1). POP1 belongs to the Popeye gene family including also the paralogs POP2 and POP3. All three paralogs encode peptides showing a perinuclear distribution. Additionally, their strong conservation and preferential expression in heart and skeletal muscle prove all three genes as promising candidates for EDMD. So far seven out of eight exons of POP1 have been tested in 47 patients. Until now one intronic DNA variant has been identified. The investigation will be extended to POP2 and POP3.

P 125

Targeted gene repair of hprt mutations by 45 base single stranded oligonucleotides

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Targeted repair of a single base in a gene of an eucaryotic cell by specific oligonucleotides is a yet controversial technique. Here, we introduce the repair of point mutations in the hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) gene as an additional model system to test targeted gene repair. In human, Hprt mutations cause Lesch-Nyhan syndrome. Using hamster V79 cells, we generated three cell lines with one hprt point mutation each. These cell lines were treated with specific single stranded 45 base phosphothioate modified oligonucleotides and selected by HAT medium. The surviving clones were investigated for the repair of the respective hprt mutation. Treatment with the oligonucleotides was successful in repairing all three hprt mutations (hprt cDNA position 74, C to T; position 151, C to T; position 400, G to A). The repair rate was very low but reproducible. We suggest that this system allows to investigate targeted gene repair in dependence of the target sequence and the oligonucleotides used.

P 126

A 5;13 translocation identified in an affected father and son is not implicated in the autosomal dominant epidermolysis bullosa

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Epidermolysis bullosa (EB) describes a group of clinically and genetically heterogenous disorders characterized by blistering of skin and other epithelia. The blisters are manifestations of a separation between the epidermis and the dermis along the basement membrane and usually result from friction or microtrauma. There are three major subtypes of EB, simplex, junctional, and dystrophic. For the autosmal dominant inherited forms of EB, 4 different genes are known, KRT5, KRT14, COL7AI, and PLEC1. We are studying a family with autosomal dominant EB of hands and feet in which the disease segregates in three generations. Conventional cytogenetic analysis by GTG-banding showed a reciprocal and apparently balanced translocation t (5;13)(q13;q32) in the affected father and affected son. However, the affected daughter shows a normal kary-

otype suggesting that the translocation is not primarily associated with the disease phenotype in the family. In order to identify the disease gene, we perform segregation analysis using polymorphic DNA markers closely linked to known EB loci. So far, the data excluded the involvement of the genes KRT5 and KRT14 that are mutated in the autosomal dominant simplex EB. Analysis of the COL7AI gene, mutated in the dystrophic form of autosomal dominant EB, is in progress. To characterize the 5;13 translocation at the molecular level, we delineated the breakpoint regions and identified an overlapping BAC clone for each breakpoint by FISH analysis. The ERBB2IP gene was found to be disrupted by the breakpoint in 5q13, and the GPC6 gene by the breakpoint in 13q32. By RT-PCR analysis of RNA isolated from lymphocytes of father and son, we identified three different fusion RNAs that consist of various exons of ERBB2IP in their 5´ region and GPC6 at the 3´ end. These data confirmed that two genes were interrupted by the translocation. The protein encoded by ERBB2IP, erbin, was shown to interact with BPAG1 and the integrin beta 4 subunit, both components of hemidesmosomes (HDs) that are cell-substrate adhesion complexes in stratified epithelia. These data suggest that erbin may be involved in the assembly of hemidesmosomes. Consequently, the ERBB2IP gene may be considered a candidate gene for epidermolysis bullosa. However, the fact that the 5;13 translocation is present in only two of three affected family members argues against its involvement in the disease in the present family. Clearly, we can not exclude that disruption of ERBB2IP may modulate the EB phenotype in the family described here.

P 127

Scanning of DDX16, Nesprin1alpha, Nesprin2alpha1 and SREBF1 as candidates for Emery-Dreifuss muscular dystrophy

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Until now Emery-Dreifuss muscular dystrophy (EDMD) has been considered as a monogenic disorder caused by mutations in STA or LMNA. But mutations in STA and LMNA account together only for 36 % of the patients, thus pointing to further genes likely to be involved. Forced by the lack of families suitable for positional cloning, we started a candidate gene approach.Emerin and lamin A/C are components of the inner nuclear membrane and the nuclear lamina. Thus it seems very likely, that defects in other genes encoding interacting peptides to emerin and lamin A/C are involved. Thus we considered DEAD/H-Asp-Glu-Ala-Asp/His-box polypeptide 16 (DDX16) and sterol response element binding protein factor 1 (SREBF1) as candidates for EDMD. Additionally, we considered genes, which are expressed specifically in heart and skeletal muscle and show a perinuclear localization such as nesprin1alpha1, nesprin1alpha2 and nesprin2alpha1 encoding nuclear envelope spectrin repeat containing peptides 1 and 2. The candidate genes were scanned for DNA variations by using primers to amplify all exons including the exon/intron boundaries in 95 patients from Germany excluded to carry mutations in STA or LMNA. The PCR fragments were studied by heteroduplex analysis. Until now we have scanned 49 exons of DDX16, nesprin1 and nesprin2. 24 aberrant heteroduplex patterns have been found so far and 18 DNA variations were validated by sequencing. Testing approx. 200 chromosomes of a German reference population, two unique variations in DDX16 (R125Q, T810M) and four in nesprin1alpha (29A>G, N323H, V572L, E646K). Interestingly, one patient carried two unique amino acid changing mutations – one in DDX16 (T810M) and one in nesprin 1 (N323H), pointing the first time to a digenic pathogenesis in Emery-Dreifuss muscular dystrophy.

P 128

Analysis of cellular processing and function of TSPY

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TSPY (Testis-specific protein, Y-encoded) shows a distinct expression pattern as well in human testis and testicular tumors, as in some prostate cancer samples and cell lines. TSPY is characterized by homology to the increasing SET/NAP gene family, which has been postulated to be involved in gene regulation due to the chromatin binding properties of the NAP-(nucleosome assembling protein) domain. We now have studied the cellular processing of the TSPY protein. Using various GFP-reporter constructs with introduced mutations or deletions we were able to demonstrate the importance of the C-terminus for the integrity and stability of the TSPY protein. While the wild-type TSPY protein is detectable in the cytoplasm and in the nucleus,e.g. mutations of the terminal CK2-Phosphorylation site led not only to an enhanced degradation of the protein, but also to the loss of the signal within the nucleus. Current pull-down assays with a cloned GST-NAP domain of TSPY should identify potential interacting proteins. To understand possible effects on genes downstream of TSPY, we applied the micro-array approach. Using the cDNA of a cell line, stably transfected with the expression plasmid pRO-K2, as a probe we started to test the Affimetrix chip, and a homemade micro-array of about 1200 genes for an altered expression pattern. Actually we are evaluating candidate genes, like MKK6, for their relevance.

P 129

Real-time RT-PCR assay for validation of cDNA array supported candidate markers in renal cell carcinomas (RCC)

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The identification of new biomarkers in renal cell carcinomas (RCC) is needed for early diagnosis and progression monitoring. Especially the molecular mechanisms for the transition from non-metastatic tumors to metastatic forms are of interest. To find and analyse these complex mechanisms high parallel analyses involving cDNA microarray data has to be used to identify up- or down-regulated genes in cancer cells, whose products may then be further validated as potential biomarkers. A widely used method for the validation of differentially expressed genes is the real-time reverse transcription polymerase chain reaction (RT-PCR) technique that monitors product accumulation and thereby allows a precise quantification. Four genes identified by previously performed cDNA microarray analysis were selected for validation. Primers flanking the appropriate cDNA sequences were selected and synthesized. The T7 amplified cDNA hybridized in the microarray analysis was used as template. The real-time quantitative PCR assay was performed with SYBR Green I dye-based detection system including product melting curve analysis to validate differentially expressed genes. In this way 20 probes (10 normal controls, 5 non-metastatic and 5 metastatic RCC) were analysed threefold. For all probes also an internal standard (PCNAb, MCPb) was co-amplified. Relative expression levels were quantified by creating a standard curve using cDNA dilutions of highly expressed genes. The whole experiment was performed on two different SYBR Green based real-time PCR cyclers (LightCycler, Roche; Opticon 2, MJ, Biozym). The amount of gene expression found by LightCycler and Opticon 2 was statistically compared and then correlated to the results found by microarray analysis. Based on the current results it can be concluded that deviation between quantification with arrays and real-time PCR are in the same dimension as between the two used real-time PCR systems.

P 130

Expression of H-/T-Cadherin during kidney development

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H-/T-cadherin (CDH13) is another member of the cadherin-superfamily of cell adhesion molecules with a postulated tumorsuppressor activity as deduced from expression studies in various primary tumors. H-/T-cadherin is an unusual cadherin molecule as an intracellular cytoplasmic domain, which is etc. a linker to the cytoskeleton, is missing. Instead it is coupled to a GPI-ancor and localizes to lipid rafts, which form so-called supramolecular activation centres (SMACs) on the apical surface of the cell. The postulated function therefore should be more cell recognition than cell adhesion. We now have generated a H-/T-cadherin-antiserum and DNA probes to study various developmental aspects. Our special interest focuses on human and mouse kidney development as we were able to show an upregulation during the differentiation of the glomeruli. In fetal kidneys no H-/T-cadherin protein expression was detectable in the early „S-shape“ stage, but it was very prominent and broad in the the early glomeruli stage, namely the inner layer of epithelial cells, before the mesangium and podocytes differentiate. This pattern changes with ongoing differentiation and becomes more punctate. Using an in situ hybridisation and immunohistochemistry approach we try to characterise the pattern of gene expression of defined members of the cadherin family and the question of a possible hierarchical order during nephron differentiation. Using the human kidney cell line HEK-293 we furthermore test for possible interactions with caveolin-1, another tumorsuppressor, which is localized

in the caveoli of lipid rafts and linked to the intracellular signalling pathway.

P 131

Expression and mutational analysis of selenoprotein phospholipid hydroxyperoxide glutathione peroxidase (PHGPx) gene

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Phospholipid hydroperoxide glutathione peroxidase (PHGPx) is an intracellular antioxidant of spermatozoa and protects it against the oxidative stress generated by reactive oxygen species. The abundance of PHGPx in the mitochondrial capsule of the spermatozoa midpiece and impaired sperm motility in selenium-deficient animals, is considered to result from insufficient PHGPx content, suggest the role of PHGPx in male fertility. In an attempt to verify the expression pattern and localization of PHGPx, immunostaining and electron microscopical studies were performed on sections and spermatozoa of different species. In mouse, PHGPx was detected in Leydig cells and spermatids. A localization of PHGPx was found in midpiece of spermatozoa of Drosophila melanogaster, frog, fish, cock, mouse, rat, pig, bull and human. This result was verified using electron microscopical analysis. Furthermore, we studied the relationship between sperm PHGPx expression, mutation in PHGPx gene and human oligoasthenozoospermia, a defect in which both the number and the motility of spermatozoa are significantly below normal. Sperm specimens from 44 infertile males were analysed for fertility-related parameters according to World Health Organisation and were classified as suffering from oligoasthenozoospermia. Two patients (4.54%) showed no expression of PHGPx and in 9 patients (20.45%) a reduced expression of PHGPx was observed. Genomic DNA sequences of PHGPx coding and its 5´flanking regions from these patients and 9 fertile volunteers were analysed for polymorphisms by PCR amplification and direct sequencing. Sequence analysis revealed no cause/effect relationship for any of the polymorphisms. From these data it can be concluded that oligoasthenozoospermia is associated with a decrease in the level of expression of mitochondrial PHGPx in mitochondria in the spermatozoa of some infertile men (24.4%) but is not linked to mutations in PHGPx gene.

P 132

Characterization of a novel brain specific transcript on human chromosome 20q13

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Based on human EST-markers we have identified a novel human gene on HSA20q13.32 whose expression appears to be restricted to the brain as Northern analysis of 8 human tissues revealed expression of a 3.2 kb and a 3.0 kb transcript in brain only. One corresponding cDNA (AJ311122) contains a 1680 bp ORF distributed on 13 exons, a second one as part of the EMBL sequence database owns a different initial exon resulting in a 1671 bp ORF. Both ORFs show a homology of the endmost 1557 bp beginning in exon 2. Multiple tissue Northern analysis of the corresponding mouse transcripts has revealed 3 major signals of 3.3 kb, 2.9 kb and 2.5 kb visible in brain only emphasizing the tissue specifity of the human expression pattern. Analysis of the 5`-end of the transcribed RNA by RACE-PCR yielded four initial exons suggesting alternative splicing, as supposed to be in human. These four mRNAs of the gene, mapping to mouse chromosome 2H4, could be verified by RT-PCR. Further on, this complex expression pattern is not restricted to the 5'-end as we could demonstrate alternative polyadenylation in hybridization experiments. The transcripts vary in ORF size but all show the 1557 bp homology as found in human. Searching the databases a striking homology of approximately 120 aa at the C-terminal end of the predicted protein was found shared with 3 other human proteins with so far unknown function, suggesting a putative domain which seems to be highly conserved down to D. melanogaster and C. elegans. Therefore further experiments should lead to characterization of the gene product, possibly relevant in brain development or function.

P 133

Molecular characterization of the novel human TRAP240-like gene which maps close to the chromosome 12 breakpoint in a patient with Noonan syndrome, and its mouse orthologue mtrap240-like

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During the characterization of the chromosome 12 breakpoint region in a patient with a balanced t (2;12)(q37;24) translocation and a clinical diagnosis of Noonan syndrome, we identified a novel human gene, thyroid hormone receptor-associated protein (TRAP) 240-like. Interestingly this gene is expressed at a lower level in the patient lymphoblastoid cell line than in control cell lines, although the breakpoint maps 28 kb to its 5´ end. We therefore investigated the human and mouse counterpart in more detail. Transcripts were cloned in silico by aligning several human and mouse expressed sequence tags (ESTs) and mRNA sequences. The complete cDNA sequences of 9546 bp and 6812 bp were determined by RT-PCR and 5´RACE experiments. The human and mouse genes span a genomic region of 310 kb and 170 kb, respectively and contain 31 exons. Their open reading frames code for proteins of 2211 and 2204 amino acids which are 87% identical. By northern blot analysis human TRAP240-like appeared to be expressed at low levels and could be detected in fetal brain, liver and kidney and adult brain, kidney, skeletal muscle, lung, placenta, pancreas and heart. The mouse ortholog showed ubiquitous expression and was particularly abundant in brain and heart. The TRAP240-like protein is 56% identical to human TRAP240, which belongs to the TRAP complex and is evolutionary conserved up to yeast. This complex is involved in transcription regulation and is believed to serve as adapting interface between regulatory proteins bound to specific DNA sequences and RNA polymerase II. Mutations in Drosophila dTRAP240 (pap) resulted in loss of segmental identity specification. Further investigations are needed to determine the biological role of the human TRAP240-like protein.

P 134

Molecular characterization of four patients with microphthalmia and linear skin defects syndrome (MLS)

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The microphthalmia with linear skin defects (MLS) syndrome is a severe developmental disorder. Major clinical signs are localized dermal hypoplasia and microphthalmia. Occasional abnormalities include agenesis of the corpus callosum, sclerocornea, chorioretinal abnormalities, congenital heart defects, infantile seizures, and mental retardation. The MLS syndrome is considered an X-linked dominant disorder with in utero lethality in males. In the majority of patients with MLS syndrome, cytogenetic studies revealed an XX complement with a variety of X-chromosomal aberrations, including deletions of Xp22-pter or unbalanced translocations between the X chromosome and an autosome or the Y chromosome, that all resulted in segmental monosomy of Xp22.3. By physical mapping, a ~570 kb minimal region of monosomy has been identified in Xp22.3 containing the genes MID1, HCCS, and ARHGAP6. We are investigating four patients (three females and one male) with MLS syndrome. The male patient is the first MLS case with a 46,XY karyotype. He carries a paracentric inversion of the short arm of the X chromosome in 15% of his blood lymphocytes with the distal breakpoint in Xp22.32~p22.33 and the proximal breakpoint in Xp22.13~p22.2. Currently, we are performing FISH experiments of fibroblast cells of the patient to detect the inversion in a second tissue. Further studies aimed at the delineation of both breakpoint regions. Two of the female patients show a de novo terminal deletion of Xp22-pter. Polymorphic microsatellite markers are being used for segregation analysis in the patients and their parents to define the extent of the cytogenetically detected deletion. In the third female patient, high resolution cytogenetic analysis revealed an apparently normal female karyotype. DNA samples of the patient and her parents were analyzed by microsatellite markers to detect a microdeletion.

P 135

Genotype Phenotype Correlation in the Noonan Syndrome

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Mutations in the gene PTPN11 have recently been identified as the molecular defect underlying Noonan syndrome (NS) in 33-50% of patients. In order to study genotype phenotype correlations, we performed mutational analysis by direct sequencing of the entire coding sequence of the PTPN11 gene in 57 patients from 50 families with the clinical diagnosis of NS ascertained according to standardized inclusion criteria. 13 known and 4 novel PTPN11 mutations could be detected in 30/50 (60%) of index cases. A causative PTPN11 mutation was found in all familial but only in 51% of the sporadic cases. Presence of pulmonic stenosis, short stature, easy bruising, and thorax deformities was significantly associated with a PTPN11 mutation, whereas cardiomyopathy was more common in patients without. Mutation-positive probands were more likely to exhibit three and more typical clinical features of NS whereas oligosymptomatic patients were found more frequently in the non-mutated group. However, more than half of the patients in the non-mutated group - among them all with cardiomyopathy - had the full clinical picture of NS indistinguishable from typical cases with PTPN11 mutation. Our findings suggest that the phenotype of NS due to PTPN11 mutations is clinically unambiguous in the majority of the patients and represents a highly penetrant trait. The higher mutation detection rate in our study compared to previous reports is most likely due to more strict clinical inclusion criteria. Individuals with the clinical diagnosis of NS but without a PTPN11 mutation presumably represent a heterogeneous group in which patients with cardiomyopathy appear to constitute an interesting subgroup for future research. The recurrent Asn308Asp mutation was associated with a very broad phenotype, but very mild facial anomalies occurred more frequently with this mutation. No PTPN11 mutation was found in 12 additional patients not fulfilling the diagnostic criteria for NS but exhibiting only single anomalies of the phenotypic spectrum.

P 136

Characterization of differentially expressed genes involved in cardiac hypertrophy

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Spontaneously hypertensive rats (SHR) provide a well known animal model to investigate hypertension and heart failure. In order to identify candidate genes participating in initiation and/or progression of the observed phenotype RNA was isolated from hearts of SHR and its normotensive genetic control, the Wistar-Kyoto rat (WKY), to screen for differentially expressed genes using a subtractive hybridization system based on cDNA selection and suppression PCR. Here we report the characterization of two candidate cDNA clones out of the group of putative deregulated genes we could neither assign a function so far nor find any homology to already known transcripts or proteins. Based on the 900 bp cDNA clone 65 and the 1000 bp cDNA clone 36 Northern Blot analysis using polyA+ - RNA from heart tissue of SHR and WKY rats has revealed transcripts of about 1,8 kb (clone 65) and 4 kb (clone 36), respectively. In silico analysis of clone 65 yielded a rat transcript of 1221 bp in size encoding 406 aa consisting of two exons spanning a genomic region of about 3,4 kb on chromosome 17 (q11). The homologous genes of mouse and human are mapped on chromosome 13 (A3.2) and 6 (p22.1). The second clone 36 shows homology to an 1456 bp rat mRNA containing 306 bp ORF (101 aa) distributed on 12 exons on chromosome 13 (q11) with a genomic length of about 27,5 kb. The homologous genes of mouse and human are located on chromosome 1 (E2.3) and 2 (q13). RACE-PCR will be performed in order to complete with cDNA sequences and refine the genomic structure. Further experiments will focus on verifying the quantitative expression data and studying the tissue specific expression pattern of the candidate genes. Quantitative trait loci (QTL) in the genomic regions of interest will be examined with respect to known genes involved in cardiac hypertrophy.

P 137

The high mobility group protein gene HMGA2 is strongly conserved among individuals

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The role of HMGA proteins in different diseases including benign and malignant tumors, and restenosis can be explained easily by their molecular functions. They act as master switches of the cell regulating a high number of target genes by modulating their chromatin. However, despite a number of interesting findings very little is known about variation of the HMGA proteins within human populations. Therefore, we have sequenced the coding region of HMGA2 of 50 healthy volunteers aimed at the detection of single nucleotid polymorphisms. The description of such polymorphisms may be of considerable interest because in most of the HMGA2-associated diseases familial cases have been reported that may be linked to mutations of HMGA2. Herein, we were able to show identical coding regions of HMGA2 in all 100 alleles analysed. Thus, HMGA2 is not only well conserved between species but there is also a high interindividual conservation of HMGA2 further supporting the important role of HMGA proteins in cellular processes.

P 138

Cloning, genomic organisation and expression pattern of a human tuberin-homology gene, the tuberin-like protein 1 (TULIP1), and its murine and rat homolog

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We have previously described the clinical and molecular characterisation of a 14q13.1-21.1 microdeletion of less than 3.5Mb in size in a patient with severe microcephaly, psychomotor retardation and further clinical anomalies. Here we report the characterisation of the genomic structure of the human tuberin-like protein gene (TULIP1) found to be deleted in our patient as well as its murine and rat homolog. The human TULIP1 gene could be mapped to the chromosome band 14q13.2 by fluorescence in situ hybridisation of BAC clone RP11-355C3 (GeneBank Accession No. AL160231), containing the 3'-end of the gene. In addition, the human genome contains a processed pseudogene of TULIP1 on chromosome 9q31.2. Expression analysis for the active locus was performed. No pseudogene could be found in the available mouse and rat sequence data. The gene is highly conserved in all three species. The function, expression profile and location of TULIP1 make it a candidate gene for at least some of the neurological findings in our patient and other 14q related cases.

P 139

Phenotypic expression in Fish-eye disease with G71R/R140H compound heterozygosity in the lecithin:cholesterol acyltransferase (LCAT) gene

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Lecithin:cholesterol acyltransferase (LCAT) is a key enzyme of lipoprotein metabolism. It catalyzes the esterification of free cholesterol (FC) in plasma to produce cholesteryl esters (CE), which represent the main core lipid components of plasma lipoproteins LDL and HDL. Primary (familial) LCAT-deficiency (LCAT-D) is a rare genetic, autosomal recessive, disease caused by the lack or near absence of LCAT activity. In a second genetic disorder known as fish eye disease (FED)(partial LCAT deficiency), LCAT esterifies FC only in LDL particles. In the present study, we describe the molecular defects in the LCAT gene of a 30 year old patient presenting with typical symptoms of FED including corneal opacity, virtual absence of alpha-LCAT activity, presence of reduced amounts of CE (low HDL-Cholesterol level), and elevated TG values. DNA sequencing revealed two separate mutations in exon 2 and in exon 4 of the patient´s LCAT gene: a G to C transversion converting Gly 71 to Arg, and a G to A transition converting Arg 140 to His. Digestion of the patient´s DNA with the restriction endonucleases StyI, and HhaI, respectively, confirmed that the patient is a compound heterozygote for both mutations. In addition a 15% mosaicism of a reciprocal 11/12 translocation was found by routine cytogenetic analysis of peripheral blood lymphocytes. To exclude chromosomal unbalances we determined the boundaries of the t (11;12)(q13.2;p13.3). Via BAC-FISH analysis we narrowed down each of

the two breakpoint regions to approximately 200-kb intervals. No loss or gain of genomic material could be identified.

P 140

Towards characterisation of loricrin keratoderma: diffuse palmoplantar keratoderma and generalised ichthyosis associated with a loricrin frameshift mutation

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We present a German family with an autosomal dominant syndrome of a mild type of generalised congenital ichthyosis and diffuse, honeycombed palmoplantar keratoderma (PPK). Some family members were born as collodion babies and some reported pruritus. The ichthyosis was overall mild but showed keratotic lichenification in particular on the flexural body parts such as the wrists, antecubital folds, popliteal folds, axillae, and around the navel. In a genome wide linkage study comprising 13 members of the family linkage was suggested with the marker at D1S498 with a maximum lod score of 2.6 (theta=0). This locus maps to a region on chromosome 1q21 containing the cluster of epidermal differentiation complex (EDC) genes. The cluster comprises many genes encoding proteins of the cornified cell envelope such as loricrin, involucrin, and filaggrin. A mutation in the gene for loricrin (LOR) was previously shown to be associated with mutilating keratoderma with ichthyosis, also known as PPK type Camisa-Vohwinkel. Because of the prominent honeycomb pattern of PPK in affected members of the family reported here resembling the PPK in Camisa-Vohwinkel disease, we considered loricrin a good candidate for the disorder in this family. We have subsequently identified a 1-bp insertion, 730insG, in LOR extending the protein by an arginine-rich domain. Interestingly, the same mutation was described in a family with mutilating keratoderma. A total of seven mutations have been found in loricrin so far underlying a heterogeneous group of palmoplantar ectodermal dysplasias nowadays referred to as loricrin keratoderma. Most of these phenotypes were initially diagnosed as mutilating keratoderma based on the finding of constrictions of fingers or toes leading to mutilation (pseudoainhum). Remarkably, there were no constricting bands visible in any affected member of our family. Hence these results demonstrate the importance of honeycombed PPK for the diagnosis but reveal that constrictions of digits are not an obligatory feature of loricrin keratoderma. Our findings allow to further delineate the clinical spectrum of the group of genodermatoses caused by loricrin mutations.

P 141

Spectrum of mutations in the epidermal lipoxygenase genes ALOXE3 and ALOX12B in patients with autosomal recessive congenital ichthyosis (ARCI)

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Autosomal recessive congenital ichthyosis (ARCI) forms a heterogeneous group of severe hereditary keratinization disorders characterized by more or less intense scaling of the whole integument, often associated with erythema, and a pronounced clinical and genetic heterogeneity. Up to the present, five loci for ARCI have been mapped to chromosomes 2q33-q35, 14q11, 17p13, 19p13, and 19p12-q12. Mutations in TGM1 on 14q11 account for approx. one third of ARCI cases with a phenotypic spectrum ranging from dark brownish bark-like skin until the so-called self-healing collodion baby. The underlying gene defect has been identified at only one of the other loci so far. Mutations in ALOXE3 and ALOX12B on chromosome 17p13, which encode two different lipoxygenases of the epidermis type, were recently found in six ARCI patients from the Mediterranean area. Here we have analyzed 150 families with ARCI without mutations in TGM1. In ALOX12B we discovered two homozygous missense mutations in two consanguineous families from Turkey. Another homozygous missense mutation was found in ALOXE3 in a consanguineous family from Southwest Germany. Patients of an extended, consanguineous Indian pedigree showed a homozygous nonsense mutation in ALOXE3. Two different mutations in two exons of ALOXE3 - one nonsense, one missense - were found in a non-consanguineous family from Czechia. In total, less than ten percent of all ARCI patients carry mutations in one of these two genes. Lipoxygenases are involved in the production of leukotrienes and various hydroxyeicosatetraenoic acids (HETE) from arachidonic acid and hydroxyoctadecaenoic acids (HODE) from linoleic acid. Exact functions, however, of the members of the epidermis subgroup exclusively found in epidermal keratinocytes and hair follicle cells are partly still unclear. In order to functionally analyze the mutations leading to ichthyosis, we are developing an in-vitro assay for these enzymes. Further analysis of the enzyme specificities and characterization of different mutations will give insight into the molecular mechanisms underlying ARCI.

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Mutation analysis in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a rare, multisystem disorder with an autosomal recessive mode of inheritance. The cardinal features are central obesity, rod-cone dystrophy, postaxial polydactyly, learning difficulties, hypogenitalism in males and renal dysplasia. The population prevalence ranges from 1:13500 livebirths among the Bedouin of Kuwait to approximately 1:160000 in Western-Europe. BBS is genetically heterogeneous with at least seven different chromosome loci linked to the disease: BBS1 on 11q13, BBS2 on 16q21, BBS3, on 3p12-p13, BBS4 on 15q22.3-q23, BBS5 on 2q31, BBS6 on 20p12, and BBS7 on 4q27. To date, five BBS genes – MKKS,BBS2,BBS4,BBS1,and BBS7 – have been identified. MKKS presumably codes for a type II-chaperonin, whereas the BBS4 gene product shows strongest homology to O-linked N-acetyl-glucosamine transferase (OGT) from several species. The BBS1, BBS2,and BBS7 proteins are similar to each other but do not display significant similarity to any known proteins or protein families.Here, we report on the identification of novel mutations in BBS genes. A 1247delG mutation was observed in exon 14 of BBS4, homozygous in an Egyptian BBS patient, who presented with the typical clinical features of the syndrome. The deletion causes a frameshift resulting in a truncated protein of presumably 467 amino acid residues. This is the first report of a homozygous single base deletion causing a truncated BBS4 gene product. In BBS1 we describe a homozygous deletion of complete exons 12 and 13. The breakpoints were identified by sequence analysis and the deletion was confirmed by MAPH. To elucidate the function of the BBS proteins, we search for potential binding partners using the yeast-two-hybrid system. Supported by: Deutsche Forschungsgemeinschaft (DFG, OE 262/1-1) and the Kempkes-Stiftung.

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Variable expression of the phenotype of a variant form of Vohwinkel syndrome caused by a small insertion in exon 1 of the loricrin gene

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Vohwinkel´s syndrome (VS) is a rare heterogenic disorder, which can be caused either by a mutation of the connexin 26 (CX26) gene or in a variant form by a mutation of the loricrin (LOR) gene. Both variants are transmitted in an autosomal dominant mode of inheritance. The two subtypes of VS are usually characterized by the presence of congenital deafness as observed in patients with CX26 mutations or absence of deafness but show different skin lesions in non-

palmoplantar areas like in patients with LOR mutations. Here we report on a 34 year old female patient with all phenotypic features of VS like hyperkeratosis and constrictions on fingers and toes with further skin lesions in non-palmoplantar areas like elbows and congenital deafness. Mutation analysis of both genes revealed a 6 bp insertion in the first exon of the LOR gene however no mutation in CX26 was found. Subsequently performed mutation analysis of her parents demonstrated that the insertion was inherited from her asymtomatic father. No other familiy member shows any symptom of VS. Such an extreme phenotype variation has not been reported thus far. The number of different mutations of the LOR gene is still very low. Therefore we assume that the new mutation described here could be responsible for variability as well as divergence in phenotype expression.

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Classical xathinuria type 2 associated with a missense mutation in molybdenum cofactor sulfurase

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The frequency of classical xanthinuria (XU) is estimated roughly to lay between 1:6,000 and 1:65,000. Clinically, XU may be fully asymptomatic or associated with more or less progressive nephropathy along with other signs due to deposition of xanthine crystals. Based on clinical laboratory findings XU can be divided into two subtypes (XU1, XU2). In XU1 but not in XU2 oxidation of allopurinol and excretion of oxypurinol can be demonstrated. XU1 is linked to mutations in XDH, the gene for xanthine dehydrogenase (XDH). XDH catalyzes both the conversion of hypoxanthine to xanthine and of xanthine to uric acid, thus being essential for oxidative elimination of terminal purine nucleotide metabolites. XDH also oxidizes purine analog allopurinol to oxypurinol that in turn blocks irreversibly XDH. Allopurinol may be oxidized alternatively by aldehyde oxidase (AO). Therefore, in XU1 oxypurinol may be detected after an allopurinol challenge. In cattle, XU2 has been shown to be linked to a deletion in MCSU, the bovine homolog of HCMS, the human gene for molybdenum cofactor sulfurase (MCS). For both XDH and AO presence of sulfurated molybdenum cofactor is functionally essential. MCS catalyzes sulfuration of the molybdenum cofactor. Therefore, it was assumed that deficiency of MCS should lead to the combined loss of activity of XDH and AO recognizable by absence of oxypurinol following an allopurinol challenge. In HCMS of two Japanese patients with XU2 the same homozygous nonsense mutation (R419X) has been reported recently (Ichida et al., Biochem Biophys Res Commun 282:1194-1200, 2001). Here we report the first missense mutation in HCMS of a German patient with XU2. cDNA (AK000740) and genomic sequence of HCMS were identified by BLAST searching GenBank using published MCSU cDNA. All 15 coding exons of HCMS from genomic DNA of our patient and an anonymous Caucasian proband were amplified by PCR and sequenced. Besides some apparently homozygous single nucleotide changes in both subjects, respectively compared to AK000740 we found a single and apparently homozygous missense mutation (T294I) in our patient only. The mutation was not present in 144 Caucasian control alleles analyzed by restriction enzyme. The patient's daughter is heterozygous carrier of the mutation. Parental DNA was not available for analysis. Therefore we cannot exclude compound heterozygosity of the missense mutation and a deletion in the patient. The residue corresponding to T294 is fully conserved among all species analyzed so far, including vertebrates, drosophila, C. elegans, bacteria, neurospora crassa, and arabidopsis. Whereas we can conclude that mutations in HCMS may be causative for XU2, this does not exclude other possible molecular causes of XU2.
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P 145

Mutations of PKHD1 and genotype-phenotype correlations in autosomal recessive polycystic kidney disease (ARPKD)

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Autosomal recessive polycystic kidney disease (ARPKD/PKHD1) is one of the most common causes of renal- and liver-related morbidity and mortality in childhood. We have recently shown, with others, that mutations in the PKHD1 gene on chromosome 6p21.1-p12 underlie ARPKD. The longest continuous open reading frame is encoded by a 67 exon transcript and predicted to yield a 4,074 amino acid protein (polyductin/fibrocystin) of thus far unknown function. Mutations were found to be scattered throughout the gene without evidence of clustering at specific sites which makes analysis extremely cumbersome. A mutation can be observed in approximately two thirds of disease chromosomes. We have compiled molecular and clinical information in a database encouraged by HUGO (http://www.humgen.rwth-aachen.de). Most PKHD1 mutations are unique to single families („private mutations") which hampers genotype-phenotype correlations. Correlations could be drawn for the type of mutation rather than for the site of the individual mutation. All patients carrying two truncating mutations displayed a severe phenotype with perinatal or neonatal demise. PKHD1 mutation screening is justified as a basis for prenatal diagnosis and genetic counselling in cases where no material/DNA of an affected child/fetus is available anymore or in cases with atypical clinical/pathoanatomical features. We provide an overview of PKHD1 mutations, review them in the context of their clinical implications and discuss putative genotype-phenotype correlations in ARPKD.

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SNP frequency, haplotype structure and linkage disequilibrium in human FBN1-gene

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The fibrillin gene (FBN1) is a relatively large (approximately 110 kb) and highly fragmented (65 exons) gene. Mutations in FBN1 cause Marfan syndrome and autosomal dominant Weill-Marchesani syndrome. Moreover, it is a candidate for association studies in complex diseases. To determine the SNP frequency and haplotype structure of the human FBN1 gene we analyzed a total of 28 single-nucleotide polymorphisms (SNPs) spanning the entire 110kb locus. 17 of these 28 SNPs were initially identified as sequence variants in Marfan patients, of which 8 were already published in the database. Additionally, 11 SNPs were selected from the genome SNP database (dbSNP), whereas heterozygosity of 0.3 was the major criteria. SNPs were genotyped on 96 unrelated individuals from Caucasian origin by sequencing the corresponding fragments. For the analysis of the linkage disequilibrium (LD) and haplotypes structure of the region we chose those SNPs, which showed a minor allele frequency of at least 9% in our control group. Only 18 of the 28 SNPs analyzed fulfilled this criteria. Of the published 11 SNPs with a given heterozygosity of more than 0.3, three were not detected in our control samples and two were found only twice. LD was calculated with ldmax using an expectation-maximization algorithm and visualized with GOLD. SNPs in a region of 110 kb covering the entire FBN1 locus are in significant pair wise LD. Haplotypes and their frequencies were estimated using the PHASE program. Within this region 5 common haplotypes were observed accounting for 91% of chromosomes. The most frequent one was found in 64% of the control samples. All but two of the rare haplotypes can be reconstructed from the three most common by chancing only one site. To review the quantity of chromosomes which are necessary for definition of LD and haplotype structure of the FBN1 locus, calculation were repeated with 48 control persons. Repeated calculation with only 48 control persons demonstrated the same LD and haplotype structure.

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Methylation status of the 5´ promoter region of the CAVEOLIN-1 gene in human prostate cancer cell lines and prostate cancer tissues

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In an ongoing study on the role of methylation of the CAVEOLIN-1 promoter for the silencing of the gene, we reported that 4 out of 7 CpGs (-881bp to -518bp relative to the translation start site) abolish gene activity when methylated. These results were based on reporter gene assays and reactivation of CAV-1 in prostate cancer cell lines by 5-aza-2´deoxycytidine. Methylation of CpG sites influences gene activity by specific binding of proteins which we tested using gel retardation assays. We report now that the PCR fragment containing the CAV-1 promoter is able to bind such a protein when methylated. This binding probably constitutes the mechanism which inactivates the CAV-1 promoter. The best known of these proteins is MECP2. Using competition experiments with MECP2, we were able to demonstrate that it does not bind to the CAV-1 promoter and that it is different from the protein which causes the band shift in the gel. All these in vitro results do not constitute direct evidence for CAV-1 being involved in

tumorigenesis in vivo. This prompted us to assess the methylation status of the CAV-1 promoter in prostate cancer directly. For that purpose, 20 prostate tumor and 4 healthy control DNAs are under investigation at present. Genomic DNA was bisulphite treated to convert unmethylated cytosines to uracils. A 363bp fragment of the CAV-1 promoter containing seven CpG sites was amplified by PCR. PCR products are cloned und will be sequenced. The sequencing results will be reported and will definitely tell how frequently methylation of the CAV-1 promoter is involved in prostate cancer. This study was aimed to reveal the possibility of an epigenetically inactivation of CAV-1 and its crucial role in the development and progression of prostate cancer.

P 148

Detection of large duplications including the PLP gene in Xq22 by comparative quantitation using real-time PCR

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Pelizaeus-Merzbacher disease (PMD) is a dys- and hypomyelinating disorder of the central nervous system. The most frequently seen X-chromosomal form is caused by mutations of the proteolipid protein gene (PLP). Typically, patients present with severe neurological symptoms and psychomotor retardation. Since there is no biochemical assay to confirm clinical diagnosis, mutation detection in the PLP gene is an important tool both for diagnostic purposes and for proper genetic counselling of the families. In about 20% of the PMD patients, point mutations (mainly missense) and smaller rearrangements affecting a few nucleotides can be found, whereas large intrachromosomal duplications including the entire PLP gene are the predominant cause of PMD (in up to 60 % of the patients). In the past, various techniques have been used to detect these duplications spanning usually 0.5-1 Mb, e.g. interphase FISH, quantitative Southern blot analysis, or quantitative multiplex PCR. None of these approaches have been found to be optimal. In our hands, the method of comparative quantitation of an amplicon from exon 4 (263 bp) of the PLP gene and that from exon 4 (189 bp) of the CFTR gene by real-time PCR offers a major break-through in detecting the duplication. The high sensitivity of the method together with the possibility to select the most appropriate parameters by monitoring the reaction „on-line“, greatly improves both reliability and reproducibility of the results. Compared to a female reference sample, DNA samples of 20 unaffected males and females showed a relative value of 0,47 (+/- 0.06) and 1.02 (+/- 0.08), respectively. In a subsequent large experimental series, we examined 115 male patients with clinical symptoms of a leukodystrophy resembling PMD. In 13 patients, data were strongly suggestive of a PLP duplication. Our preliminary results also suggest that the method is sensitive enough to reliably detect carriers, i.e. to discriminate between two and three gene copies. In conclusion, the method of comparative quantitation by real-time PCR is a fast and convenient way to genotype PMD patients for the duplication in the PLP gene.

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Frequent allelic Variants of the Myocardial Ikr-alpha-Subunit Gene KCNH2 modulate the Duration of the QT Interval in the General Population

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The QT interval in the surface ECG is a sensitive and specific indicator of myocardial repolarization. QT prolongation is indicative for repolarization disturbances potentially leading to Torsades de Pointes tachycardia and sudden cardiac death. We investigated the influence of minor gene variants in known monogenic long QT syndrome genes on the QT interval. We analyzed gene variants and surface-ECG recordings from participants of a population based epidemiological survey in the Augsburg region (n=4149). Patients on cardiac medication or those showing signs of any disease pathology in their ECGs were excluded (remaining n=1035). QT correction parameters for heart rate (RR interval, +0.148 ms/ms) age (+0.245 ms/y) and sex (-7,70 ms for female) were determined by linear regression and were applied to transform measured values to heart rate-, age- and sex- independent QTc values. Mean QTc was 409,5 ms (SD +/- 15,1 ms) standardized to a 45 yr old male with a heart rate of 60/min.Four of five SNPs in KCNH2 exhibited strong pairwise LD and showed the most significant association to the QTc interval. The strongest association was at rs740952 (p=0,011) by ANOVA analysis of the SNP ‘s three genotypes. QTc values according to genotypes were in GG-homozygotes (n=634): 408,1±15,4 ms, in AG-heterozygotes (n=304): 410,0±14,9 ms and in AA-homozygotes (n=41): 414,6,1±17,3 ms. If analyzed for each sex separately the effect was significant and more pronounced in men (p=0,015) and was not significant but had the same trend in women (p=0,228).Haplotype analysis revealed the presence of 5 major haplotypes (AF>0,05) in KCNH2.Haplotype association analysis gave similar results but with a loss of significance due to increased degrees of freedom in the statistical analysis.

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Gene constructs for xenotransplantation

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The presence in humans of xenoreactive antibodies directed against swine Gal antigen present on the surface of xenograft donor cells, leads to the complement activation and immediate xenograft rejection – as a consequence of hyperacute immunological reaction. The Gal antigen (Gala1,3Gal) is synthesized by the a1,3-galactosyltransferase. In aim to prevent hyperacute rejection it is possible to modify swine genome by transfection of human genes - controlling enzymatic cascade of complement or modifying set of the cell surface proteins of the graft donor. For this purpose genetic constructs containing human CD59, CD55 and CD46 genes and human gene encoding a1,2-fucosyltransferase were prepared. CD59 blocks formation of the membrane attack complex. CD46 blocks formation of C3 convertases in the classical and alternative pathways of complement activation. CD46 has Factor I cofactor activity, which cleaves and inactivates C4b and C3b compounds. CD55 accelerates decay of C3 convertases in the classical and alternative pathways. It dissociates C2 and C2a from C4b and dissociates B and Bb from C3b. Introduction of additional copies of human gene encoding a1,2-fucosyltransferase into the swine genome leads to reduction of the Gal antigen level on the surface of donor cells. This results in reduced binding of xenoreactive natural antibodies to endothelial cells of xenograft and protection from complement mediated lysis.

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Studies on the gene expression profiles in patients with abdominal aortic aneurysm (AAA)

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Abdominal aortic aneurysm (AAA), a localized abnormal dilatation of aorta, is a life-threatening condition affecting 4-9% of population with a risk increasing with age. Other risk factors include hypertension, atherosclerosis and smoking. Familial occurrence of abdominal aortic aneurysm indicates involvement of genetic factors in development of AAA however no single gene was shown to be responsible for it. Based on the results of the studies done so far, it is believed that pathogenesis of abdominal aortic aneurysm is a complex and probably multifactorial process. Since gene expression profiling of abdominal aortic aneurysm tissues in comparison with clinically unchanged aorta may help to understand complex biological processes responsible for pathogenesis of AAA we applied DNA array technique in our studies. The expression profiling experiments were performed on cDNA arrays supplied by Clontech covering 588 genes from Atlas (Tm) Human Cardiovascular Array and 234 genes from Atlas (Tm) Human Stress Array. We have identified several up-regulated and down-regulated gene expression changes in abdominal aortic aneurysm tissues when compared to control unchanged aortas.

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Rapid and highly sensitive pharmacogenetic diagnostics using real-time PCR

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Adverse drug reaction can be frequently observed during treatment of several diseases and may occur due to genetic reasons. According to a meta-analysis published by Lazarou et al. (1998) drug-induced site reactions rank on place four of death rate. However, it is not known which of these cases of death have a genetic cause. A huge number of single nucleotide polymorphisms have been described in the last ten years. Many of these SNP's were identified within drug receptor, phase I and phase II genes. They may alter enzyme activity and thereby have influence on the individual drug metabolism. It has been described that drug treatment of patients can lead to severe adverse drug reactions including death due to enzyme deficiency from genetic alterations. In the last decade the direct detection of single nucleotide polymorphism using the versatile polymerase chain reaction or PCR-related methods has revolutionized the field of medical diagnostics. Nevertheless, conventional PCR-based methods require a post-reaction analysis by gel electrophoresis, which is time-consuming and prone to cross-contamination. Also, carrier of a single nucleotide polymorphism need to be identified by further steps, for example RFLP.A highly specific detection system is required to identify doubtless heterozygous and homozygous carrier of a genetic polymorphism. The artus GmbH is specialised in the field of real-time PCR and offers pharmacogenetic assays for use with the LightCycler(R) instrument (Roche Diagnostics), which allows genotyping of four important genes. The exon 14 skipping within the dehydropyrimidine dehydrogenase gene (DPD), three SNP's of thiopurine S-methyltransferase (TPMT), two SNP's of methylenetetrahydrofolate reductase (MTHFR) and seven of N-acetyltransferase 2 (NAT2) can be analysed. Using these assays the number of adverse drug reactions due to genetic reasons can be reduced, when substrates like 5-fluorouracil, thiopurines, methotrexate or isoniazid are administered. In addition, genotyping of these genes can help to optimise drug dosage and duration before starting patients therapy. All of these assays have been developed according to the requirements of the technical documentation and are CE-certified.

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No evidence of submicroscopic deletion or segmental uniparental disomy within the candidate regions 7p11.2 and 7q31-qter in a series of non-UPD Silver-Russell syndrome cases

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Different authors demonstrated that 7-10% of patients with the phenotype of Silver-Russell syndrome (SRS) have maternal uniparental disomy for chromosome 7. No single genetic cause for this syndrome has been found but the evidence of maternal uniparental disomy of chromosome 7 in several cases suggests that at least one imprinted gene on chromosome 7 is involved in the pathogenesis of SRS. 7p11.2-p13 and 7q31-qter might be separate candidate regions for a gene or genes involved in SRS. We re-investigated 107 patients with the clinical diagnosis SRS with a panel of more than a dozen markers from the 7p11.2-p13 and 7q31-qter regions. 95 families were informative for the region 7p11.2-p13 and 83 for the region 7q31-qter. The markers tested did not show evidence for microdeletion, duplication or uniparental maternal disomy for the critical segments 7p11.2-p13 and 7q31-qter in our patients. Although the markers used were specific and the method sensitive, it should be considered that low-level mosaicism in UPD is often difficult to detect. More cases of SRS and additional markers within the critical regions should be investigated for segmental matUPD7.

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Ext1 regulates chondrocyte differentiation

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Hereditary multiple exostoses (HME) syndrome is an autosomal dominant inherited human disorder, which is characterized by the formation of multiple cartilaginous capped benign tumors (exostoses) that develop from the growth plate of endochondral bones. So far HME has been linked to missense or frameshift mutations in the tumor suppressor genes Ext1 and Ext2. Both are glycosyltransferases involved in heparan sulfate (HS) biosynthesis.It has been shown that in Drosophila the homolog of Ext1, tout velu (ttv), is required for transport of hedgehog (Hh). One of the vertebrate homologs of Hh, Indian hedgehog (Ihh), is a key regulator of endochondral ossification.Ext1 knockout mice are embryonic lethal due to gastrulation defects. We are analyzing a mouse line carrying a hypomorphic allele of Ext1 obtained from a genetrap screen (Ext1-GT). Since Ext1 mutations lead to skeletal deformations in human patients we started to analyze the role of Ext1 and HS-chains during endochondral ossification focussing on the potential role of Ext1 in mediating the Ihh signal.Analysis of the Ext1-GT mutants revealed a severe delay in chondrocyte differentiation. We could show that less amounts of HS in these mutants allow further diffusion of Ihh whereas treatment of limb explants in a limb culture system with heparin leads to a restriction of the Ihh signal. These experiments implicate an important role of HS in establishing a gradient of Ihh signalling in cartilage thereby regulating chondrocyte differentiation.

P 155

No evidence for an increased malignancy risk in LGI1-caused epilepsy

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The LGI1 gene (Leucine-rich Glioma Inactivated-1) was first cloned from a glioblastoma cell line, where it was interrupted by a t (10;19)(q24;q13) translocation event. Additional evidence for a possible role of LGI1 in tumorigenesis came from the observation that its expression is reduced in low grade brain tumors, and significantly reduced or absent in advanced gliomas. It was therefore proposed that LGI1 is a tumor suppressor gene involved in the formation and malignant progression of glial tumors. Surprisingly, it has been recently shown that LGI1 is mutated in families with autosomal dominant lateral temporal lobe epilepsy (ADLTE). An urgent question soon emerged: are LGI1-mutation carriers in ADLTE families at a higher risk for brain tumors and other malignancies? The mutations found so far either cause a truncation of the LGI1 protein or affect structurally or functionally important sequence motifs. It can therefore be predicted that the individuals carrying one of these mutations express either a markedly reduced amount of LGI1 protein in their brain and other tissues, or an LGI1 protein with impaired function. We have now studied the comorbidity in the largest ADLTE family described so far, a 5-generation Norwegian family in which 17 individuals are known to carry an LGI1 mutation. Our analysis showed that in only one individual a malignancy had been diagnosed. This individual underwent surgery for gastric cancer 18 years ago and is now in good health. Sudden death had occurred in two individuals with epilepsy. One young man with sporadic seizures from age 12, but otherwise in good condition, was unexpectedly found dead in bed in his usual sleeping position with no signs of a preceding seizure. His sister suffered from multiple chronic disorders and died suddenly at age 64. The obligate carriers in generation II and the likely carrier in generation I all reached an above-average age (90-95 years). None of them had a known history of cancer. Neuroimaging in 11 of the subjects with clinical ADLTE revealed no signs of intracranial neoplasms. The present study demonstrates that the heterozygous LGI1 mutation C46R does not increase the number of malignancies or lower their age of onset, two features which are typical for familial cancer syndromes caused by germ line mutations of tumor suppressor genes. Thus, our clinical findings do not support the hypothesis that carriers of an LGI1 mutation might have a higher risk of malignant disease.

P 156

Familial Amyotrophic Lateral Sclerosis (FALS): Pathogenic Mutations and Non-pathogenic Variants in the Gene for Cu/Zn Superoxide Dismutase (SOD1)

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults. The typical age of onset is between 50 and 60 years. Loss of motor neurons in the brain and spinal cord give rise to muscular weakness and wastage, leading to paralysis. The disease manifests itself in different ways depending on which muscles weaken first. In 5 to 10 % of cases, ALS is inherited in an autosomal dominant manner. The discovery of mutations in the gene for Cu/Zn superoxide dismutase (SOD1) prompted to oxidative damage as a mechanism contributing to the disease. We have performed genetic testing on probands from 22 clinically diagnosed ALS families (FALS), and 3 apparently sporadic cases (SALS). Sequencing of intronic and exonic sequences associated with exons 1 to 5 of the SOD1 gene revealed a number of intronic sequence variants (e. g. T359A, A738C, G1107A, A1119T, C1152G, T1460C, G1399A, A3536G), and three ?approved? pathogenic missense mutations (R115G, E100K, I104F) that are listed in the ALS mutation data base (www.alsod.org).

SOD1-associated ALS is commonly assumed to result from a toxic gain of function process whereas most of the detected intronic mutations could only operate via a loss of function mechanism. The intronic mutations, therefore, most probably represent non-pathogenic sequence variants. This conclusion is substantiated further by estimating allele frequences among FALS probands and normal controls.

P 157

DIAGNOSTIC PITFALL: SOMATIC MOSAIC FOR HETEROZYGOUS SMN1 DELETION

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Objective: Infantile spinal muscular atrophy (SMA) is caused by a homozygous deletion of the SMN1 gene in nearly 95% of patients. Identification of carriers for the SMN1 deletion can be done by quantitative analysis of the SMN1-gene copies or via linkage studies in close relatives. We herewith present a family consisting of an affected boy with a proven SMN1-gene deletion, whose paternal uncles wanted to clarify their carrier status. Methods and results: Linkage analysis for the SMN-region flanking markers (D5S681, D5S629, D5S557 and D5S610) revealed identical haplotypes for the patient's father and his youngest uncle, while the second uncle was excluded as carrier. By analysing the multicopy-markers C212 and AG1-CA only one marker copy each on the deletion-carrying chromosome could be detected in the father in contrast to two copies on the identical haplotype of his youngest brother. To clarify the situation, the paternal grandmother was investigated and showed two marker copies on either chromosome but with different intensity on the deletion-carrying chromosome. The second copy, which was clearly present in the youngest uncle, gave a very weak signal in the grandmother's lymphocytes. Thereupon, a quantitative PCR based on a Real-Time-PCR (TaqMan technology) was performed to calculate the gene copy number in the family members. In the father a heterozygous SMN1 deletion could be confirmed, while his youngest brother had two SMN1 copies and was not classified as a carrier. The grandmother however showed an amplification-pattern corresponding to 1,34 SMN1 copies lying between the clear-cut ranges for one (0,6-1,2) and two (1,56-2,2) SMN1 copies (Anhuf et al., Hum Mutat, in press). Conclusions: The results can be explained by a somatic mosaic for a heterozygous SMN1-deletion in the grandmother. The correct evaluation of the SMA carrier status in this family was only realized by the combination of linkage analysis and quantitative determination of SMN1 copy number. Further investigations (e.g. FISH-analysis) are in progress.

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Mutations in the DJ-1 gene (PARK7) are not a common cause of early onset Parkinson´s Disease.

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Background: Parkinson´s disease (PD) is the second most common neurodegenerative disorder characterized by tremor, rigidity, bradykinesia, and postural instability. Genetic and/or environmental factors are discussed as disease cause. In the past seven years, five genes and six gene loci (PARK1-10 and Nurr1) have been shown to be associated with inherited forms of PD. Monogenic forms of PD were mostly identified in patients with an early onset of the disease. While dominant forms of PD are very rare, recessively inherited forms, such as Parkin (PARK2), appear to occur much more frequently. Recently, two different homozygous mutations (a point mutation and a large deletion) in a second recessive PD gene, DJ-1 (PARK7), were identified in two families.Patients and Methods: We performed mutational analysis of all six coding exons of the DJ-1 gene in 37 patients with early-onset PD. This included 33 patients from a cohort of patients previously studied for mutations in the Parkin gene (Group 1) and four patients referred for genetic testing of recessively inherited PD genes (Group 2). For the detection of point mutations and small deletions, we used denaturating high performance liquid chromatography (dHPLC) on the WAVE system (Transgenomics). In addition, we developed a quantitative duplex PCR assay on the LightCycler (Roche Diagnostics) for the detection of large deletions (exon rearrangements). Results: We screened 37 patients with an age of onset < 40 years (32.0 +/- 6.4 years; range 15-39 years) of different European ethnic origin, but mostly German (n = 34). In Group 1, no DJ-1 mutation was identified, however, a heterozygous sequence alteration was found in intron 6 (IVS6+38T>C) in one case. One patient of Group 2 carried a heterozygous 11-bp deletion involving the highly conserved intronic splice site (IVS5+2-12del). RNA of this Russian patient with an age of onset of 17 years was not available. Neither exon rearrangements nor homozygous mutations were detected in our patient sample. Discussion: We identified only one heterozygous DJ-1 mutation in 37 patients with early onset PD, whereas mutations in the Parkin gene occurred more frequently (n = 6; Group 1: one compound heterozygous, two heterozygous; Group 2: one compound heterozygous). Interestingly, the identified DJ-1 mutation that is expected to affect splicing and predicted to result in a truncated protein occurred in the heterozygous state only. It remains to be investigated whether this patient carried a second mutation in another region of the gene (e. g. promoter or introns) or in another as yet unidentified (recessive) PD gene. In conclusion, mutations in the DJ-1 gene seem to be a rare cause of early-onset PD, and the role of single heterozygous mutations in this gene remains to be elucidated.

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Point mutations in the genes for Friedreich ataxia and the ataxia-oculomotor apraxia syndrome

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Friedreich ataxia (FRDA OMIM 229300) characterised by juvenile onset, progressive ataxia, sensory loss and absence of tendon reflexes, is the common autosomal recessive neurodegenerative disorder in Caucasians. In about 95% of patients the disease is caused by a GAA triplet expansion in the first intron of the FRDA gene. In 2% to 5%, FRDA alleles are created by point mutations. The analysis of 6 patients with symptoms of FRDA and heterozygosity for the repeat expansion identified point mutations in two cases. In one patient the second allele shows an Asn->Lys mutation, in the other person the repeat expansion is combined with a Leu->Arg exchange.Recently, the investigation of DNA samples from patients with FRDA-like clinical presentations, hypoalbuminemia, and oculomotor apraxia identified recessive mutations in the aprataxin gene APTX (AOA1: ataxia-oculomotor apraxia syndrome OMIM 208920). We investigated 165 patients with FRDA phenotypes but missing GAA repeat expansions. Two patients revealed homozygosity for the Trp279stop mutation, in three patients a heterozygous Ser->Tyr change was found. Our results show that point mutations in the FRDA- and APTX-gene are present in ataxia patients of German origin.

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Heterogeneity of breakpoints in non-LCR mediated large constitutional deletions of the 17q11-q12 NF1 tumor suppressor region

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Deletions of the NF1 region at 17q11.2 most frequently span 1.4 mbp and are caused by meiotic recombination between low copy repeats (NF1 LCR) derived from the WI-12393 gene and sequences with homology to chromosome 19. Several NF1 patients with even larger deletions at 17q have been reported, but the position of their breakpoints within a contiguous BAC/PAC contig were not determined. Therefore it remained unclear whether deletions larger than the common 1.4 mbp deletions are also LCR mediated. Here, we describe the molecular characterization of the deletion of an NF1 patient encompassing 4.7 mbp and compare it with those of four other NF1 patients with very large deletions in 17q which have previously been characterized by marker analysis and Southern hybridisations. Both the proximal and distal breakpoints of all five patients clearly fall at different locations and are not flanked by LCRs. The centromeric break in patient BUD was mapped to BAC 271K11 at 17q11.2 between a partial SMS-repeat and the WI-12393 derived LCR, whereas the distal break was located between the two SCHLAFEN (SLFN) genes SLFN1 and SLFN3 at 17q12. Comparisons between the otherwise conserved human and mouse segment revealed major differences in the number and orientation

of SLFN genes. Thus the distal breakpoint of patient BUD is located in a region containing multiple evolutionary breakpoints. We show that the deletion was paternally inherited and occurred by an intrachromosomal mechanism. Since 3 out of 4 previously analyzed NF1 deletions that were also not bordered by LCRs were also found to be of paternal origin, non-LCR-triggered deletions are most likely mitotic events during spermatogenesis. Patient BUD as well as patient UWA106-3, who also has a large deletion extending beyond the distal NF1 LCR at 17q11.2 suffer from multiple bilateral spinal neurofibromas. We suggest, that their deletions include a modifier locus which predisposes these patients to the development of multiple spinal neurofibromas.

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Detection of a private SPG4-nonsense-mutation in a large German pedigree by using dHPLC

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Spastic paraplegia type 4 (SPG4) is the most frequent form of autosomal dominant hereditary spastic paraplegias (AD-HSP). Mutations in the SPG4 gene, which encodes spastin, an ATPase belonging to the AAA protein family, are scattered along the 17 exons of the coding region. We investigated a large German family with AD-HSP. Among the 27 members of this pedigree 11 individuals show clinical evidence of SPG4. We mapped the causative region for this family to chromosom 2p21-p22 which is identical to the SPG4 locus. Interestingly four out of seven gene carriers in one generation were unaware of symptoms. Initial sequencing analysis of all exons did not detect a mutation. By screening the 17 exons with the new technology of dHPLC DNA analysis we were able to find a novel mutation in exon 5 of the SPG4 gene. This mutation, which leads to an early stop codon on one allel, has not been reported so far.

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Spinocerebellar ataxia type 5 (SCA5) in a German kindred

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Spinocerebellar ataxia type 5 (SCA5) is an autosomal dominant neurodegenerative disorder linked to chromosome 11. To date, only two SCA5 families have been published, the underlying mutation is still unknown. We here report on a large 5-generation family with autosomal dominant cerebellar ataxia tightly linked to the SCA5 locus. Linkage analysis yielded a maximum two-point lod-score at D11S1889 (Z=4.21 at theta=0.0). All affected individuals in the family shared a common haplotype between markers D11S1883 and D11S4136, spanning an interval of approximately 6.5 cM, resp. 6.35 Mb. The clinical phenotype is characterised by a purely cerebellar syndrome with a downbeat nystagmus occurring prior to the development of other cerebellar features. In accordance to the clinical phenotype, imaging studies demonstrate pure cortical cerebellar atrophy. Progression is slow even in patients with a disease onset during the second decade. The age of onset varied from 15 to 50 years with no statistical evidence for anticipation.

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Identification of an atypical duplication in Charcot-Marie-Toth disease type 1A

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A Duplication of a 1.4-Mb region in Chromosome 17p11.2-12 that is delimited by two 24-kb low copy number repeats (CMT1A-REPs) represents frequent genomic rearrangements resulting in two common inherited peripheral neuropathies, Charcot-Marie-Tooth disease (CMT1A) and hereditary neuropathy with liability to pressure palsy (HNPP). CMT1A is the most frequent inherited peripheral neuropathy, with an estimated prevalence of 1 in 2500. About 70% of the cases of the demyelinating form of Charcot-Marie-Tooth disease (CMT1A/HMSN) are caused by the autosomal dominantly inherited 1.4-Mb submicroscopic duplication, encompassing the peripheral myelin protein 22 (PMP22) gene. Additional small repeats within the 1.4-Mb Region have been proposed to originate in atypical rearrangements leading to alternatively sized duplications also causing the HMSN I phenotype. We screened 252 Patients suspected for HMSN I / HNPP by means of polymorphic markers in-between the repetitive elements flanking the CMT1A region, fluorescence in situ hybridisation (FISH) with the PMP22 overlapping probe p150M21 and multiplex quantitative real-time-PCR using a FAM labelled Taqman-probe from PMP22 exon 3. One patient suspected for HMSN I due to distal muscle weakness, abandoned reflexes and reduce nerve conduction velocities of the nervus tibialis, ulnaris and medianus, showed a normal polymorphic marker pattern excluding the standard CMT1A-Duplication recognized with a likelihood of 95.6%. Multiplex quantitative real-time PCR in quadruplicates was repeatedly carried out and resulted in a DDCt ratio within the predefined duplication area, indicating a duplication of the PMP22 gene. FISH analysis was at first inconsistent, but repeated in higher resolution assumed a duplication of the PMP22 gene, as well. Southern-hybridisation (EcoRI/SacI) using probe pLR 7.8 resulted in a normal pattern, excluding a standard 1.4-Mb duplication. Southern-hybridisation specific for PMP22 revealed a novel junction fragment due to a smaler duplication including the PMP22 gene. Implications for routine analysis of patients with the clinical diagnosis of HMSN I include the application of PMP22 specific hybridisation methods to detect atypical duplications.

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Quantitative expression analysis of neutral endopeptidase (NEP) in CRPS patients

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Neurokinins play an important role in the neurogenic inflammatory process which characterizes the complex regional pain syndrome (CRPS). The release of calcithonin gene related protein (CGRP) and substance P (SP) from nociceptive C-fibers results in vasodilatation and protein-extravasation in the impaired tissue region. This release is followed by edema, increased skin temperature and pain. After noxious events, serum concentrations of CGRP and SP are increased in CRPS patients. They also display hyper-responsiveness to experimentally applyed substance P, even in none-acute disease states. One of the reasons for the SP hyperreactivity could be a decreased proteolytic activity keeping the SP level high. The patient collective under investigation consisted of 6 familial cases and 13 sporadic patients who suffered from CRPS once or repeatedly. In a first approach we analyzed the neutral endopeptidase (NEP) gene as a key enzyme for SP inactivation for SNPs. Overall 7 NEP gene variations could be located in exon 1, intron 6, 17, 19, 22 and exon 23. These variations allowed the construction of haplotypes suitable for association studies in the future. In total 13 different haplotypes were identified for the families. A female Met8Val variation carrier was identified in one CRPS family. Her yet unaffected mother showed the identical variation, but not her repeatedly affected father. We used quantitative PCR to measure NEP expression in peripheral blood cells in order to analyse a (hypothetical) influence of the variations on NEP expression. The control group (n=7) showed homogeneous NEP expression in a narrow range defined as 1. For 9 patients the expression level was decreased down to half of the control group value, for 12 CRPS patients the expression was in the range of the control group. Interestingly, the Met8Val carrier and her not yet affected mother exhibited the highest NEP expression with an increase up to 3.4 fold. The functional consequences of the Met8Val variation are not yet clear, but the increased expression may indicate that an impaired proteolytic activity may increase the intracellular NEP level by an unknown feed back mechanism. No single haplotype could be associated with the decreased expression level. Our results indicate that NEP plays a role in CRPS which deserves further investigation.

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Cloning, Physical Mapping and Expression Analysis of the Human Serotonin Receptor Genes HTR3C, HTR3D and HTR3E

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For more than 50 years the serotonin system has been the subject of intense research. This has provided an exciting insight and led to the discovery of multiple drugs targeting serotonin receptors, metabolising enzymes and re-uptake sites. During the past few years researchers focussed especially on elucidating the complexity of different physiological actions in the serotonergic network. We have identified two novel human serotonin 5-HT3 receptor genes, HTR3D and HTR3E, by performing homology searches using the public human sequence databases and subsequently cloned the full length cDNAs

by 5’ and 3’ RACE. Mapping of HTR3D and HTR3E by hybridisation, PCR and FISH revealed that both genes together with HTR3C are clustered in a subinterval of less than 100 kb on chromosome 3q27. Comparative expression analysis of all HTR3 genes, namely HTR3A, B, C, D and E showed HTR3D expression to be restricted to kidney, colon and liver and HTR3E expression to colon and intestine, whereas all other genes are widely expressed in many tissues including brain.

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A female patient with Rett syndrome carrying a de novo t (1;7)(p22;q32) balanced translocation

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Rett syndrome (RTT) is a severe neurodevelopmental disorder affecting young girls. In approximately 80% of patients mutations in the MECP2 gene encoding the transcriptional repressor methyl-CpG binding protein (MeCP2) have been identified.We have investigated a female patient with Rett syndrome carrying a de novo t (1;7)(p22:q32) balanced translocation but no obvious MECP2 mutation. Systematic FISH analysis of both breakpoints identified an overlapping BAC clone (AL513187) on chromosome 1. This clone contains the Netrin-G1 gene. By semiquantitative RT-PCR we could show that in lymphoblastoid cells of the patient, Netrin-G1 expression levels are markedly reduced compared control cell lines. Detailed FISH studies and in silico sequence analysis region failed to identify an obvious candidate gene for the chromosome 7 breakpoint. NetrinG-1 belongs to the UNC-6/netrin family, a small phylogenetically conserved protein family playing a role in axon guidance. However, it has characteristics which differ from those of classical netrins: it is predominatly linked to the plasma membrane via a glycosyl phosphatidyl-inositol anchor and lacks affinity to receptors for netrins (Nakashiba et al., 2000). In both fetal and adult mouse netrin-G1 is predominantly expressed in the brain.These findings suggest the possibility that mutations in Netrin-G1 give rise to a RTT phenotype. Therefore, we are performing mutation analysis in RTT patients without MECP2 mutation.

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Trinucleotide repeat expansions in TBP is the second most common monogenic cause for a Huntington's disease-like phenotype in Caucasians

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Expansion of a CAA/CAG trinucleotide repeat in the TATA-binding protein (TBP/spinocerebellar ataxia 17, SCA17) has recently been involved in autosomal dominant cerebellar ataxias. SCA17 is a rare cause of dominant cerebellar ataxia in Caucasians with particular interesting clinical features. Beside cerebellar signs psychiatric disturbances such as psychosis, depression, and dementia may be first symptoms of the disease. Moreover, within SCA17 families, affected members may present only with psychiatric symptoms while others show a cerebellar phenotype with dementia. Therefore, a remarkable overlap between SCA17 phenotypes and Huntington's disease-like (HDL) phenotypes exists. To investigate whether a CAA/CAG repeat expansion in the TBP/SCA17 gene may underlie the HDL features in Caucasian patients we analysed the CAA/CAG repeat in a large group of 1.323 patients who were referred for Huntington's disease (HD) testing to DNA laboratories in Germany and Austria. None of the patients carried an expansion of more than 38 CAG repeat units in the IT 15 gene, thus excluding HD as the cause for the symptoms. More than 45 CAA/CAG repeats in the TBP/SCA17 gene were detected in nine independent patients. Their phenotypes were indistinguishable from HD although cerebellar ataxia was noticed in many of these patients during disease progression. In conclusion, repeat expansion in the TBP/SCA17 gene can be found in HDL phenotypes. Particularly, the co-occurence of psychiatric and cerebellar symptoms in addition to choreatic disturbances in different members of a pedigree are suggestive for this differential diagnosis of HD.

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ASSOCIATION AND NON-ASSOCIATION OF NMDA RECEPTOR 2B SUBUNIT POLYMORPHISMS WITH SOME ASPECTS OF ALCOHOLISM

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The ionotropic glutamatergic N-methyl-D-aspartate receptors (NMDAR) have been implicated as main target sites for acute and chronic effects of ethanol. The ethanol sensitivity seems to depend on the composition of the heteromeric receptor. Those receptors which contain the NMDAR2B subunit are known to be the most sensitive to ethanol1. In an earlier study2 we showed an association of the silent C2664T polymorphisms in exon 8 of the gene of the NMDAR2B with some aspects of ethanol dependence Recently, Miyatake et al3 reported a novel T-200G variant located in one of the SP1 binding sites of this gene. By reporter gene assays they found functional consequences of this variant. We investigated, whether this new polymorphism is associated with ethanol dependence and if there is an linkage to the previously studied polymorphism in exon 8. The study included 367 alcohol dependent individuals and 335 control individuals of German origin in the case control study. Additionally trios of Polish origin were recruited for the family based study. Genotyping was done using FRET-probes in a PCR/melting curve analysis. For the promoter polymorphism, there was no association to alcoholism or some related traits. In the case control study we found a trend to the personality scale „reward dependence" and an association of the G allele prevalence and an alcoholic mother. Evaluation of the exon 8 polymorphism revealed a significantly reduced prevalence of the T allele in patients with an early age at onset (chisquare=4.130, df=1, p=0.042), and in Cloninger type 2 alcoholics (chisquare=5.939, df=1, p=0.015). Furthermore, patients carrying the C-allele showed higher rates in the personality trait subscale of impulsivity compared to those lacking it (p=0.012). Our family based study revealed a preferred transmission of the C allele by fathers to the affected offspring with an early onset. Our results show an association of the NMDAR gene and alcoholism. The recently reported functional T-200G polymorphism in the promoter region seems not to contribute to this association. Acknowledgements: This study was supported by the German (BMBF, #Pol 01/63) and Polish (KBN, #Po5D 14622) governments.References:1Lovinger D. Alcohol Clin Exp Res (Suppl) 2000; 24: 183A.2Wernicke C et al. Biol Psychiat 2003; in press, available online.3Miyatake et al. Mol Psychiat 20027: 1101-1106.

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Further evidence for genetic heterogeneity in restless legs syndrome

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The restless legs syndrome (RLS, OMIM 102300) is a neurological disorder characterized by dysesthesias, usually located in the lower limbs, associated with an irresistible urge to move these limbs. The symptoms occur predominantly at rest in the evening or at night, seriously interfering with sleep onset and disruption. Limited population based surveys revealed an age dependent disease prevalence of 5 - 10%. Clinical surveys showed that 40 - 60% of RLS patients report a positive family history. A segregation analysis in 238 families suggested evidence for a single major gene acting dominantly in early age-at-onset families, but with an additional

multifactorial component and a high phenocopy rate. So far, two major susceptibility loci for RLS in two large pedigrees have been published. In a French Canadian family, linkage was shown to chromosome 12q12-q21. For this family a recessive pattern of inheritance was suggested. Recently, a second RLS locus was described in a large Italian family. A genome-wide linkage analysis in this family with an autosomal dominant pattern of inheritance led to the identification of a locus on chromosome 14q13-q21. We report a large family from Bavaria with 113 individuals, 32 of them being affected. Genotyping of the published RLS loci on 12q and 14q was performed in 46 family members using microsatellite markers. To save costs, PCRs were performed with three primers, one fluorescent dye labeled universal primer and two microsatellite specific primers. One of the specific primers was designed with a universal sequence tag at the 5´end. Altogether, 18 markers (12q: 11 markers, 14q: 7 markers) spanning these RLS susceptibility loci were analyzed. Linkage analysis was performed with different mode of inheritance models, including the recessive model of the French Canadian pedigree and the autosomal dominant model of the Italian family. Both, two-point and multipoint LOD scores, excluded linkage of our family to the 12q and 14q loci. This indicates further genetic heterogeneity of RLS with a locus outside the two published susceptibility regions on chromosomes 12q12-q21 and 14q13-q21.

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Characterisation of a complex chromosomal rearrangement (CCR) including a t (2;12)(q21;q12) and a pericentric inversion of chromosome 2 associated with mental retardation

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We report on a five year old patient with a 2/12 translocation and a pericentric inversion of chromosome 2 leading to the following karyotype: 46,XY,t (2;12)(q21.1;q12) inv (2)(p13.3;q14.3) de novo, who was referred for cytogenetic analysis because of a relatively mild mental retardation, mainly an impaired speech development, characterised as orofacial dyspraxia and motor retardation. In addition mild dysmorphic facial features were observed. By fluorescence in situ hybridisation (FISH) using a panel of mapped bacterial artificial chromosome (BAC) clones, we narrowed down the breakpoint regions to about 100kb respectively. No chromosomal unbalances were found up to that resolution. BACs spanning all four breakpoints have been identified. Up to now only one known transcript, reported as DKFZp434K2435 from the 12q12 region could be found to be disrupted by the complex rearrangements. A detailed characterisation of this gene was performed.

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DHPLC as a diagnostic method in hereditary motor and sensory neuropathies

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Hereditary motor and sensory neuropathies (HMSN; CMT) are the most common inherited peripheral neuropathies in humans. A wide range of clinical as well as genetic heterogeneity has been observed. In up to 50% of the cases HMSN is associated with a duplication of chromosome 17p11.2-12 (containing the peripheral myelin protein-22 gene; PMP22). In most of the other cases mutations in 17 genes [e.g. the PMP22, the myelin protein zero (MPZ) and the gap junction protein 1 gene (GJB1 or Connexin 32)] causing a variety of HMSN phenotypes have been described. In this study we investigated sporadic and familial HMSN cases after excluding the dup17p11.2-12, for mutations in the PMP-22, MPZ, Cx 32 and the early growth response 2 (EGR2) genes by the denaturing high pressure liquid chromatography (DHPLC) method. DNA samples of several patients showed aberrant elution pattern on the DHPLC run. Upon sequencing sequence variants were identified in these patients. We detected several mutations and non-pathologic sequence variants. Furthermore we could describe novel mutations that were confirmed by co-segregation analysis and that were absent in 94 healthy controls. We found that under optimized conditions, DHPLC screening had the same sensitivity as DNA sequencing. Our results suggest that DHPLC is an easy and reliable method of screening for mutations in several genes that have been described as causing HMSN. Findings of novel mutations point out the complexity of the genetic background of the disease.

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A diagnostic procedure in two steps for DM2 (PROMM)

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Recently, myotonic dystrophy type 2 (DM2, PROMM, MIM #602668, MIM#600109) has been described as a separate disease entity that is distinctive from classical Steinert's disease DM1, since it lacks a CTG repeat expansion on chromosome 19q. A gene locus for DM2 has been mapped to chromosome 3q, and recently it was demonstrated, that a CCTG repeat expansion in the first intron of the ZNF9 gene is the causative mutation. The repeat track is transcribed in DM1 as well as in DM2 and is detectable in muscle nuclei in form of ribonucleoprotein inclusion bodies, probably disturbing the transcription-splice-machinery.The repeat expansions in DM2 are huge, extending more than 10000 repeats in many patients. Due to the extensive mitotic instability during the whole life, each patient presents an extreme mosaic hardly to detect by routinely used lab methods. To overcome this problem we developed a two step procedure for routine molecular diagnosis of DM2. In a first step the polymorphic marker CL3N58 which includes the CCTG repeat tract is typed. It sorts out all persons with two alleles having no CCTC expansion and has a positive predictive value of 0.81 in the German population. Additionally analysis of CA-repeatmarker CL3N59 was performed, which is in a strong linkage disequilibrium with the repeat expansion (77.5% of German PROMM patients have a special allele with 27 CAs). A patient having this allele and being homozygous for CL3N58 has a probability of about 97% that he is affected by PROMM. In a second step, a special developed long range PCR for all homo/hemizygous patients was performed. The procedure is described and the results are compared with those of Southern blots. There was no contradiction between both methods. The PCR test has a sensitivity and specifity of nearly 100%, shown in selected members of definitely 3q-linked PROMM families. So, our procedure done in more than two hundred independent patients, seems to be feasible and reliable.

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Plexin B3 mediates neurite outgrowth and homophilic binding

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Searching genes contributing to neurological disorders linked to Xq28 we identified PLXNB3, the gene encoding plexin B3 (B3). Plexins are multifunctional neuronal transmembrane receptors implicated in semaphorin mediated axon guidance. In mammals there are known nine plexins. Their biological activities and signal transduction mechanisms are poorly understood. We have cloned human PLXNB3 and characterized its structure and expression. It is strongly expressed in the developing and adult CNS with a preferential neuronal expression pattern (see poster of S. Veske et al.). After transfection of NIH-3T3 cells with PLXNB3 cDNA, B3 strongly stimulates neurite outgrowth of cultivated murine cerebellar neurons. Using different cell aggregation assays and various PLXNB3 deletion constructs we could show that B3 exhibits Ca2+-dependent homophilic binding in trans mediated through the sema domain. Furthermore we could demonstrate that human B3 interacts with its murine homolog. Most likely this interaction is responsible for stimulation of neurite outgrowth in our model. These data suggest that PLXNB3 may be essential in neurodevelopment and maintenance of CNS functions. Therefore, PLXNB3 appears to be promising candidate gene for X-linked neurological disorders. However, up to now we did not find mutations associated with clinical phenotypes (see poster of S. Veske et al.). [Supported by DFG, SFB444, C3; correspondence: finckh@uke.uni-hamburg.de]

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Mutations in the set binding factor 2 (SBF2) gene are a cause of the rare autosomal recessive form of Charcot Marie Tooth neuropathy

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We have recently shown, with others, that mutations in the set binding factor 2 (SBF2) gene on chromosome 11p15 are responsible for autosomal recessive Charcot Marie Tooth disease (ARCMT)type 4B2. So far, three pathogenic mutations have been identified in the SBF2 gene (Hum Mol Genet 2003;12:349-356, Am J Hum Genet 2003 72:1141-53). We screened the coding region of the SBF2 gene in a Turkish consanguineous Charcot Marie Tooth disease family compatible with linkage to chromosome 11p15. A homozygous duplication of 5 bp, leading to a frameshift, was detected in exon 38 of the SBF2 gene (F1765fsX1769). This mutation is predicted to result in a truncated protein with deletion of the C-terminal pleckstrin homology (PH) domain. The PH motif, putatively involved in binding to lipids or proteins, is highly conserved in SBF2 and its orthologues across species. Our results provide further evidence that mutations in SBF2 are responsible for ARCMT in some individuals. While locus heterogeneity and the usually small size of families have so far hampered genetic analysis of ARCMT, progress in the identification of disease genes, e.g., SBF2, will facilitate future molecular diagnostics.

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Chromosomal Aberrations in Patients with Motor Neuron Disease

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In amyotrophic lateral sclerosis (ALS) we most recently found an increased rate of constitutional chromosome aberrations (5.9%, compared to 0.05-0.1% in the normal population). ALS is a common neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons in the brain and spinal cord, typically resulting in an adult-onset motor syndrome. The paralytic disease is uniformly fatal within five years. 5-10% of the patients are familial cases, and different human gene loci have been implicated in this disorder. In contrast, the cause of the far more common sporadic form of ALS and its relation to similar forms of motor neuron disease is still unknown. Here we report on a sporadic patient with late-onset lower motor neuron disease (LMND) and an apparently balanced chromosomal rearrangement. Karyotype analysis revealed a constitutional rearrangement inv (10)(p11q21). At the age of 49 years, the patient had developed a slowly-progressive lower motor syndrome with prominent peroneal lesions. 15 years later, the motor syndrome is still confined to the lower extremities. The clinical diagnosis is a progressive motor neuropathy of the lower limbs (LMND), a heterogeneous disorder encompassing pure motor Charcot-Marie-Tooth (CMT) disease, distal spinal muscular atrophy (dSMA) and hereditary motor neuropathy (HMN). Ongoing FISH mapping experiments have already led to the identification of a breakpoint-spanning BAC clone which contains several possible candidate genes. Analysis of this and other patients with balanced chromosome rearrangements may provide new insight into the etiology of ALS and clinically related forms of motor neuron disease such as LMND.

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Variable clinical spectrum of X-linked lissencephaly with abnormal genitalia (XLAG) in three families with identified ARX mutation

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Nonsense mutations and missense mutations in the highly conserved homeodomain of the aristaless-related homeobox gene (ARX; Xp22.13) have recently been reported in males with the clinically distinct phenotype of X-linked lissencephaly with abnormal genitalia (XLAG, OMIM 300215, Kitamura et al., 2002). Typical clinical features of XLAG include lissencephaly with neonatal-onset intractable epilepsy, agenesis of the corpus callosum as well as ambiguous or underdeveloped genitalia. Female mutation carriers might present with hypoplasia of the corpus callosum.Here we present the clinical data of three male patients with identified ARX mutation and clinical features of the XLAG spectrum. One previously reported frameshift mutation (nt790delC) in exon 2 of the ARX gene was identified in the index patient with XLAG, resulting in premature truncation of the predicted gene product with complete loss of homeodomain and aristaless domain. The second XLAG patient was found to carry a hemizygous ARX missense mutation R332C in the highly conserved homeodomain of the predicted protein. Both mothers were found to be heterozygous mutation carriers with hypoplasia of the corpus callosum on MRI, but without obvious neurological abnormalities on clinical examination. In a subsequent pregnancy of the mother of proband two the male fetus was found to carry the normal allele.In addition, a 2 bp duplication within exon 4 of the ARX gene (nt1416_1417dupAC) was identified in another male patient with neonatal onset intractable epilepsy and agenesis of the corpus callosum (ACC), but without typical imaging signs of a neuronal migration defect. A positive family history suggests the presence of female carriers including the mother of the index patient. Our data further contribute to the clinical spectrum observed in probands with identified ARX mutation, which also includes the allelic disorders Partington syndrome, X-linked West syndrome (ISSX) and X-linked mental retardation with myoclonic epilepsy (XMESID).

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Two new mutations in the 3'coding region of the LIS1 gene cause different grades of lissencephaly

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Disorders of neuronal migration are an important cause of mental retardation and epilepsy. Classical lissencephaly is a severe human brain malformation characterized by an absence or reduction of the normal cerebral convolutions (agyria / pachygyria). Mutations in the LIS1 gene (chromosome 17p13.3) and the DCX gene (Xq22.3) have been found to cause classical lissencephaly as well as subcortical band heterotopia. The sequencing of both genes has revealed two new mutations in the 3' end of the LIS1 gene in patients with different degrees of lissencephaly. Patient AG, a 2-year old female, is severely developmentally delayed with muscle hypotonia and a highly pathological EEG which shows an increased tendency for seizures. Brain MRI showed severe grade 1 lissencephaly with almost no cerebral gyration. We detected a heterozygous C to T transition mutation in codon 399 of the LIS1 gene (S399R), which is neither present in either of the patients' parents, nor in 50 healthy control individuals. This new mutation is the most 3' mutation yet to be described and alters one of the amino acids in the 7th WD40 repeat of the LIS1 protein.The second mutation, the heterozygous deletion of a single nucleotide (1050delG) results in a frameshift from codon 350 ending with a stop codon at amino acid position 354. This mutation would result in the LIS1 protein being truncated in the 6th WD40 repeat. The patient (HH), now 3 years old, is severely developmentally handicapped, is blind and deaf, and has epilepsy. Brain MRI showed lissencephaly of approximately grade 3, with occipital and temporal pachygyria and mild cerebellar hypoplasia. The mutation was not found in the patient's parents.The clinical severity of the mutations in these patients reinforces the suggestion that all seven WD repeats are necessary for the proper folding of the LIS1 protein. Functional analysis of the mutations may help to explain the difference in grade of lissencephaly between the patients and which LIS1 interactions are crucial for proper cortical layering.

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CGH-detectable losses of 9p21 are embedded in highly complex but consistent patterns of genomic imbalances in head and neck squamous cell carcinomas (HNSCC)

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The deletion of 9p21 in human neoplasias has attracted increasing interest because not only the cell cycle control genes CDKN2A and B but also the gene of methylthioadenosine phosphorylase (MTAP)are localized on this chromosomal band. The latter seems to be associated with certain phenomena of therapy sensitivity or resistance in human neoplasia. In addition, prognostic value has been attributed to this deletion in HNSCC. Considering the complex patterns of genomic imbalances found previously in these carcinomas, 74 head and neck cancers (35 oral, 20 oropharynx, 14 larynx and 5 hypopharynx carcinomas) analysed by comparative genomic hybridisation (CGH) were re-examined for visible losses including 9p21.In 13 of the oral and in 10 of the non-oral tumors dim (9p21) was detected. Tumors carrying this loss were characterized by a higher number of additional DNA copy number alterations (CNAs) as compared to the tumors of the same localization but without the deletion. In addition, the complex pattern of CNAs in the deletion-positive tumors was more consistent than in the deletion-less tumors. Nevertheless, in oral carcinomas this associated pattern was very similar to that found in a subgroup of deletion-less tumors characterized by a high number of CNAs. In the non-oral cancers the overall pattern of genomic imbalances was not only more heterogenic than in the oral carcinomas but differed more distinctly between dim (9p21)-positive and negative tumors.The only clinical associations between presence or absence of dim (9p21) and clinical parameters in oral carcinomas was a shorter disease free survival (dfs) in the tumors carrying the deletion and a surplus of pT4-class tumors in this group. Beside the dim (9p21)-associated patterns of genomic imbalances, some additional minimal combinations of specific CNAs seemed to be associated with short dfs. In the non-oral HNSCCs, in contrast, no evident association could be found between presence of 9p21 loss and any clinical parameter.Therefore, the clinical and prognostic impact of del (9p21) in HNSCC cannot be separated from the complex pattern of genomic imbalances accompanying this deletion in human HNSCC. It is this pattern which, interacting with a complex network of genetic alterations not visible by CGH, apparently is of high impact on the clinical features of this type of tumors.Supported by Deutsche Krebshilfe.

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DNA copy number changes in Thyroid Carcinoma of children

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The genetic changes leading to thyroid cancer in children are poorly characterized. We studied DNA copy number changes by comparative genomic hybridization (CGH) in 29 thyroid carcinoma in collaboration with the multicentric therapy study for malign endocrine tumors; GPOH-MET-97. All 20 papillary carcinoma (PTC) showed 1 to 10 individual DNA copy number changes (median= 3). Gains and losses affected 11 different chromosomes. The most frequent aberrations were +17 (18/20), +15q11.2-q15 (13/20), +1q32.2-qter (8/20) and +6p22.1-p23 (7/20). In follicular carcinoma (FTC), we found chromosomal imbalances in all but one of the 7 tumors. Each showed 1 to 14 individual DNA copy number changes, respectively (median= 4). Gains and losses affected 16 different chromosomes. The most frequent aberrations were +17 (6/7), -1p22.1-q32.1 (4/7) and +1q32.2-qter (3/7). Therefore, PTC and FTC showed common (+17,+1q) as well as different chromosomal anomalies. Aberrations in FTC are spread on a larger number of different chromosomes than in PTC. PTC and FTC are often grouped together as differentiated thyroid cancers but the present results indicate some genetic based differences. Evaluation of CGH findings and the clinical data is part of ongoing work. The results of this analysis will be discussed in the poster.

P 180

High amplification of c-myc in a patient with a carcinoma of unknown primary site (CUP)

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CUP is a carcinoma with metastatic invasion but without known localisation of the primary tumour. The incidence is between 0,5% and 10% of all malignant diseases. It is only possible to diagnose the primary tumour in 10% to 20% during the course of diagnostic investigations. The problems of CUP are difficulty of diagnosis, heterogeneity of manifestation, often an atypical way of metastasis and an adverse clinical course of a subgroup. Changes of chromosome 1 are often observed in CUP. Furthermore, an increased expression or a high amplification of several proto-oncogenes are observed in CUP, such as c-myc, ras, c-erbB-2. We report about a 54 years old male patient with the diagnosis of CUP. Our patient had an adverse clinical course. He died three weeks after diagnosis. Firstly, as primary site the head of the pancreas was favoured because of increased CA 19-9 level, swelling of a lymph node in the epigastric region and an enlarged pancreatic duct. However, no imaging technique could confirm this suspicion. Thus, a reliable diagnosis was not possible. Conventional cytogenetic analysis (CCA) and fluorescence in situ hybridisation (FISH) were performed on unstimulated bone marrow cells. These cells showed besides normal metaphases two different hyperdiploid aberrant karyotypes with a chromosome number of 48 and with trisomy of chromosome 2 and 7 in both clones. Differences between the aberrant clones are a variable number of „double minute“ chromosomes (dmin) and the presence of additional material of unknown region on the short arm of chromosome 1 [add (1)(p)]. Dmin chromosomes are frequently associated with the amplification of proto-oncogenes. Such an amplification has been correlated with a poor clinical outcome in a variety of human tumour types. We detected a high c-myc amplification on the dmin chromosomes after FISH with a locus-specific probe for the proto-oncogene c-myc [Vysis].

P 181

Breakpoint differentiation in chromosomal aberrations of hematological malignancies – identification of 33 previously unrecorded breakpoints

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Routine cytogenetic analysis provides important information of diagnostic and prognostic relevance for hematological malignancies. In spite of this, poorly spread metaphase chromosomes and highly rearranged karyotypes with numerous marker chromosomes, are often difficult to interpret. In order to improve the definition of chromosomal breakpoints multicolor banding (MCB) was applied on 45 samples of patients suffering from hematological malignancies like myelodysplastic syndrome (MDS), acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML) or acute lymphoblastic leukemia (ALL). The breakpoints defined by GTG banding were confirmed by MCB in only 8 cases, while in the remaining 37 the breakpoints had to be redefined. In 20/45 cases the breakpoints could only be characterized after application of MCB. In summary, 73 different breakpoints were characterized, thereof 33 were not described up to present. 11 cases showed known acquired aberrations and 21 cases had previously described aberration types like del (5q-), del (7q-), del (13q-) or t (1;5) as sole rearrangement or in connection with other complex ones. In 11 cases in summary 19 breakpoints described before in hematological malignancies were involved, while in 14 cases 33 up to present not recognized breakpoints were identified. Thus, MCB has proven to be a powerful and reliable method for screening of chromosomal aberrations, which considerably increased the accuracy of cytogenetic diagnosis. Supported by the Wilhelm Sander-Stiftung (99.105.1-2) and the EU (QLRT-1999-31590).

P 182

Cryptic chromosomal changes in the non-proliferating cells of acute myeloid leukemia with monosomy 7

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Mosaic Monosomy 7 is one of the most frequent abnormality in myeloid disorders. However, for cases with pure monosomy 7 no clear clinical correlation is available up to now. In the present study 6 AML cases with pure monosomy 7, as identified in GTG-banding were analyzed. Multicolor banding (MCB) using a probe set for chromosome 7 excluded additional cryptic rearrangements on the remaining chromosome 7 prior to micro-CGH analyses (Heller et al., Int J

Oncol 2000, 16: 461-468). For the latter, bone marrow interphase cells of the six mentioned AML cases, were collected using an extended glass needle under microscopic view. In each case ten to fifteen nuclei were collected and their DNA was amplified and labeled separately by DOP-PCR. In divergence to previous micro-CGH protocols, in this approach specifically those interphase nuclei were collected, which were significantly larger in diameter than the average, with the aim to microdissect a specific subpopulation – the malignant cells. In 5 of the 6 studied cases the monosomy 7 was identified in CGH. Additionally, in one of those five cases (case 1), gain of whole chromosome 9 was present according to the CGH-profiles. In the sixth case (case 6) the monosomy 7 could not be detected in micro-CGH, however, gain of whole chromosome 15 was detectable. Both, the gain of chromosome 9 and of chromosome 15, respectively, could be confirmed by interphase FISH using the corresponding centromere-specific probes. Trisomy 15 was found in 5/60, trisomy 9 in 4/100 interphase nuclei, indicating to malignant subclones in cases 6 and 1, respectively. These subclones escaped conventional analyses as they are only present in a small and – presumably in cell-culture - non-proliferative subpopulation. In this pilot study, micro-CGH identified a biclonality in AML case 6 and a karyotypic evolution in case 1. Thus, cryptic chromosomal changes in the non-proliferating cells in 2 out of 6 AML cases were identified. It can be speculated that this is an up to present overlooked more general mechanism obscuring clear clinical correlations. Supported by the Wilhelm Sander-Stiftung (99.105.1-2).

P 183

Is NBS1 a melanoma suceptibility gene?

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Introduction: Nijmegen Breakage Syndrome (NBS) is a rare chromosomal instability disorder associated with microcephaly, immune deficiency, growth retardation, hypersensitivity to ionizing radiation, cutaneous manifestations and an increased incidence of malignancies. The NBS1 gene product Nibrin as part of the Mre11/RAD50/Nibrin complex is involved in DNA-repair mechanisms and recombination, and thus it has been suggested that NBS1 might have a tumor suppressor gene function. Since UV-induced damage plays a major role in melanoma development and NBS patients display frequently sun sensitivity and various skin pigmentation abnormalities, the aim of this study was to elucidate the potential involvement of NBS1 in the pathogenesis of melanoma. Patients and Methods: We screened samples of 376 melanoma patients for mutations in exon 6 of NBS1, where the most frequent 657del5 mutation occurs. Among 376 patients we defined four groups: 50 familial melanoma patients with at least one first degree relative suffering from either melanoma or pancreatic cancer (FM), 27 patients with multiple melanomas (MM), 119 melanoma patients with at least one first degree relative suffering from any other cancer (FDRC) and 180 patients with none of these features, diagnosed with sporadic melanoma (SM). The average age of primary diagnosis of melanoma in these cases was 50 years. Methods: DNA was extracted from peripheral blood lymphocytes and exon 6 of NBS1 was amplified by means of the polymerase chain reaction (PCR). All samples were analyzed by denaturing high performance liquid chromatography (DHPLC) and those samples showing shifts were directly sequenced. Results and Conclusions: Three coding sequence alterations could be identified including one novel amino acid substitution not published to date. One patient was heterozygous for the major NBS1 mutation 657del5, which causes a frameshift and thus a premature truncation of the protein. The frequency of the 657del5 mutation in our study population was less than 1%. We therefore presume that this mutation does not play a major role in the development of melanoma. A second case was heterozygous for the missense mutation V210F (628G->T), which has recently been found in patients with non-Hodgkin- lymphoma (NHL) and Acute Lymphoblastic Leukaemia (ALL). Finally, in one patient a novel amino acid substitution F222L (664T->C) was detected. Since this mutation could not be shown in approximately 4,000 newborns of Slavic origin and among 800 German newborns around Berlin, it is most likely that this sequence alteration is not a polymorphism. Loss of heterozygosity (LOH) analysis of tumor tissue in NBS1 mutation carriers as well as functional experiments will be performed to further assess the causative nature of the amino acid substitutions found here. Since only exon 6 of NBS1 was screened for mutations it remains to be investigated if there are other changes in this gene leading to melanoma predisposition.

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Gains of 2p involving the REL locus correlate with nuclear c-Rel protein accumulation in neoplastic cells of classical Hodgkin`s lymphoma

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Structural aberrations of the short arm of chromosome 2 mostly resulting in gain of 2p13~16 have recently been described as being highly recurrent in Hodgkin/Reed-Sternberg (HRS) cells of classical Hodgkin`s lymphoma (cHL). In cHL cell lines and primary cases with available metaphase spreads, sequential chromosome analyses and fluorescence in situ hybrididization (FISH) have shown that such gains in 2p are frequently associated with segmental chromosomal aberrations, which constitute a novel genetic mechanism leading to gene copy gain. As the gains in 2p consistently lead to increased copy numbers of the REL oncogene locus, we investigated the expression of the c-Rel protein in a series of 30 cHL cases with known genomic REL status as determined by comparative genomic hybridization and interphase cytogenetics. Expression of the c-Rel protein was investigated in 26 biopsies by immunohistochemistry. Distinct patterns were observed in HRS cells with no staining, cytoplasmic, and/or nuclear staining for c-Rel. All 13 samples with additional copies of the REL locus displayed nuclear staining for c-Rel while 13 cHL lacking 2p gains displayed a significantly lower proportion or complete absence of HRS cells with nuclear c-Rel expression. Detailed analysis using combined immunophenotyping and interphase cytogenetics of individual HRS cells demonstrated that REL gains correlated with the presence of nuclear c-Rel staining. Additionally, in two cHL with translocation breakpoints in 2p13~16, nuclear staining of c-Rel was observed, in one of them with a staining pattern indicative of a truncated c-Rel protein. The correlation between structural aberrations involving the REL locus and nuclear c-Rel accumulation in HRS cells qualifies REL as a target gene of the frequent gains in 2p in cHL. The data suggest that REL aberrations are a genetic mechanism contributing to constitutive NF-kB/Rel activation in cHL.

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Deletions in the HLA class II locus in chromosomal band 6p21 in primary lymphomas of the central nervous system

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Primary lymphomas of the central nervous system (PCNSL) belong to the heterogenous group of diffuse large B-cell lymphomas (DLBCL). In contrast to extracerebral DLBCL, the molecular pathogenesis of PCNSL particularly in immunocompetent patients is poorly understood. It was recently shown, that HLA-expression is absent in more than 50% of DLBCL in the immune privileged organs CNS or testis but only in about 10% of nodal DLBCL. In part of the cases loss of HLA-expression was associated with deletion in the HLA gene cluster, particulary with deletions of the HLA class II genes. Only very few PCNSL have been hitherto analysed for such HLA class II deletions. Thus, we performed FISH with two FISH probes for the HLA class II genes DQA1 and DRA on stereotactic biopsies of a series of 19 PCNSL. A FISH probe for the centromeric region of chromosome 6 served as a control. Signal constellations indicating a deletion of both HLA genes were detected in 5 of 19 PCNSL. In one additional case a deletion of only the HLA-DQA1 gene was detected. Thus, a total of 6 of 19 PCNSL displayed deletion in the HLA class II gene cluster. In 2 of these 6 cases the losses were biallelic. These data indicate that a considerable percentage of PCNSL shows dele-

tions of HLA class II genes. The pathogenetic meaning of loss of HLA expression in immune privileged organs warrants further investigations. This study was supported by Deutsche Krebshilfe (10-1641-De 1).

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Identification of gene dosage alterations in the progression of retinoblastoma

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Mutations that inactivate both alleles of the retinoblastoma gene (RB1) are a prerequisite for the initiation of retinoblastoma, a childhood malignant tumor of the eye. As cytogenetic analyses have shown multiple recurrent chromosomal alterations, additional mutations seem to be required for progression of this tumor. Comparative genomic hybridization has shown that gains involving chromosomes 6p and 1q and losses of chromosome 16q material are frequent in tumors from patients with late age at diagnosis of unilateral retinoblastoma whereas tumors from patients with early diagnosis have only few DNA copy changes. The minimal region of gain on the short arm of chromosome 1 is 1q31-q32. This region contains the GAC1 gene, which is a putative oncogene and was found to be 5-fold amplified in one retinoblastoma. We have chosen this region for high-resolution analysis with Matrix-CGH and selected and verified 48 BACs that are located in this region. The Matrix-CGH slides also contain DNA from 219 clones representing various regions of the human genome. In order to confirm the Matrix-CGH data and to determine minimal regions affected by dosage changes we have adapted a quantitative assay based on Real Time PCR of CA-repeat loci. As these loci have a common core sequence a universal TaqMan probe composed of multiple TGs can be used to monitor the reaction. We have chosen CA-repeat loci from the 1q31-q32 region including one locus located in the GAC1 gene and have analyzed a set of tumors with known CGH status. Concordant with CGH data, all tumors without gains of the 1q31-q32 region in CGH show no increased dosage in real time PCR. However, of 13 tumors with gains of 1q material in CGH, 5 show no increased dosage. Interestingly, dosage of CA-repeat locus in the GAC1 gene is not amplified in all of the 8 tumors that are positive in Real Time PCR.

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Characterization of the OSTL gene which is located near the breakpoint of the t (6;12)(q23;p13) that involves ETV6 in a B-ALL cell line: possible role in B cell receptor signalling and survival

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Reciprocal translocations affecting band 12p13 are found as recurring chromosomal changes in myeloid and lymphoid malignancies. We have cloned and characterized a new gene (OSTL), that is involved in the translocation t (6;12)(q23;p13). This translocation results in the fusion of ETV6 with STL in a B-cell acute lymphoblastic leukaemia (ALL) cell line (Suto et al., Genes Chromosomes Cancer 18:254-268 (1997)). OSTL shares the first exon with STL but is transcribed in the opposite direction (OSTL = Opposite STL). Since the ETV6/STL fusion gene encodes only a very small protein, which lacks any known functional domains, we hypothesize that the main leukemogenic effect of this translocation might be the deregulation of OSTL.The human STL/OSTL genomic locus spans more than 450 kbp on 6q23 and there are several alternative splice forms of OSTL. OSTL encodes a protein of 307 amino acids containing a RING finger related motif, a modified B-box domain and a RING finger motif.We have sequenced the complete cDNA of both the human and the murine OSTL gene. The homology between the mouse and human protein is about 99%. There is also an 83% homology between human OSTL and a puffer fish protein over a 250 amino acid region. Indicating that this highly conserved protein has an important function. A green fluorescent protein OSTL fusion protein expressed in mouse fibroblast cells (NIH3T3) was localized to the cytoplasm. Multiple tissue Northern blot analysis showed high expression of human OSTL in testis and skeletal muscle. Northern analysis of different mouse tissues revealed high expression in liver, ovary and testis. Whole mount in situ hybridization experiments were performed to investigate the temporo-spatial expression pattern of Ostl during mouse embryogensis. There was distinct expression of Ostl in the somites (myotome), first and second branchial arches, optic and otic vesicles, limb buds and in the hair follicles of the vibrissae in mouse embryos of embryonal days 9.5 to 13.5. This expression pattern suggests an important role for Ostl in the early development of these structures.Interestingly, a yeast two hybrid screen identified two OSTL interacting proteins that are important in B-cell receptor signalling and B-cell survival (SIVA, HAX-1). These results support our hypothesis that deregulated OSTL expression can lead to hematologic malignancies.

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Prognostic impact of increased copy number of the MALT1 locus detected by fluorescence in situ hybridization in surgically resected t (11;18)–negative gastrointestinal B-cell lymphomas

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Gastrointestinal B-cell lymphomas lacking the t (11;18)(q21;q21) are heterogeneous with regard to biology and clinical outcome. Among the various genetic aberrations in t (11;18)-negative gastrointestinal B-cell lymphomas, trisomy of chromosomal band 18q21 containing the MALT1 locus is a common event and has been suggested to be an alternative means of MALT1 activation. Using dual color interphase fluorescence in situ hybridization on isolated nuclear suspensions we investigated retrospectively the prognostic importance of MALT1 gene dosage in surgically resected primary gastrointestinal B-cell lymphomas with a median follow-up of 56 months. From the 29 t (11;18)-negative lymphomas evaluated, four cases showed complete trisomy 18 and three cases partial trisomies 18q21. Increased MALT1 gene dosage was detected in 2 of 10 low grade marginal zone lymphoma of MALT type and in 5 of 19 gastrointestinal B-cell lymphomas with large cell component. Increased MALT1 gene dosage was associated with a significantly shorter disease specific survival time in the complete group (p=0.0458) as well as in the subgroup of gastrointestinal B-cell lymphomas with large cell component (p=0.0447). These data suggest increased MALT1 gene dosage to be a predictor of poor outcome in gastrointestinal B-cell lymphomas.

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Clonal chromosome imbalances in bone marrow cells of Fanconi anemia patients: gains of 3q26q29 as an adverse risk factor

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Fanconi anemia (FA) is a heterogeneous autosomal recessive disease characterized by chromosome instability, progressive bone marrow failure and an increased susceptibility to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). FA cells have a reduced fidelity in processing DNA double strand breaks (DBS) which leads to unbalanced chromosomal aberrations such as deletions, insertions and translocations. This specific intrinsic susceptibility might, together with extrinsic factors, influence the course of the disease resulting in the outgrowth of clones with chromosomal aberrations in bone marrow cells (BM). We report on a high incidence of expanding clonal aberrations with partial trisomies and tetrasomies of chromosome 3q in bone marrow cells and peripheral blood cells of 18 out of 53 FA-patients analyzed, detected by conventional cytogenetics and specific molecular cytogenetic approaches (Tönnies et al. 2003; Blood 101:3872-4). To determine the clinical relevance of these findings, we compared the cytogenetic data, the morphologic features of the bone marrow, and the clinical course of these patients with 35 FA patients without clonal aberrations of 3q. Both groups did not differ significantly in respect to age, gender, and complementation group. There was a significant survival advantage of patients without abnormalities of chromosome 3q. Even more pronounced was the risk assessment of patients with gains of 3q material in respect to the development of morphologic MDS and AML. Thus, our data of patients with 3q aberrations reveal that gains of 3q are strongly associated with a

poor prognosis and represent an adverse risk factor in FA. Supported by a grants from the Deutsche Fanconi Anämie Hilfe e.V. and the Charité Research Fund (No.2000-627), Humboldt-University, Germany.

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Determination of the heterozygote frequency of the 657del5 mutation in the NBS1 gene in saxon population and multi tumor families

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Introduction: The Nijmegen Breakage Syndrome (NBS) is an autosomal-rezessive hereditary disorder characterized by dysmorphic features, immunodeficiency and radiation sensitivity. The gene product, Nibrin, plays an important role in DNA double-strand repair. NBS heterozygosity is supposed to be associated with an increased cancer risk. The most frequent mutation is 657del5. Heterozygote frequency for this mutation was estimated to be 1 to 154 (Varon et al., 2000) in Czech population. However the frequency in Germany was just 1 to 866 (Carlomagno et al., 1999). Saxony is a close neighbour to Slavic population. Therefore it should show a higher heterozygous frequency than the remainder of Germany. Aim of the study was to estimate the frequency of the 657del5 mutation in the NBS1 gene in Saxony. Method: We tested DNA probes of 552 anonymous and unrelated individuals and 171 patients with a family history of cancer. All probands have originated from Saxony. We amplified the 111 bp region carrying the mutation within exon 6 by standard PCR using a labeled primer set (FAM-5"-TGTTTTG-GCATTCAAAAAT-3" and 5"-CCCAC-CTCTTTGATGA-ACCAT-3" (Matsuura et al., 1998)). PCR products were sized using the GeneScan Analysis Software. DNA of probands with heterozygous fragments were sequenzed using the ABIPrism 377 Sequencer (Applera), and the 5-bp deletion in one allele was demonstrated. Results and conclusion: Among the 552 anonymous DNA probes we could identify three heterozygous carriers of the 657del5 mutation giving an estimated frequency of heterozygotes of 1 to 184 in our population. Within the 171 familial cancer patients there was one heterozygote carrier. The frequency in both groups - Saxon population and multitumor families - corresponds well to the estimated frequency of the mutation in the Czech population (1:156) and is much higher compared to other parts of Germany. In view of this high heterozygous frequency we suggest that the 657del5 mutation should be considered if clinical findings such as microcephaly, growth retardation, immunodeficiency and predisposition to cancer as well as high sensitivity towards radiation are found in patients originating from Saxonia. Our data do not suggest a close relationship between hereditary cancer syndromes and the common NBS1 gene mutation. Further studies should be performed analysing the relevance of heterozygous NBS1 mutation for the incidence of radiation induced cancer.

P 191

Higher incidence of malignancies in Peutz-Jeghers patients with specific missense mutations in the STK11 gene

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Peutz-Jeghers syndrome (PJS) is a cancer associated disease and most often caused by STK11 mutations. The aim of the present study was to identify specific mutations associated with an increased tumor risk in PJS patients. STK11 mutation analysis was performed in 41 PJS patients by PCR-SSCP and DNA sequencing. For genotype-phenotype correlation studies we have reviewed the literature and have added STK11 mutations from 25 references. These included 22 PJS patients with tumors, 47 PJS patients without tumors and 28 patients with sporadic tumors. By mutational screening we have found germline mutations in 27 of our 41 patients (66%). They consisted of seven nonsense, ten deletions, five insertions, one splice mutation and four missense mutations. In nine of the 27 cases malignant tumors have occurred in the index patient and/or in affected relatives. Together with the data from the literature we found correlations between the STK11 genotype and the tumor frequency. Missense mutations in the part of the gene corresponding to functional protein domains VIB-VIII were associated with a high incidence of malignancies. In contrast, in-frame deletions and splice mutations were only rarely associated with tumor development suggesting a lower tumorigenic potential. In conclusion this demonstrates that the mutation type and position of the STK11 mutation in the gene may have a prognostic significance for tumor development. This observation has to be verified in a larger patient cohort. The clinical significance of screening for cancer predisposing mutations is to identify mutation carriers at an early age and thus to improve disease management.

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Early age of Wilms' tumor onset in patients with WT1 germline mutations

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Germline alterations in the WT1 gene are found in a subset of WTs. We report here a combined database containing our 24 new cases with germline WT1 alterations and our previously described cases as well as literature cases for whom age of tumor onset, gender and laterality were known. This combined database contains 117 patients with and 165 patients without WT1 germline alterations. Using this information we have determined the median age of tumor onset for patients with (13 months) and without WT1 gene alterations (36 months). When patients were grouped according to their type of mutation the earliest onset was found in patients with nonsense/truncation (12 mo, 66 patients), followed by missense mutations (18 mo, 30 patients) and deletions (22 mo, 21 patients). Patients with the two most frequent nonsense mutations R362X and R390X and the Denys-Drash syndrome hot spot mutation R394W/Q/L had a very early onset (9 mo, 12 mo and 18 mo, respectively). The highest number of bilateral tumors was observed in the group of nonsense/truncation mutations, with a higher percentage of bilateral tumors when the stop codon occurred in the 5' half of the WT1 gene. There was no significant difference in age of tumor onset between males and females either with nor without WT1 mutations, although a slightly younger age was observed in males in all subgroups. In addition to genital tract anomalies and stromal-predominant histology, tumor bilaterality and early age of tumor onset may indicate a risk for carrying a germline WT1 mutation.

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Hyperdiploidy defines a distinct cytogenetic entity of aggressive meningiomas

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Although meningiomas are usually benign WHO grade I tumors, a minority of meningiomas are more aggressive (grades II or III) and tend to recur. The principal cytogenetic feature of all grades of meningioma is monosomy 22, whereas tumor progression and recurrence is usually associated with stronger hypodiploidy, i.e., monosomy of further autosomes and, most frequently, heterozygous loss of chromosome 1p. Within a series of over 400 cytogenetically characterized meningiomas, we identified a subgroup of 15 cases without the common chromosome aberrations but instead a strikingly similar pattern of hyperdiploidy. In the absence of any structural chromosome rearrangements, these meningiomas each have between 49 and 56 chromosomes, with trisomy12 (13/15 cases), trisomy 20 (12/15 cases), trisomy 5 (11/15 cases), and trisomy 17 (9/15 cases), along with variable trisomies of all other autosomes except #1, #2, and #21. Chromosome losses are rare, affecting #7, #22, and Y each in only 1/15 cases. Histomorphologically, the hyperdiploid meningiomas do not show distinct peculiarities as compared to usual meningiomas. However, their growth potential in terms of the Ki-67 proliferation index is significantly elevated; 14/15 hyperdiploid meningiomas were assigned to WHO grade II. We conclude that hyperdiploidy constitutes a small but clinically relevant entity of unusually aggressive meningiomas which, although lacking distinct histomorphological features, are cytogenetically unrelated to the majority of common-type meningiomas.

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Cytogenetic Characterization of Vulva-Melanoma

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Melanomas develop in different organs and are characterized by different clinical and biological features. Cutaneus-melanomas grow localized for a relatively long time before they develop lymphogenic secondary. Uveal-melanomas tend to set hematogenic secondary after a long period of local growth. Primary mucosa associated melanomas have a very poor prognosis due to local invasive growth and/or early hematogenic secondary. Probably mucosa associated melanomas evolve from multifocal origin. The most important pathogenetic factor of cutaneus- and uveal-melanoma is UV light exposure whereas mucosa-melanomas are UV independent. Vulva-melanoma is a rare condition of mucosa associated melanoma. Cytogenetics, pathophysiology, and oncogenesis of vulva-melanomas are widely unknown. We characterized cytogenetically three cases of vulva-melanomas and revealed complex aberrations in all cases. Molecular cytogenetic analysis using multicolour in situ hybridization was performed in two cases for detailed characterization.The results of combined analysis of G-banded metaphases and M-FISH staining were drawn into an aberration map for efficient comparison of breakpoints gains and losses. So far only two independent cases have been analysed in detail, but a high grade of similarity of breakpoints gains and losses, with hotspots on chromosomes 1, 6, 8, 10, 12, 13, 17 and 21, have been found.

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Interphase FISH using YAC-derivated probes reveals losses on 4q (4q32->4qtel) in high grade lesions and in cervical cancers

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In ongoing functional studies we have demonstrated that putative senescence genes are located on the long arm of chromosome 4 (4q32->4qter). We are now evaluating the potential significance of loss of gene function (LOH) in this region by interphase FISH of paraffin sections using YAC-derivated probes on high grade lesions and cervical cancer. Our final aim is to find useful genetic markers involved in cervical tumour progression.Sections of paraffin-embedded cervical cancers (21 cases) and high grade cervical intraepithelial neoplasias (CIN 2/3) (20 cases) were selected. After slide preparation Fluorescence- in situ- hybridisation (FISH) was performed. Specific FISH-probes binding to 4q were generated from YAC-clones (MPI Berlin). The centromeric chromosome 4-probe (Qbiogene Heidelberg) served as control probe.Interphase FISH analyses revealed allelic losses in the 4q terminal region at frequencies ranging from 23% to 47% in cervical cancer and from 12% to 20% in high-grade lesions, depending on the probe used. These findings underline the significance of our functional data obtained by microcell-mediated chromosome transfer and data from LOH-analyses, thereby suggesting that inactivation of genes within this region on chromosome 4 may represent an essential step in cervical carcinogenesis.

P 196

Mitotic instability of different chromosomes in glioma cell lines

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Mitotic chromosome malsegregation produces aneuploidy, which is commonly detected in cancer cells. In glioma cells, especially in glioblastoma multiforme, a high number of numerical chromosomal aberrations are found and especially gains of chromosome 7 and losses of chromosome 10 are very characteristic. Although chromosomal loss and gain is well known, only little is known about the mechanisms leading to specific recurrent aneuploidies. Abnormalities in the chromosomal segregational apparatus are very likely to play a role. These include centrosomal defects, defects in kinetochore microtubule attachment, and movement of the chromosomes relative to the poles. In a recent study we investigated frequent mitotic errors in glioblastoma cells, where we found losses of chromosome 7 and gains of chromosomes 10 and 17. Chromosomes 8, 12 and 18 were either lost or gained. We now analyzed whether these different types of chromosomal malsegregation are due to a defect of the mitotic spindle apparatus in glioma cells. We therefore investigated 6 glioma cell lines by combined immunohistochemistry with an alpha-tubulin specific antibody and fluorescence in situ hybridization with centromere specific probes for chromosomes 7, 8, 10, 12, 17 and 18. We found an equal distribution of the chromosomes 10, 12, 17, and 18 in mitotic cells. Chromosome 8 was mostly distributed equally but also unequally by spindle microtubules, whereas surprisingly chromosome 7 was not distributed correctly to the opposite spindle poles in most of the investigated cells. The mitotic instability of the chromosomes 7 and 8 does not seem to derive only from a defect of the spindle apparatus, because the mitotic spindle seems to be intact in 5 of 6 investigated cell lines. Furthermore chromosomes 10, 12, 17 and 18, which are often malsegregated in glioma cells, are separated correctly to the opposite spindle poles during mitosis. Therefore, an unknown mechanisms has to determine the numerical aberrations of the investigated chromosomes.In one glioma cell line we found an increase of the number of spindle poles, which could not be detected in the other cell lines. These multipolar spindle apparatus might cause mitotic instability at least in some tumors. We suppose at least two different mechanisms of chromosomal malsegregation in glioma cell lines.

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Evaluation of the potential significance of a senescence gene locus on chromosome 4 involved in cervical carcinogenesis by LOH analyses

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Using microcell fusion we had demonstrated that the loss of putative senescence genes is essential for HPV-induced cervical tumorigenesis. These genes may be located on the long arm of chromosome 4 (4q34.1->4qtel). In the present study we evaluate the potential significance of loss of gene function in this region by microsatellite-PCR.Histologically verified microdissected areas consisting of 10<hoch>3<hoch>-10<hoch>4<hoch> dysplastic or tumour cells were studied for loss of heterozygosity (LOH). Twenty-five cases of cervical cancer and 25 cases of cervical intraepithelial neoplasia II/III (CIN; 2 to 5 areas per individual tumour) were examined. DNA from microdissected specimens was amplified by DOP-PCR. Microsatellite PCR was performed on DOP-PCR products using highly polymorphic repetitive sequences located on chromosome 4.Highest frequencies of allelic loss in cervical carcinomas were observed for the loci 4q35.1 (47%), 4q28.2 (43%), 4q32.1 (40%) and 4p16.3 (42%). LOH was more frequent in the q arm as compared to the p arm. Studies of CIN II/III are in progress. Complementation assays and methylation studies will be performed to map the putative senescence gene locus more precisely.

P 198

Characterisation of a Complex Chromosome 3 Rearrangement in Familial Renal Cell Carcinoma

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Hereditary renal cell carcinoma (RCC) is a rare disease and most commonly associated with the von Hippel-Lindau (VHL) syndrome. In addition, a small number of families with constitutional chromosome 3 translocations and dominant inheritance of clear cell RCC have been described. Identifying genetic changes in these patients may help to understand the molecular pathogenesis of the common sporadic clear cell RCC. We have identified a novel family affected by early onset clear cell RCC. Cytogenetic analysis showed a so far undescribed constitutional

chromosome rearrangement. Involvement of the VHL gene in renal carcinogenesis was excluded by sequence analysis, fluorescence in situ hybridisation (FISH) applying a VHL-specific probe and expression studies. Here we present additional data from cytogenetic, molecular cytogenetic and molecular studies. The karyotype present in two members of the family shows a complex chromosome 3 rearrangement with more than two different breakpoints on chromosome 3. Multiplex fluorescence in situ hybridisation applying 19 band-specific probes for chromosomes 3 and 8 have been performed. These results allow to narrow down the breakpoints of the chromosomal regions affected by the rearrangement: 46,XY, der (3)t (3;8;3;8)(3pter->p12.1::8p21.1->8p21.3 or 8p21.3->8p21.1::3p13.1->3p12.1::8p21.3-8pter), der (8)t (3;8)(q13.1;p21.1). Data of the deletion mapping on chromsomes 3 and 8 will be presented.

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INTERPHASE-CYTOGENETIK BEI CHRONISCH LYMPHATISCHER LEUKÄMIE (B-CLL) &#8211 KORRELATION MIT ZAP-70 mRNA-EXPRESSION

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Die chronisch lymphatische Leukämie (B-CLL) ist die häufigste bei Erwachsenen diagnostizierte Leukämieform. Da die CLL-Zellen durch einen sehr niedrigen Proliferationsindex charakterisiert sind, ist die Chromosomenbandenanalyse aus unstimulierten Kulturen nur in 40-50% der Fälle erfolgreich. Einige B-CLL-spezifische Chromosomenaberrationen haben sich jedoch als wichtige unabhängige Prognosefaktoren erwiesen, weshalb es für ein Risiko-adaptiertes klinisches Management der Patienten von großer Bedeutung ist, bereits bei Diagnosestellung eine cytogenetische Analyse mittels Interphase-FISH durchzuführen. Wir haben mononukleäre Zellen von mehr als 60 CLL-Patienten auf folgende Aberrationen untersucht: Deletionen der Chromosomenbanden 13q14 (Rb1-Gen bzw. D13S272-Locus), 11q22~23 (ATM-Locus) und 17p13 (p53-Gen), Trisomie 12 bzw. der Bande 12q13 und Translokationen mit Bruchpunkt in 14q32 (IgH-Gen). Mit diesem Sonden-Panel konnten wir in knapp 80% der Fälle eine oder mehrere Veränderungen nachweisen.Die häufigste Anomalie war die Deletion in 13q14 (47,8%), gefolgt von der Deletion in 11q22~23 (39,1%) und der Trisomie 12 (28,2%). In rezenten Untersuchungen konnte gezeigt werden, dass die mRNA-Expression des den IGVH -Gen Mutationsstatus anzeigenden Markerproteins ZAP-70 signifikant mit der prognostischen Bedeutung der beobachteten Chromosomenaberrationen korreliert und somit einen weiteren potentiellen Prognosefaktor für die B-CLL darstellt.

P 200

Identification of fusion genes involving a novel gene called THADA in benign thyroid tumors with 2p21 rearrangements

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Cytogenetic aberrations occurred in about 20% of benign thyroid tumors and were divided in different subgroups. Of these, numerical aberrations of chromosome 7 i.e. trisomy 7 (alone or with additional trisomies), structural rearrangements involving 19q13.4 and chromosome 2 are the three main cytogenetic subgroups. Recently, we have narrowed down the 2p21 breakpoint region to a segment of about 450kb. Further sequence- and RT-PCR-analyses revealed a candidate gene located within the breakpoint region. In two cell lines derived from benign thyroid tumors with the translocations t (2;20;3)(p21;q11.2;p25) and t (2;7)(p21;p15) we have detected two fusion genes both involving the candidate gene called THADA (thyroid adenoma associated). In both fusion genes (THADA-FUS3p and THADA-FUS7p) the 3'-part of THADA is truncated. Due to the possible function of THADA as a death receptor interacting protein the loss of the 3'-part may lead to an altered apoptosis induction resulting in an increasing of the population of epithelial cells of the thyroid. Thus, we presume that the translocations in benign thyroid tumors involving the chromosomal region 2p21 affecting THADA are an important event in the development of these lesions.

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Cytogenetic and molecular genetic characterisation of six malignant thyroid carcinoma cell lines

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Thyroid goiters and adenomas are frequent diseases of the follicular thyroid epithelium, whereas follicular thyroid carcinomas are rather rare. In contrast to the malignant thyroid tumors, follicular thyroid goiters and adenomas are cytogenetically well examined epithelial tumors. So far, cytogenetic studies of about 500 thyroid goiters and adenomas have revealed that in addition to cases with normal karyotype in 20% of them recurrent clonal changes are found. The aberrant cases were divided in different cytogenetic subgroups. One of these subgroups is characterized by structural rearrangements involving the chromosomal band 2p21. Recently, we mapped the breakpoints in two established cell lines from benign thyroid tumors showing translocations in this region within a segment of about 450 kb flanked by BAC-clones 339H12 and 1069E24. By 3´-RACE analyses on these cell lines we have identified fusion genes consisting of the thyroid adenoma associated gene (THADA) located in 2p21 and unknown genes located in 3p25 and 7p15. We have analysed the follicular thyroid carcinoma cell lines FTC133, FTC238, and WRO and the anaplaslic thyroid carcinoma cell lines C643, HTH74, and S277 by karyotyping of 20 metaphases of each cell line. The cytogenetic investigation revealed complex karyotypes in all investigated cell lines. To investigate the question of an involvement of the thyroid adenoma associated gene (THADA) in these cell lines, we have performed FISH with two BAC clones that covered this gene and the breakpoint region of 2p21 in benign thyroid adenomas. Interestingly, we found no rearrangement of THADA in the malignant thyroid tumors analysed.

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Identification and application of stage-specific genetic markers for molecular cytogenetic diagnostics of breast tumors based on fine-needle aspiration

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It is well established that the early detection of breast tumors is essential for enabling an effective treatment of the patients. However, the many genetic aberrations identified for breast cancers result mostly from studies of invasive carcinomas or of earlier lesions located in the close vicinity of invasive carcinomas. Therefore we are in the process of analyzing 20 cases of ductal carcinoma in situ (DCIS), 20 cases of lobular carcinoma in situ (LCIS) and 10 cases of atypical ductal hyperplasia (ADH) to detect genomic changes (gains, losses, amplifications) in non-invasive breast tumors. The approach includes laser capture microdissection (LCM) of paraffin-embedded tumor material, whole genome amplification by ligation-mediated PCR and comparative genomic hybridization (CGH).BAC-contigs have been designed and established for all known genetic markers, including chromosomal regions on 1q32, 8q24, 11q13, 16q22, 17p13, 17q12 and 20q13. These FISH-probes have been successfully used for tests on imprints and paraffin sections obtained from invasive breast carcinomas, from DCIS and LCIS cases, as well as from fibrocystic mastopathies. The identified genetic changes mirrored the CGH-results. Based on our own CGH-data we will include additional markers for early lesions. The aim of our project is the identification and validation of stage-specific genetic markers for the diagnostics of early breast tumors as well as invasive carcinomas based on the analysis of fine-needle aspirates (FNA). Fine needle aspiration causes less traumatic stress to patients compared with conventional core biopsies. However, cytological diagnostics of FNA is rather difficult to perform. We therefore combine molecular pathology and molecular cytogenetics that may allow for the introduction of fine needle aspiration into routine clinical diagnostics of breast tumors.

P 203

Molecular diagnostics of medullary thyroid cancer in Polish patients

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Medullary thyroid carcinoma (MTC) occurs in familial and sporadic forms and is the major feature of the multiple endocrine neoplasia type 2 syndromes (MEN 2). Hereditary form of MTC may occur as familial medullary thyroid cancer (FMTC) or more commonly is associated with phaeochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2A (MEN 2A) and also with mucosal neuromas, ganglioneuromatosis of the gastrointestinal tract in multiple endocrine neoplasia type 2B (MEN 2B). It is an autosomal dominant cancer syndrome, caused by mutation in RET protooncogene, mapped to the centromeric region of chromosome 10q11.2. Protooncogene RET encodes a member of the receptor tyrosine kinase family of transmembrane receptors and is expressed in various tissues as thyroid, adrenal, developing kidney, nerve tissue and in some human neoectodermal tumors as well. It is possible that RET play also a role in the differentiation and proliferation of neural cells. In case when the medullary thyroid cancer is recognized, genetic tests are performed, independently from data obtained on basis of family interview and physical recognition, indicating hereditary form of cancer. It is estimated that individuals with negative interview in direction of hereditary form show 10% probability of genetic predisposition. Genetic tests include analysis of mutation in RET protooncogene in DNA obtained from whole blood lymphocytes. Positive results prove directions to testing patient's families. In our laboratory at Institute of Human Genetic in Poznan we perform screening analysis of six exons of RET gene (10, 11, 13, 14, 15, 16) by PCR-SSCP analysis with using fluorescent-labeled primers as it enabled analysis of many samples in relatively short time. Every detected change in SSCP band pattern predisposed to sequencing. Group of 168 individuals was analyzed, including patients diagnosed and hospitalized in Clinics of Endocrinology at University of Medical Sciences in Poznan and their families. The group included families with FMTC, MEN 2A syndrome. The most frequently identified changes occurred in exon 11 (26 changes) and in exon 10 (21 changes). Occasionally we detected irregularities in exon 13, 14 and 16. Virtually all patients with FMTC, MEN 2A and MEN 2B develop MTC, what is a clear rationale for performing thyreoidectomy as soon, as RET mutation has been identified. Statistically in families of patients with hereditary form of MTC, 50% of 1st degree relatives are carriers of mutations, and genetic analysis should be performed for all 1st and 2nd degree relatives. Consequently full medical characteristics describing development of the disease should be performed immediately to consider necessity of surgery. In case when clinical symptoms are not present prophylactic thyroidectomy should be considered, as much better solution than frequent monitoring of blood calcitonin level.

P 204

Incidence of t (12;21)(p12;q22) in the Polish population of children with diagnosed acute lymphoblastic leukemia

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The reciprocal translocation t (12;21)(p12;q22) constitutes the most frequent chromosomal lesion observed in acute lymphoblastic leukemia and leads to the fusion of TEL (12p12) and AML1 (21q22) genes. We performed the nested RT-PCR study on 24 children with ALL in order to distinguish the children with the expression of TEL/AML1 fusion gene and for the positive cases, the FISH analysis with ETV6/AML1 probe was done to confirm the RT-PCR results. The samples of bone marrow and/or peripheral blood from 24 patients and healthy donor (negative control) accounted for the material. The total RNA was isolated with the usage of QIAGEN commercial kit. The specific fusion sequence TEL/AML1 on cDNA was amplified using nested RT-PCR reaction and PCR products were analysed on 2% agarose gel with ethudium bromide staining. The REH line made up the positive control (product PCR – 181 bp). The incidence of t (12;21)(p12;q22) on der (21) was studied by means of fluorescence in situ hybridization with ETV6/AML1 probe on interphase nuclei. For each patient, at least 200 interphase nuclei were estimated. Among 24 analyzed patients with the help of nested RT-PCR, 10 showed the presence of gene fusion transcript TEL/AML1. All of them had PCR product of 181 bp (standard) in the bone marrow and/or in the peripheral blood. In one case the product length 142 bp (variant) occurred in the bone marrow, whereas these two different size products (181 bp and 142 bp) appeared simultaneously in one patient's peripheral blood. After applying the FISH for these 10 patients, it turned out, that 6 of them showed the fusion signal on der (21) and also del (12p12) and amplification of AML1 gene. These changes constituted 3%, 3.4%, 2.9% of evaluated interphase nuclei, respectively. In the remaining 4 patients, the nested RT-PCR outcomes were not in agreement with the FISH results, but additional chromosomal abnormalities presented above emerged. The incidence of t (12;21)(p12;q22) in the examined group of children was 42% on the basis of nested RT-PCR and 25% on the basis of FISH. This discrepancy might result from the fact, that sensitivity of nested RT-PCR reaction is higher in comparison with FISH. This points at the strict correlation between molecular and cytogenetic methods, which complement each other and in connection with it, they should be applied simultaneously at the time of diagnosis. Moreover, the small group of patients underwent analysis might be a reason for these divergences in RT-PCR and FISH results.

P 205

Hereditary non-polyposis colorectal cancer: Detection of large genomic deletions in MSH2 and MLH1 genes by MLPA (multiplex ligation-dependent probe amplification)

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Hereditary nonpolypolis colorectal cancer (HNPCC) is an autosomal-dominant disease, that is based on germline mutations in DNA mismatch repair genes, mainly in MSH2 and MLH1. A significant percentage of mutations in these genes consists of large genomic deletions encompassing one or several exons. Large deletions have been identified by Southern hybridization and by semiquantitative multiplex PCR methods. Recently, MLPA (multiplex ligation-dependent probe amplification), a new method for relative quantification of DNA sequences, has been developed for deletion screening in HNPCC patients (J. P. Schouten, MRC-Holland).In this method each probe consists of two oligonucleotides that hybridize to adjacent sites of the target sequence; hybridized probe oligonucleotides are ligated, permitting subsequent amplification. All ligated probes have identical end sequences, permitting simultaneous PCR amplification using only one pair of primers. With the MLPA test probes for all 16 exons of MSH2, 19 exons of MLH1, and 7 control probes from other genes can be amplified in a single PCR reaction, separated on a sequencing gel and quantitatively evaluated for the presence of deletions.By using the MLPA test, we examined 6 patients with known large deletions that had been previously detected with multiplex PCR; all 6 deletions were confirmed by MLPA. We then examined 107 patients by MLPA who were suspected of HNPCC but in whom no point mutation had been detected by using DHPLC and sequencing, for deletions in MSH2 and MLH1 genes and identified 9 deletions. The size of deletions ranged from one exon to a deletion of a whole gene. 6/9 (67%) deletions were located in MSH2 and 3/9 (33%) in MLH1. We conclude that MLPA is an efficient and easy to perform method for detection of unusual copy numbers of genomic sequences. It is at least as sensitive as the semiquantitative multiplex PCR method used before. In addition, results are easier to evaluate by this method when compared to the multiplex PCR method.The overall frequency of large deletions in patients examined for mutations in MMR genes within our study was about 10%: Of 618 unrelated colorectal cancer patients fulfilling the Bethesda criteria for HNPCC, 296 exhibited microsatellite instability in their tumor tissue or met the Amsterdam criteria for HNPCC. Among these pre-selected patients 108 disease-causing point mutations (36%) had previously been detected in the MSH2 and MLH1 genes by SSCP, heteroduplex analysis or DHPLC followed by direct sequencing. 185 of the remaining patients were examined for large genomic deletions by semiquantitative multiplex PCR and / or MLPA. Overall, we identified large genomic deletions in 28 patients (28/296=9%), of which 17 (61%) are located in the MSH2 gene and 11 (39%) in the MLH1 gene.Supported by the Deutsche Krebshilfe

P 206

Cytogenetic analyses refine the impact of INK4a/ARF defects on chemoresistance of primary lymphomas in vivo

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The INK4a/ARF tumor suppressor locus has been identified as one of the most frequently mutated gene loci in human cancer entities. Both products ARF and INK4a are implicated in stress responses and cellular growth control. Using the Eμ-myc transgenic lymphoma model, INK4a was recently found to mediate a cytostatic response to anticancer treatment that displays features of premature senescence and contributes to the outcome of cancer therapy. Accordingly, treatment-mediated selection against INK4a genes in tumors that became ARF deficient during tumor development can compromise senescence and render these tumors resistant to therapy.We applied spectral karyotyping (SKY), comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analyses to untreated primary lymphomas with defined genetic defects at the INK4a/ARF locus, and correlated the cytogenetic results with clinical responses of these lymphomas following anticancer drug therapy in vivo. Moreover, relapsed lymphomas underwent exon-specific re-evaluation of the INK4a/ARF locus. CGH analysis unveiled that virtually all myc-driven lymphomas acquired additional chromosomal gains or losses, irrespective of their INK4a and ARF expression status. Remarkably, a gain of chromosome 14 was detected in the vast majority of ARFnull lymphomas, while this observation was never made in INK4a/ARF null lymphomas. ARFnull lymphomas with gain of chromosome 14 achieved a much better long-term outcome as compared to those with no detectable gain. In contrast, gain of chromosome 6 in untreated ARFnull lymphomas which do express INK4a was found to precede therapy-induced loss of INK4a encoding alleles, previously shown to disable drug-induced senescence and to compromise long-term outcome to therapy.In conclusion, our data suggest that disruption of the ARF-p53 axis is not sufficient to allow accelerated tumor formation in the context of activated Myc, since INK4a/ARF deficient lymphomas typically acquired additional alterations. Moreover, cytogenetic lesions were identified that refine the prognostic impact of INK4a/ARF defects on treatment outcome. Linking cytogenetic data to functional genomics of treatment responses in vivo, our approach outlines the technology to screen for chromosomal aberrations that mimic, compensate for or that are incompatible with defects at a key locus controlling tumorigenesis and chemosensitivity.

P 207

Recurrent APC gene mutations in Polish FAP Families

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Familial adenomatous polyposis (FAP) is a genetically determined disorder, inherited in an autosomal dominant manner. The occurrence of FAP is associated with mutations in the adenomatous polyposis coli (APC) gene described in 1991. APC gene mutations arise de novo in 1 per 10,000 newborns. The APC gene is localized on chromosome 5q21 and consists of 21 exons. Mutations of the APC gene, in most cases, are small deletions or insertions with the most frequent mutations the AAAGA deletion at codon 1309, which occurs in 10% of FAP families and the ACAAA deletion at codon 1061, which occurs in 5% of FAP families. The dysfunction of the APC gene causes the accumulation of B-cathenin and the expression of genes, which promote cell division. The FAP syndrome constitutes a relatively low percentage of colorectal carcinomas (1-2%) and is characterized by the presence of numerous (at least 100) polyps lining the mucosa of the large intestine and rectum. The DNA bank of Polish FAP families was established in 1997. This study is a report presenting a spectrum of mutations of the APC gene in Polish FAP patients. One hundred twenty unrelated Polish FAP families were screened for mutations in the APC gene using HA and SSCP methods. Most frequent mutations in Polish FAP families were 3927-3931delAAAGA, which occurred in 15 families (12.5%), while 3183-3187delACAAA was observed in 6 families (5%). The frequency of these mutations was similar to other populations. In exon 15, we observed another two recurrent mutations and each of them was observed in two families. One of them was a known 2626C>T substitution and the second one was a new mutation 3202-3205delTCAA. Also in exon 11 we observed the 1491insT in two families. It should be mentioned that in studied group of 120 families, the recurrent mutation were observed in 27 (22.5%) Polish FAP families.

P 208

RET Proto-oncogene Polymorphisms in the Context of C-Cell hyperplasia and Sporadic and Hereditary Medullary Thyroid Carcinoma

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RET protooncogene mutationscreening of patients suffering from MTC or CCH, assumed as precancerosis, is widely acknowledged to elucidate hereditary thyroid carcinoma. We have tested 415 patients suffering from sporadic disease and 40 index cases with hereditary MTC and determined SNPs in exons 11, 13, 14 and 15. We could not find any significant difference of frequencies to controls, excluding a disease predisposing function of any polymorphism. We found however allelic colocalisation of the Ser691 variant located in RET exon11 on the exon15 codon 904 C allele. Our analysis confirmed also the 836 T base exchange to be located exclusively onto the 769 G allele.We examined 199 subjects for their histopathological data in relation to SNPs presence. Thereby we could find a higher frequency of the 769 G variant in the group of patients without CCH (P=0,0499), however exon 13 SNP frequencies of subgroups with and without CCH did not differ significantly. Other SNPs did not show any significance with regard to C cell disease or additional presence of NMTC (FTC, PTC). We found however neoplastic CCH more frequent in males (P<0,0001) and found an additional NMTC 3,7 times more frequent in males than females with C cell disease (P=0,0136), which is clearly distinct from NMTC male/female ratio of 1:9,7. Thus C cell disease seems to change pathophysiological base of NMTC inverting sex ratio / influence of gender.

P 209

Gonadoblastoma and mixed germ cell tumor in dysgenetic gonads of XY female

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Gonadoblastoma is a neoplasm defined histologically by the occurrence of discrete aggregates of germ cells and epithelial cells resembling immatureSertoli or granulosa cells. In some cases more malignant germ cell tumors, such as dysgerminoma, occur with gonadoblastoma in dysgenetic gonads. We report 25-year-old XY female patient with clinical signs of pure gonadal dysgenesis. She underwent a laparoscopic gonadectomy, and histological examination showed gonadoblastoma on the left side and mixed germ cell tumor in the right gonad. The karyotype of fibroblasts in both gonads was 46,XY. The SRY gene was apparently normal in lymphocytes of peripheral blood as well as in gonadal fibroblasts. PCR analysis showed no deletion of Y chromosome specific sequences. We recommended early gonadectomy in XY females with gonadal dysgenesis.Supported in part by grant No PO5E 135 21 from the Polish Committee for Scientific Research

P 210

Prenatal Diagnosis of Chondrodysplasia Punctata

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Several different disorders with similar punctate cartilaginous changes are known. We report on

prenatal findings in two brothers with rhizomelic chondrodysplasia punctata (RCDP) and in a female fetus with Conradi-Hünermann-Happle syndrome (CDPX2). In the two male fetuses (week 22 and week 14/3 of gestation) prenatal sonographic examinations revealed shortening of the long bones and punctate calcifications. The diagnosis of RCDP (autosomal recessive) was established by X-ray findings and the biochemical verification of a marked deficiency in peroxisomal plasmalogen synthesis. In the female fetus (week 32 of gestation) prenatal sonographic examinations showed shortening of long bones and a complex malformation of the spine. The diagnosis of Conradi-Hünerman-Happle syndrome (X-linked dominant) was established by X-ray findings and the molecular verification of a mutation in the gene encoding delta (8)-delta (7) sterol isomerase emopamil-binding protein (EBP gene). We want to underline the importance of biochemical or molecular examinations for accurate diagnosis of RCDP.

P 211

A prenatal case of a X-autosome translocation in a male fetus with a normal phenotypic outcome

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Cytogenetic investigation of cultured amniotic cells after amniocentesis at 14+4 weeks of gestation showed a reciprocal translocation between the X-chromosome and one chromosome 3 in a male fetus. At a band resolution of 400 bands no chromosomal imbalance was detected. Ultrasonographic examination of the fetus at 14+4 and 20+4 weeks of gestation showed no abnormalities. Cytogenetic investigation of the parents revealed the same translocation in the mother also in her first son. At the age of 11 years this boy presented no particularities in his physical development as in his mental status. After extensive genetic counselling the parents decided to prolonge the pregnancy until birth. A boy was born at term without any abnormalities. At the age of 7 months the boy only showed hypertonus and neurodermitis. This case presents a rare familiar X-autosome translocation without any phenotypical abnormalities in both sons of the carrier mother until now.

P 212

Ultrasound biometry of the fetal nose in the second trimester: Physiologic variability and preliminary evaluation of its value in the screening for fetal aneuploidy

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Objective of the present study was to define the optimal conditions for fetal nose ultrasound assessment and to calculate the range of physiologic variability of examined parameters. Preliminary assessment of fetal nose biometry value in the screening for fetal aneuploidy, by comparison of the aneuploid and healthy fetuses. The study group consisted of 529 pregnant women with euploid singleton fetuses between 14 and 21 weeks of gestation, and 7 fetuses with chromosomal aneuploidies. Ultrasound measurements of fetal nasal bone length and nasal width were performed in both groupsResults: The influence of gestational age and fetal position upon the nasal measurements was assessed. The range of physiologic variability of every parameter was calculated. Influence of fetal position on measurements was noticed. Decrease of nasal bone length and increase of nasal width in the group of fetal trisomy 21 was noted, but scarcity of the aneuploidy group does not let to draw statistically significant conclusions.Conclusions: Fetal nose biometry seems to be a valuable additional marker of fetal trisomy 21 in the second trimester, requiring further studies. The study was supported in part by the grant of the Polish State Committee for Scientific research no 3 PO5E 156 22.

P 214

Quality Assurance in Assessment of Growth Characteristics of Complete Cell Culture Media in Routine Diagnostic Caryotyping – A Comparison Study of a New developed Synthetic Safety Medium with Conventional Non-synthetic Media in Amniotic Fluid Cell and CVS Cultures regarding to the European In Vitro Diagnostic Device Directives to meet the Requirements for CE Mark Conformity

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Since the European In Vitro Diagnostic Device Directives and quality assurance regulations for laboratory work (cGLP) request co-operation in production (cGMP) and routine diagnostic cytogenetic analysis of chromosomes (caryotyping) R&D is focussed on standardised material and methods. Fetal bovine serum (FBS) was used as a main component of "ready-to-use"complete cell culture media to iniiate cell growth since nearly 20 years. The fluctuation of the characteristics of such biological source material and and the risk of unexpected effects caused by virus or endotoxin contamination (non-specific stimulation, chromosomal aberrations, pseudomosaicism) forced the efforts to develop a new generation of fully synthetic cell culture media to replace FBS-containing products in cytogenetics. First in-house pilot studies indicate and an external multicenter study shall confirm the possibility to grow amniotic fluid cells and CVS without FBS within a propriate short time. If further results confirm the feasibility of usage of such type of material, cell culture in cytogenetics could be safer and done under better defined conditions. The new developed Safety Medium could be an advantage to meet the requirements of high quality standards in laboratory work.

P 215

A Novel Type of Autosomal Recessive Syndactyly: Clinical and Molecular Studies in A Family of Pakistani Origin

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Non-syndromic syndactylies have been classified into five major types (I-V), all showing autosomal dominant mode of inheritance. Later, the classification was extended and three additional variants (VI-VIII) were defined. Type VII, the Cenani-Lenz syndactyly, is the only non-syndromic, autosomal recessive type. It is characterized by fusion of all phalanges with metacarpal synostosis, dislocated and dysplastic carpals and infrequently, radio-ulnar fusion. Here, we present a Pakistani family with a novel non-syndromic autosomal recessive syndactyly manifesting a unique combination of clinical features. In both hands, reduction of certain phalanges is evident. Radiological examination shows synostosis of third and fourth metacarpals bearing single phalanges. The first three toes are webbed, with hypoplastic terminal phalanx in all the toes. The phenotype segregating in our family is the second well documented autosomal recessive, non-syndromic syndactyly. Since the clinical features had minimal overlap with syndactyly types I, II and III, we have performed microsatellite marker screening to look for the cosegregation of this phenotype with any of the known loci for these respective types. We show that the phenotype in our family is not linked to chromosomal regions 2q34-q36, 2q31 and 6q22-q23 encompassing loci for syndactyly types I, II and III.

P 216

Disruption of the Pelota Gene Causes Early Embryonic Lethality and Defects in Cell Cycle Progression

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Mutations in either the Drosophila pelota , pelo, or the Saccharomyces cerevisiae homologous gene, DOM34, cause defects of spermatogenesis and oogenesis in Drosophila, and delay of growth and failure of sporulation in yeast. These phenotypes suggest that pelota is required for normal progression of the mitotic and meiotic cell cycle. To determine the role of the pelota in mouse development and progression of cell cycle, we have established a targeted disruption of the mouse Pelo. Heterozygous animals are variable and fertile. Genotyping of the progeny of heterozygous intercrosses shows the absence of Pelo-/- pups and suggests an embryonic lethal phenotype. Histological analyses reveal that the homozygous Pelo deficient embryos fail to develop past day 7.5 of embryogenesis (E7.5). The failure of mitotic active inner cell mass (ICM) of the Pelo-/- blastocyts to expand in growth after 4 days in culture and the survival of mitotic inactive trophoplast indicates that the lethality of Pelo null embryos is due to defects in cell proliferation. Analysis of the cellular DNA content reveals the significant increase of aneuploid cells in Pelo-/- embryos at E7.5. Therefore, the percentage increase of aneuploid cells at E7.5 may be directly responsible for the arrested development and suggests that Pelo is required for the maintenance of genomic stability.

P 217

Identification of an novel Lim domains containing gene that interacts specifically with Ror2

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ROR2 is a membrane-bound receptor tyrosine kinase, that belongs to a family of receptor tyrosine kinases with a conserved cytoplasmatic kinase domain, distally located serine/threonine-rich and proline-rich domains and several distinct extracellular domains, including an immunoglobulin, a cysteine-rich and a kringle domain. Ror2 has been implicated in chondrogenesis as shown by the severe chondrodysplasia phenotype in Ror2 knock out mice. However, the signalling pathways in which Ror2 participates remain unidentified to date. Mutations in ROR2 have been described to be responsible for autosomal dominant brachydactyly type B (BDB) and the autosomal recessive Robinow syndrome (RRS). We used the cytoplasmatic part of ROR2 to perform yeast two hybrid-assays. We phosphorylated Ror2 by coexpression of constitutively active src kinase. In a second set of experiments we used unphosphorylated cytoplasmatic Ror2 as bait. Screening was performed against a cDNA library obtained from mouse embryos stage E9.5 to E10.5. In both screens we were successful in identifying several clones that are potential interaction partners. In particular, we succeeded in identifying a novel Lim domains containing protein that was shown to interact with ROR2 in a phosphorylation independent manner. The identified Lim protein interacts specifically with Ror2 but not with other tested receptor tyrosine kinases. Interaction could be confirmed by co-immunoprecipitation in 293-cells and was located to the distal serine/threonine- and proline-rich domains. This Ror2 interacting partner is a novel member of the Lim protein gene family. It locates to mouse chromosome 7 and consists of 9 exons. Lim domain proteins belong to a diverse family that all share different numbers of Lim domains, which have been shown to mediate protein-protein interactions. This and other identified potential targets may help to elucidate Ror2 signalling pathways.

P 218

TBX5, essential for heart formation, is not associated with the etiology of transposition of the great arteries (dTGA)

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Cardiac development is a complex process requiring the integration of several signaling factors and cascades. TBX5, a member of the T-box gene family, has turned out to be a major player in the patterning of the heart during early embryonic development, being involved in compartmentalization and septation processes. We therefore addressed the question whether defects in TBX5 are causative for the phenotype in a group of patients displaying a transposition of the great arteries (TGA). TGA reflects a septation defect of the common outflow tract of the heart, affecting 0.2 per 1000 live births. Despite this high incidence very little is known about the regulating mechanisms. Screening 37 patients with isolated TGA for mutations within the TBX5 gene by means of direct sequencing, we identified one missense mutation in exon 4, Asp111Tyr, and two polymorphisms in intron 4 and the 3´ UTR, that were detected in similar frequencies in control individuals. Our data suggest that TBX5 mutations are not causative for TGA.

P 219

Novel genes expressed in human fetal growth plate cartilage – characterization of candidate genes for skeletal disorders

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Based on a unique human fetal growth plate cartilage cDNA-library an EST-project was initiated in the context of the German Human Genome Project. The project aims at the identification of genes and pathways involved in bone growth and differentiation. Candidate genes are expected to be of value for diagnosis and treatment of monogenic and multigenic heritable disorders of the skeleton. Here, we describe the further characterization of two promising candidates from this EST-project.Clone 86H12 corresponds to a novel gene located on chromosome Xq, which is represented only four times in the NCBI EST-database. The 13 exons of the gene could be verified by RT-PCR. Northern blot analysis showed a fairly low expression in various tissues. A strong expression was detected in placenta and fetal cartilage. Analysis of the deduced putative protein structure anticipated 12 leucine-rich repeats. A significant sequence homology to biglycan and ECM2 as well as the fact that the only four ESTs in the databases are from two different osteoarthritic cartilage and a chondrosarcoma cDNA libary, make this clone an excellent candidate for further analysis. Consequently, first expression studies have been performed in different tissues including osteoarthritic cartilage using real time-PCR. The data suggest that 86H12 is strongly expressed in some normal and arthritic cartilage samples. The second clone, 68F08, corresponds to a novel hypothetical protein in man and mouse. We verified the complete cDNA sequence by RT-PCR. The main transcript detected by Multiple Tissue Northern blot analysis is about 9 kb in size. Protein domain analysis (SMART) revealed a Thrombospondin N-terminal-like domain (TSPN), a triple-helical domain with 335 Gly-X-Y-repeats and a fibrillar collagen C-terminal domain (COLF1). The domains are highly conserved between man and mouse and indicate that this gene most probably encodes a novel fibril-forming collagen. Meanwhile the gene, which is located on chromosome 9q, has been submitted to the databases by P. Byers/Seattle and was named COL27A1. The gene shows a ubiquitous expression in several tissues and a strong expression in developing skeleton. The high homology to other fibrillar collagens involved in skeletal development together with it's expression pattern makes COL27A1 an excellent candidate gene for skeletal dysplasias.

P 220

Novel mutations of the androgen receptor gene in patients with androgen insensitivity syndromes

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The androgen receptor gene, mapped to chromosome Xq12, belongs to the subfamily of steroid hormone receptor genes within a large superfamily of ligand-responsive DNA-binding transcription factors. Mutations within the androgen receptor gene cause a spectrum of X-linked recessive disorders that range from complete androgen insensitivity (CAIS) (MIM 300068) over partial androgen insensitivity (PAIS), such as Reifenstein syndrome (MIM 312300) to minimal androgen insensitivity syndrome (MAIS) or infertile male syndrome (MIM 308370). So far, only few recurrent mutations have been reported while most patients have individual mutations, the vast majority of them being missense mutations. Nonsense-mutations and insertions or deletions are rare. Here, we report on eight novel mutations, six of which are missense mutations (C614Y, I680T, P682A, M745I, M761T, A870Q) identified in five patients being affected with CAIS and in one patient with PAIS, respectively. In a patient affected with CAIS a duplication of a AGG triplett following nucleotide 2726 (2726_2727dupAGG) was detected. In a further CAIS family DNA sequencing revealed a 13 bp deletion from nucleotide 2461 to 2473 (2461_2473delTGCTCTCTAGCCT), altering the reading frame and leading to a premature termination codon at position 783. In a ninth patient being affected with MAIS we found a missense mutation (R840C), that was so far only reported in patients with PAIS.

P 221

Effect of age on the frequency of aneuploidies in human sperms

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Inherited numerical chromosomal aberrations can be deleterious to human reproduction and to the viability and health of the offspring. Maternal age has been known to be the most important aetiological factor implicated in human trisomy formation. In order to assess the possible risk of chromosomal abormalities in offspring from older fathers, we investigated the effects of age on the frequency of chromosomal aneuploidy rates of human sperm, using the fluorescence in-situ hybridization (FISH) for the chromosomes 13, 16 and 21. Semen sample were collected from 16 men aged > 40 years and from 11 men aged < 38 years as controls. Multicolour fluorescence in-situ hybridization was used to determine the aneuploidy and diploidy frequencies of chromosome 13, 16 and 21 using directly labelled DNA probes on decondensed sperm nuclei. A minimum of 500 sperm per donor and > 13000 sperms in total were evaluated. The average aneuploidy rate was 1,5% in spermatozoa of older men and 1,4% in younger men. The diploidy rate in spermatozoa of older and younger men were 0% - 1,43% and 0% - 1,4%, respectively. The incidence of aneuploidy was

not significantly higher in older men (Wilcoxon test p=0.21). The results suggest that men of advanced age still wanting to become father do not have a significantly higher risk of procreating offspring with chromosomal abnormalities compared with younger men.

P 222

Expression and functional analysis of ADAM 27

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ADAM 27 (also known as ADAM 18) is a membrane-anchored sperm protein belonging to the ADAM (A Disintegrin And Metaloprotease) family of the proteins, which are proposed to be involved in gamete interactions and membrane fusion in mammals. This gene shows testis specific expression restricted to germ cells. Generated antibodies detect the protein on the posterior sperm head. Interestingly, protein seems to be relocated after the capacitation process, initially concentrated protein is present in a spot-like structures on the surface of posterior sperm head, than becomes diffused but still restricted to the same area. Since ADAM 27 protein is detected on the sperm it is proposed that this protein is taking part in the complicated process of fertilization as an adhesion molecule. To determine what role ADAM 27 plays in fertilization, we have expressed the putative extracellular domains of mouse ADAM 27 in bacteria as a fusion protein with maltose-binding protein and used in in vitro assays to characterize its binding to mouse eggs. In order to elucidate the physiological function of ADAM 27 gene in vivo we have disrupted its locus by homologous recombination. In the present study we report generation of knock out mouse lacking functional protein and its phenotype. Human orthologue gene hADAM 18 was shown to be functional in contrast to three other non-functional well known ADAM family genes.

P 223

Molecular and cellular approaches for study of proliferation and differentiation of spermatogonial stem cells

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Male reproductive success ultimately depends on the ability of the testis to produce prodigious numbers of sperm at a consistent rate during the spermatogenesis process. Spermatogonial stem cells (SSC) initiate and maintain spermatogenesis in the seminiferous tubuli of the testis. To perform this role, the SSC must self replicate as well as produce daughter cells that can expand and differentiate to form spermatozoa. The aims of this study are to study the molecular and cellular aspects of SSC proliferation and differentiation. We started with expression analysis of SSC expressed genes Tex18 and Stra8. Tex18 is a novel gene which is localized on chromosome 10 of mouse and consists of only one exon. 1191 bp long mRNA of this gene is coding for 80 aa protein. Cellular localization of the gene is not known yet. Stra 8 gene is localized on the mouse chromosome 8. Its mRNA is 1455 bp long and includes 9 exons. Expression of Stra 8 is induced by retinoic acid. Stra 8 protein is 393 aa long and is localized in cytoplasm. Using RT-PCR analysis it was shown that expression of these genes start in prenatal male germ cells (as for now we know, that expression occurs at the 15,5 dpc) in the mouse and correlates with the appearance of spermatogonial stem cells.Expression was also shown after birth (days 5-30) and in the adult mice.Expression of both genes was observed in mutant mice, Tfm/y, Insl3-/-, olt/olt, qk/qk, but not in w/wv mice, which lack any germ cells. Detailed functional and expression analysis of genes therefore provide an important step toward the elucidation of molecular mechanisms underlying spermatogonial stem cell division and differentiation. In second approach, we generated transgenic mouse lines expressing a selective marker protein which is specific for spermatogonial stem cells. For this purpose we used the promoter region of Stra8 gene to direct expression of EGFP protein, as a selective marker, respectively. Different transgenic lines were produced. These mice provide a useful source for isolation and purification of SSC by FACS sorting. Using differential screening of gene expression in purified SSC and testis of germ cell-deficient mutant mice, SSC specific genes should be identified and analysed.

P 224

Low level gonosomal mosaicism in women undergoing ICSI cycles

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Cytogenetic analyses of couples presenting with male factor infertility show a higher frequency of chromosomal abnormalities in infertile males compared to unselected male newborns. There is also an increased risk of low-level X-chromosomal mosaicism in female partners of couples undergoing intracytoplasmic sperm injection (ICSI). 891 consecutive females scheduled for ICSI treatment had routine cytogenetic analysis in our Institute from 1996-2001. 46 of them showed chromosomal abnormalities, from which 35 were low-level gonosomal mosaicisms. For these 35 women a control cohort of females with normal karyotypes also scheduled for ICSI was matched. To compare the incidence of low-level gonosomal mosaicism in the group of ICSI females to other females without fertility problems due to male factor infertility, two further groups were analysed: 294 consecutive females with recurrent abortions and 104 consecutive women with clinical or cytogenetical affected children who also had routine chromosome analysis. The incidence of low-level gonosomal mosaicism in the group of females with recurrent abortions (25/294; 8.5%) was significantly higher than in the group of females undergoing ICSI (35/891; 3.93%) ($p<0.01$). The incidence of low-level mosaicism was similar between the groups of ICSI females and females with cytogenetical or clinical affected children (6/104; 5.76%). Regarding anamnestic factors like menarche, menstrual cycle stability, treatment parameters and pregnancy rates no significant difference for the females undergoing ICSI with gonosomal mosaicism and the females undergoing ICSI without chromosomal abnormalities was found. We can conclude from this case-control study that low-level gonosomal mosaicism has no impact on the fertility rate of females undergoing assisted reproduction. Data about the incidence of low-level gonosomal mosaicism of the general population of females in the reproductive age without fertility problems or abortions are not known. It is possible that there might be an increased risk of habitual abortions in conjunction with low-level gonosomal mosaicism. This, however, should be addressed in a separate study.

P 225

A case of an unusual familial reciprocal translokation 46,XX,der (14;15)(q10;q10),der (14)t (14;15)(q10;p12)

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We report a case of a de novo reciprocal translocation. We obtained a blood sample of a 32 yaer old woman and her husband from a centre of reproduction for cytogenetic analysis. The couple is over three yaers childless and plan to undergo ICSI-therapie. The male karyotyp (46,XY) was normal. The female had the karyotyp: 46,XX,der (14;15)(q10;q10),der (14)t (14;15)(q10;p12). At first it seemed to be a normal Robertsonian translocation with an additional very small marker chromosom. But after further investigation with NOR-banding, CBG-banding and FISH analysis the result was an reciprocal translocation between the centromeric regions of the chromosoms 14 and 15. To decide whether the small chromosom obtains euchromatic material or not a cytogenetic analysis of the parents and the three brothers of our patient was performed. The results: the mother and one of the brothers were carriers of the same rearrangement as the daughter respectively the sister; the second brother had only the small chromosom 47,XY,+der (14)t (14:15)(q10;p12) and the third brother was carrier of the Robertsonian translocation 45,XY,der (14;15)(q10;q10). Because of the random segregation of the translocated chromosoms in healthy family members we can assume that the small marker chromosom does not carry euchromatic material.Therefore we suppose that there is no increased risk but that for missed abortion and uniparental disomy 14;15 (UPD). Adjacent segregants are invariably lethal in utero (translocation trisomy 14, translocation trisomy 15). UPD 14 and UPD 15 are possible outcomes, although the only proven case is a child with UPD 15 (mat) (Smith et al., 1993)(R. J. M. Gardner, G. R. Sutherland, 1996). At this moment our patient is pregnant after ICSI-therapie and wants to undergo prenatal diagnosis.

P 226

Characterization of a conditional mouse-model for alpha-synucleinopathies

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The presynaptic protein alpha-synuclein is a major component of Lewy bodies in the substantia nigra and cortex of patients with alpha-synucleinopathies, i.e. Parkinson´s disease, dementia with Lewy bodies and other diseases. Pathogenetic research of these diseases has been rapidly advanced by the development of animal models. By using the inducible tetracycline-responsive system, we have created a conditional mouse model of alpha-synucleinopathies. This system allows expression of transgene, in this case human wildtyp or mutated [A30P]alpha-synuclein, to be turned off by oral administration of tetracycline-analogs. In order to direct the expression of human alpha-synuclein to the brain, we used both the prion protein promoter and the CamKII-alpha promoter. We investigated the conditional overexpression by performing Western blot analysis with protein extracts from several tissues and immunostaining of paraffin-embedded brains. These analysis showed, that double-transgenic mice express human alpha-synuclein in a brain-specific manner, whereas little alpha-synuclein was detected in tissues of single-transgenic mice, carrying only the alpha-synuclein construct. This pattern was mainly due to cross-reaction of the ?human-specific? antibody. Histological analysis of transgenic mice at different ages showed aberrant expression of the human alpha-synuclein protein in cell soma but no Lewy body-like alpha-synuclein-inclusions could be identified, yet. Administration of doxycycline, a tetracycline-analog, downregulates alpha-synuclein expression to basal levels in the brain of double-transgenic mice. We currently investigate if elderly transgenic mice show a neuropathological or a behavioral phenotype. Our mouse-model may help to gain insights to the precise role of human alpha-synuclein in the development of synucleinopathies and will be used to demonstrate if neuropathological symptoms are reversible.

P 227

Molecular characterisation of the canine HMGB1

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As witnessed by a number of recent articles a growing number of scientists predict that human genetics will be ”going to the dogs” in this century. Due to the emerging advantages of numerous canine diseases as a genetic model for human orthologs, the dog could join the mouse as the species of choice to unravel genetic mechanisms of e.g. cancer predisposition, development, and progression. A very interesting gene in terms of oncology is the high mobility group protein gene B1 (HMGB1). This best analysed member of the HMGB protein family, HMGB1 (synonymously known as HMG1 or amphoterin), can modify chromatin structure by bending DNA thus influencing the transcription of a number of target genes. Beside its function as an architectural transcription factor, HMGB1 can also be secreted by certain cells, e.g. macrophages. As an extracellular protein HMGB1 is a ligand for the receptor for advanced glycation end products (RAGE) thus activating key cell signalling pathways, such as p38MAPK, JNK, and p42/p44MAPK and playing an important role in inflammation and tumour metastasis. In our studies we characterised the canine gene HMGB1 on various levels. The canine cDNA sequence consists of 2236 bp spanning five exons with a total homology of 90.8 % to its human counterpart. The genomic structure of the gene consists of the five exons and four introns of which exon 1 (76 bp) and a contig spanning exon 2 – exon 5 (3959 bp) were characterised. Homology comparison to the human ortholog revealed 98.7 % similarity for exon 1 and 73.9 % for the contig spanning exon 2 – exon 5. The protein deduced from the generated cDNA sequence is a 215 amino acid (AA) molecule with a weight of 24892.67 Daltons. Homology comparison to the human counterpart showed 100% homology of the molecules. The chromosomal locus was mapped by FISH to CFA 25. Expression analyses by northern blot revealed two transcripts of approx. 1.4 and 2.4 kb in various tissues.

P 228

Runx2 and Runx3 Cooperate During Cartilage Differentiation

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Runx genes encode evolutionary conserved transcription factors, that are involved in a variety of embryonic cellular differentiation pathways. Runx2 is an essential factor for bone formation as demonstrated by the absence of osteoblasts/bone in Runx2 (-/-) mice. Runx2 also is known as a positive regulator of cartilage differentiation. Runx3 is expressed in TrkC positive neurons and is essential for their survival. Additionally, Runx3 has been involved in intestinal development and human gastric cancer. We have shown previously, that Runx3 is expressed in cartilage in distinct but partially overlapping domains with Runx2. Here we show, that in the chick Runx2 is expressed in domains comparable to the mouse. In chicken micromass cultures, Runx2 and Runx3 both weakly stimulate chondrogenesis. Both proteins strongly induce differentiation to Alkaline phosphatase positive chondrocytes. These results indicate, that Runx2 as well as Runx3 positively influence chgondrogenesis but play their main role in later steps of chondrocyte differentiation. To asess the role of Runx3 in vivo, we crossed a Runx-dominant negative transgene on the Runx2 (-/-)-background. Double mutant mice exhibit a complete block in chondrocyte differentiation in skeletal elements that are only mildly affected in Runx2 (-/-) mice. Thus, we demonstrate that Runx3 is a positive regulator of chondrocyte differentiation in vivo, acting in concert with Runx2.

P 229

Microarray analysis of Calcium channel (P/Q type) mutant mice (B6.D2-Cacna1atg/tottering)

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Calcium controls diverse cellular processes, which include muscle contraction, hormone or neurotransmitter release, gene expression and cell proliferation. To evoke these responses, multiple voltage-dependent Ca2+ channel types form major Ca2+ entry pathways. Of these, P/Q type Calcium channels are multisubunit complexes and the channel activity is directed by a pore-forming alpha-1 subunit, which is often sufficient to generate voltage-sensitive Ca2+ channel activity. Spinocerebellar ataxia-6 is caused by expansion of a CAG repeat in the coding region of the alpha-1A voltage-dependent calcium channel subunit (Cacna1a). We analysed mice with a mutation in Cacna1a (tottering mutant mice) by microarray analysis.Tottering mice have a nucleotide substitution in the calcium channel alpha 1a gene, Cacna1a, which alters pore function of the protein product. So the RNA level of Cacna1a is not affected in the microarray results. RNA for microarray analysis was isolated from total mouse brains of homozygous mice for the tottering mutation (Cacna1atg). Because C57Bl6 is the genetic background of the tottering mice, we used this mouse strain as a control. Three 3 month-old males were analysed both for the tottering and the wild type mouse strain with the Affymetrix U74A array. For data analysis of the microarrays we used the Affymetrix software tool (MA
S 5
.0, MicroDB, DMT 3.0) and the internet portal NetAFFX. We found 207 genes differently regulated, which we could divide into 12 functional groups. Only those genes, which were found in at least 7 of 9 comparisons similarly adjusted, were defined as differently expressed genes. We will present the complex pattern of altered expression of neuronal genes in the tottering mice to gain insights into the pathogenesis of cerebellar diseases in humans.

P 230

Microarray analysis to define expression networks altered in alpha-synuclein knock-out mice (SNCA -/-)

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Alpha-synuclein plays a central role in the development of Parkinson´s disease (PD) and represents the main component of intracellular protein aggregation (Lewy bodies) in PD patients. The currently unknown function of alpha-synuclein and the complexity of the affected pathways led us to employ Microarray expression profile analysis to gain insights into the pathomechanism underlying PD. RNA for chip-analysis was isolated from total mouse brains of SNCA-/-mice (Abeliovich, et al. 2000 / Jackson

Laboratories). As controls we used mouse strain C57Bl6 which is the genetic background of the knock-out mice. Three 3-month-old males were analysed for each model using the Murine genome U74v2 oligonucleotide chip (Affymetrix, USA).By comparing the microarray-data of SNCA-/- mice with wild-type we used the following conditions: At least 7 of 9 comparisons must show a signal log ratio (SLR) of min +/- 0,75 (which means a Fold Change of +/-1.6). In consideration of these points we found about 25 genes which are differently regulated in the SNCA-/- mice. Most of the genes differently regulated between controls and synuclein knockouts are involved in vesicle transport. This is in particular intriguing as alpha-Synuclein is thought to be involved in vesicle transport in dopaminergic neurons. Interestingly, one of these down regulated genes, which also is part of the vesicle transport cascade, is the Ataxia telangiectasia mutated (ATM) gene. In current studies we investigate, which of the down regulated proteins interact directly with alpha-synuclein. Furthermore, it will be interesting to learn the dependence of expression regulation between the differentially regulated genes. Using this approach we hope to establish expression profile networks to further gain insights into the role of alpha-synuclein in the pathogenesis of PD.

P 231

A TSPY transgenic mouse line

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We have generated a transgenic mouse line harbouring a complete structural human TSPY gene. FISH analysis shows that the human TSPY transgene is integrated to a single chromosome of the transgenic mice and the transgene integration site maps ectopically to the distal long arm of the Y chromosome. The human TSPY construct was introduced into the mouse genome in approximately 50 copies. The transgene is correctly transcribed and spliced according to the human pattern and is mainly expressed in testes. Within the testes we identified spermatogonia and early primary spermatocytes as TSPY expressing germ cells. With the establishment and molecular genetic characterization of a transgenic TSPY mouse line, an easy to manipulate in vivo model for further investigations of regulation, expression and function of human TSPY is available.

P 232

Searching for maternal uniparental disomy of chromosome 11 (UPD11) in Silver-Russell syndrome patients

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Silver-Russell syndrome (SRS) is characterised by pre- and postnatal growth restriction (PNGR) and a characteristic small, triangular face accompanied by other dysmorphic features including fifth finger clinodactyly and skeletal asymmetry. The syndrome is genetically and clinically heterogeneous and various genetic findings have been associated with SRS. However, only chromosomes 7 and 17 have been consistently implicated in SRS patients. In up to 10% of SRS maternal uniparental disomy for chromosome 7 has been demonstrated. As SRS seems to have a heterogeneous background other chromosomes may also be involved. We focused on chromosome 11 as a possible candidate region in the aetiology of SRS on the basis of the following observations: Firstly paternal UPD (11p15) is associated with Beckwith-Wiedemann syndrome (BWS) resulting in overgrowth. Furthermore three cases of maternal duplication of 11p15 were recently described in association with growth retardation, two of which showed a SRS-like phenotype.To elucidate whether maternal UPD11 is a common finding in growth retardation and SRS, we screened 46 SRS patients and their parents for mUPD (11). Hereby we focused on the region 11p15 in which the BWS critical region is localised. Six microsatellite markers between the markers D11S922 and D11S1758 were analysed by fluorescent PCR and subsequent gel electrophoresis. Data were evaluated by using the ABI377 DNA sequencer. No case of maternal UPD (11) could be detected in any of the 46 cases by the use of the aforementioned methods. However, partial UPDs of other regions of chromosome 11 could not be excluded. Nevertheless, UPD of the chromosome 11 region which appears to be involved in growth is not a major cause for SRS.

P 233

Dysproportional growth retardation and multiple bone dysplasias in three different highly consanguineous sibships

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In the course of population genetic studies on the island of Nias/North Sumatra/Indonesia we came across familial disproportionate short stature and bone dysplasias such as mid-face hypoplasia, scoliosis, bowing of the long bones, rhizomelic shortening of the arms, incomplete elbow extension, and brachymetapodia. As expected in a highly inbred family of more than 100 members we could also find the same malformations in both sexes in sibs of two other distant branches. This suggests it to be an autosomal recessive entity. Two of the 3 sibships could be studied with 4 of 7 and 4 of 5 affected individuals, respectively. Within a sibship phenotypic expression is quite similar but differs in some aspects in the other sibship. Mid-face hypoplasia is common in all affected probands of both sibships whereas in the second sibship there was no scoliosis, growth retardation was less and the hands and feet were milder affected. This is compatible with endogenic and/or exogenic modifiers. A genome wide scan is in progress.

P 234

Identification of the second mutation in the grey lethal gene in human malignant infantile osteopetrosis

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Malignant infantile osteopetrosis is characterized by osteosclerosis, hepatosplenomegaly and pancytopenia, becoming apparent during the first months of life. The pathophysiology is due to an osteoclast dysfunction leading to an inadequate bone resorption. Mutations have been found in the human a3 subunit of the H+-ATPase and the voltage-gated chloride channel CLCN7. In a small inbred family with malignant infantile osteopetrosis no mutation was found in the a3 subunit of the H+-ATPase nor in CLCN7. In this family, we excluded several candidate gene loci which in mice cause severe osteopetrosis by linkage analysis, among them c-FOS on 14q24, c-SRC on 20q11, OPGL on 13q14, CSF-1 on 1p13. We also investigated a locus on chromosome 6q21 flanked by the genes Fyn and Ros1, which is syntenic to a region of mouse chromosome 10, where the causative gene for the osteopetrotic mouse grey lethal has been mapped. In our family, we detected homozygosity for a region spanning approximately 15cM between the markers D6S1717 and D6S287. Interestingly, the comparison of the grey lethal region between both species revealed an inversion on the human locus putting the human grey lethal locus centromeric to Fyn. We conducted a mutation analysis on several functional candidate genes in this critical region, which revealed no causative alterations. Recently mutations in a novel gene of unknown function in this region were described in the grey lethal mouse and in one human family. Based on the published data, we performed a mutation analysis in all six exons and the corresponding intron/exon boundaries of the grey lethal gene. A homozygous two-base pair deletion was found in the child in exon 2, both parents were heterozygous for this deletion. In summary, we describe here the identification of the second human family carrying a mutation in the grey lethal gene responsible for autosomal recessive malignant infantile osteopetrosis.

P 235

Is intrauterine growth modulated by variations in the insulin gene variable number of tandem repeats locus and a variation in the gene for insulin-like growth factor-1?

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Background: Intrauterine growth retardation (IUGR) affects approximately 10% of all newborns. Associations between IUGR and increased risks for type 2 diabetes and cardiovascular disease later in life are well established. Insulin and insulin-like growth factors 1 and 2 (IGF-1 and-2) are essential intrauterine growth factors. Common genetic variations in their encoding genes might explain the association between low birth weight and the predisposition for metabolic syndrome. An association between

birth weight and genetic variations in the insulin (INS) gene variable number of tandem repeats (VNTR) locus has been described. Newborns with genotype III/III, which is present in 10% of all Caucasians, were shown to be longer and heavier at birth (Dunger, Nature Genetics 1998). In vitro, it could be shown that the length of the INS VNTR locus influences transcription of INS, which might explain this association (Bennett, Annu Rev Genet 1996). Absence of the wild-type allele of a polymorphism in the promotor region of the IGF-1 gene is associated with low birth weight (Vaessen, Lancet, 2002) as well as with diminished insulin-secreting capacity, and an increased risk of type 2 diabetes and myocardial infarction (Vaessen, Diabetes 2001). Aims of the study: We thus investigated in 1400 six-year-old Caucasian children living in the region of Giessen, Germany, whether an association exists between birth weight and the polymorphisms in the INS VNTR region and the IGF-1-promotor gene.The secondary aim of the study was to investigate whether children with IUGR show an increased rate of obesity and an increased blood pressure as early symptoms of metabolic syndrome. Furthermore we aimed to study whether there is an association between these symptoms and the polymorphisms under study. As the datasets of the questionnaires and the genotype information are currently transferred to the database, the results of this study will be presented at the meeting.

P 236

Relationship between different clinical diagnoses of short stature: implications for genetic studies

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Short stature is a common concern in pediatric practice and stature is known to be highly heritable (heritability approx. 80%). Constitutional delay in growth and puberty (CDGP, defined via small stature - less than 3rd age- and sex-specific percentile -, bone age retardation of at least one year and the absence of any other specific underlying disorder), familial short stature (FSS, short stature and lower confidence limit of adult target height as determined from parental heights also below 3rd percentile) and idiopathic short stature (ISS, short stature, no underlying disorder) are considered normal variations in growth and seem to cluster in families. To investigate whether these clinical definitions represent useful entities for molecular genetic studies, we retrospectively collected clinical and anthropometrical data on 304 children and their parents who presented because of short stature at the endocrinological out-patient unit of the University Childrens Hospital Gießen in 2000 - 2002. We find that 24.5% of these children were diagnosed with CDGP, 7.5% with FSS, 29.5% with both and 8.2% with ISS. When comparing the two differentiating features, bone age retardation (CDGP vs. FSS and ISS) and target height (FSS vs. CDGP and ISS), we do not find bimodal distributions which would be expected if the conditions are clearly distinct. In the CDGP and ISS groups, parental heights are mostly just above the threshold for FSS and in the FSS and ISS groups many children also have delayed bone age, but less than one year. Growth and pubertal development of parents were not different in the groups. The large overlap of 29.5% with CDGP and FSS also underlines the close relationship between these conditions. We conclude that the clinical differentiation as based on the thresholds for target height and bone age retardation does not reflect a truly different underlying etiology. Based on these retrospective analyses, we decided to use an affected sib pair design and prospectively recruit CDGP, FSS and ISS patients in order to perform a linkage genome scan scheduled for the end of 2003 in an attempt to identify chromosomal regions and subsequently genes related to growth and development. Detailed physical examination and laboratory tests are used to exclude any other disorders underlying the short stature (such as growth hormone deficiency, Turner's syndrome, dystrophy). For inclusion, one sib has to be below the 5th height percentile, the other below the 15th. Bone age and data on body weight and eating behavior are collected as secondary phenotypes. Acknowledgements: We thank the patients and their families for their participation. This study is supported by the National Genome Network (NeuroNetz Marburg; BMBF).

P 237

Two-step inactivation of the tissue non-specific alkaline phosphatase (ALPL) gene during progression of meningiomas by deletion plus promotor

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Microscopic deletions of chromosome 1p including bands 36.1-34 and loss of expression of tissue non-specific alkaline phosphatase (also called liver/bone/kidney-type ALPL; gene located on 1p36.1-34), i.e., inactivation of both alleles in the tumor tissue are strongly associated with progression of meningiomas, indicating a growth-suppressing function of ALPL or in combination with a closely linked gene in normal meninges and low-grade meningiomas. We investigated different possible mechanisms for the somatic inactivation of this enzyme. One possibility is inactivation by mutation of the ALPL gene. ALPL sequencing showed indeed a mutation known from hypophosphatasia patients in 1 out of 10 meningiomas. A second possibility is down-regulation of ALPL transcription by DNA methylation. The ALPL gene has two promotors (liver-type and bone-type) which are active in a tissue-specific manner. In all analyzed meningiomas only the bone-type ALPL gene promotor, which is CpG-rich, was active. CpG-rich promotors can be regulated by DNA methylation. By high-resolution bisulfite genomic sequencing of six meningiomas, we found elevated levels of methylation in the meningioma-specific bone-type ALPL gene promotor. In primary cell cultures of meningiomas treated with 5-azacytidine - an inhibitor of DNA methylation - for 6 days, we found increased amounts of ALPL transcripts, supporting the notion of in vivo down-regulation by promotor DNA methylation. We conclude that the tissue non-specific alkaline phosphatase gene can be somatically silenced in a two-step process through deletion of one allele plus methylation of the second allele, constituting a crucial step in the progression pathway of meningiomas.

P 238

Identification of a novel retina-specific gene, C12orf3, encoding a putative transmembrane protein.

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Age-related macular degeneration (AMD) is a degenerative retinal disease of multifactorial genesis ultimately causing severe, irreversible loss of visual acuity. To date, little is known about the genetic factors conferring susceptibility to AMD. In our effort to identify candidate genes, we cloned C12orf3, a novel gene of 26 exons localized to chromosome 12p13.3. Quantitative reverse transcriptase (qRT)-PCR and Northern blot analysis demonstrate that this gene is exclusively expressed in the retina suggesting an important role of this protein in the retinal tissues. The 3950 bp mRNA of C12orf3 is coding for 1002 amino acids with a calculated mass of approximately 114 kDa. Transmembrane prediction programs suggest a secondary structure with eight transmembrane helices. Searches in protein databases identified at least three hypothetical proteins with high similarity to C12orf3 indicating the presence of a novel family of transmembrane proteins. As a first step to address functional aspects of C12orf3 and its possible implication in retinal disease, we first aimed to establish the cellular and subcellular localization of C12orf3. Towards this goal, polyclonal antisera were raised in rabbits and mice immunized against fusion proteins representing various C12orf3 epitopes. Sera have been used for Western blotting analysis as well as for immunocytochemical studies. In addition, heterologous expression of C12orf3 in 293-EBNA cells facilitated the subcellular localization of the protein. Immunofluorescence microscopy on mouse eye sections show that C12orf3 is expressed mainly in both rod and cone outer segments. This indicates that C12orf3 may play a role in the structural organization of the photoreceptors or, alternatively, may be involved in the phototransduction pathway.

P 239

Expression analysis of DNA-repair related genes in Fanconi anemia fibroblasts using a medium density cDNA microarray

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Microarrays can be used for simultaneously quantifying the expression of multiple genes in parallel, thus providing the basis for identifying genes that are differentially expressed in distinct cell types, developmental or disease stages. We have developed a specialized cDNA microarray with approximately 500 genes involved in the different DNA repair pathways. This DNA repair chip allows us to monitor the mRNA expression levels in a complex disorder such as Fanconi anemia (FA). FA-patients are characterised by congenital abnormalities, bone marrow failure, and a high risk of developing acute myeloid

leukaemia and squamous cell carcinomas. Seven FA genes have been cloned, and some or all of their products were found to interact with well-known DNA damage response proteins, including BRCA1, ATM, BLM and NBS1. Thus, FA proteins are likely to be involved in cell-cycle checkpoint (s) and DNA repair. The cellular sensitivity of FA cells towards DNA crosslinking agents and the discovery of BRCA2 as a bone fide FA-gene suggest a role for FA proteins in homologous recombination-mediated repair. Disruption of the FA pathway results in chromosome instability, a cytogenetic hallmark of many human cancers. To evaluate the effects of FA mutations on DNA-repair gene expression, the mRNA expression patterns of fibroblasts from six different FA complementation groups were analysed. The gene expression patterns were found to differ between complementation groups, suggesting separate or additive roles of the various FA-proteins in specific DNA repair pathways.

P 240

Towards the functional analysis of innate host defense peptides on human chr. 8p23

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Defensins (DEF) are small disulfide-bridged, cationic peptides contributing to innate host defense of higher organisms against microorganisms and enveloped viruses. They are characterized by typical disulfide patterns, and classified as alpha- and beta-defensins (DEFA, DEFB), and the cyclized rhesus-theta-defensin (RTD). Five alpha- (DEFA1-4, HE2/EP2), four beta-defensin genes (DEFB1-4), and RTD pseudogenes are clustered on human chr. 8p23, we have characterized on two contigs of 0.7 and 0.3 Mb (http://genome.imb-jena.de).The defensins permeabilize cell membranes mediated by the positively charged and amphipathic peptide backbone. However, they show little primary structure conservation, that presumably accounts for their high variation in antimicrobicidal capacity and in salt-resistance. By random and site-directed mutagenesis of recombinantly expressed DEFB3 (1, 2) we have constructed variants with a different relation of positive charges and hydrophibicity in the DEFB3 backbone, which are tested against E. coli, Pseudomonas aeruginosa, Salmonella spec., and Staphylococcus aureus to understand the structure-function-relationship of the broad-spectrum antimicrobicidal activity and salt resistance.

P 241

Extended in vitro lifespan of porcine hepatocytes by transfection with the gene for the human catalytic subunit of telomerase reverse transcriptase

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As in vitro maintenance of primary hepatocytes is difficult the availability of liver cells is limited. Improvement of the expansion of hepatocytes in cell culture would provide a new source of liver cells for several downstream applications. It has been shown for some human somatic cells that the reconstitution of telomerase only resulted in infinite proliferation without oncogenic transformation. Thus, as an initial model, we tried to stimulate the proliferation of porcine hepatocytes by ectopic expression of the gene for the human catalytic subunit of telomerase reverse transcriptase (hTERT). For that, porcine primary hepatocytes were obtained by perfusion the liver with collagenase after total hepatectomy. The cells were transfected in several attempts with hTERT cloned in an eukaryotic expression plasmid. In two independent approaches transfected cells still proliferate since now 5 months under specific culturing selection whereas the non transfected control cells died in the meanwhile. Determination of the telomerase activity by TRAP assay revealed active telomerase in these cells in comparison to untransfected control cells. The maintenance of the hepatocytic character and the telomere lengths are currently under investigation. The establishment of immortalized hepatocytes by ectopic expression of telomerase without oncogenic transformation could be helpful for the development of a human bioartifical liver.

P 242

Functional characterisation of the nuclear localisation signal in the bHLH transcription factor TWIST

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TWIST, a bHLH transcription factor, regulates its target genes as a heterodimer with other HLH proteins. Mutations in the TWIST gene are described to cause the Saethre-Chotzen syndrome (SCS; MIM #101400), an autosomal dominant craniosynostosis syndrome. It ischaracterised by the premature fusion of coronal sutures which leads to skull deformation, accompanied by limb abnormalities of variable severity and facial dysmorphisms.Several domains of the TWIST protein, i.e. the glycin-rich domain, the basic domain and two conserved classical NLS motives (NLS1 and NLS2), have previously been discussed as possible nuclear localisation signals. In an immunofluorescence assay, several myctagged deletion constructs of the TWIST protein were analysed in order to determine, which of the mentioned motives are responsible for nuclear localisation/import. Human U2OS osteosarcoma cells were transiently transfected with the generated deletion constructs. The transfection experiments indicated that the region upstream of NLS1 is not necessary for nuclear localisation. The other constructs, however, were localised in the cytoplasm. In order to analyse the functionality of the classical nuclear localisation signals in more detail, NLS1 and NLS2 were destroyed by site-directed mutagenesis. Transfection experiments were performed again. Only NLS1 seems to be essential for nuclear transport of TWIST. Subsequent cotransfection analysis of the NLS1-mutated construct and E12 - a putative heterodimerisation partner – led to a compensation of the mislocalisation: nuclear localisation of TWIST could be observed in spite of the non-functional NLS1. Therefore we conclude that there is a co-import of TWIST and E12 as a heterodimer using the functional NLS of E12.

P 243

Identification and Characterization of the Human OSF-2-Promoter as a Potential Target for Regulation by TWIST

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We have identified the human homolog of the mouse Periostin Gene by BLAST searches and literature research. The homology of the human and mouse genes was subsequently confirmed by alignments of proteins, mRNA and the promoter sequences of both the human and the mouse gene. Two pieces of different lengths of the human OSF-2 promoter – one 1000 bases upstream of the mRNA start, the other 500 bases upstream of it – were cloned into pGL3-Basic, and the effect of cotransfection with a TWIST overexpressing vector carrying a CMV-promoter or an Actin-C5-promoter – depending on the cell line – was measured by dual luciferase assay in OHS, a human osteosarcoma line, and SL-2, a Drosophila embryonic mesoderm-derived cell line, the latter being used to prevent unspecific background effects found in human systems. The results indicate that OSF-2 is upregulated by TWIST after at least 48 hours of incubation if cells are not confluent. In all other cases, we see a downregulation as it is usually reported for TWIST. By a consensus search (CANNTG) we have found four potential E-Boxes in the putative promoter area of OSF-2, at least one of which we suspect to be a TWIST box. Site-directed mutagenesis was performed to generate point mutations in all four potential E-Boxes. Luciferase assays with the mutated promoters show an effect of some mutations on regulation by TWIST. Furthermore, cotransfection with overexpressed TWIST and E12 showed a very strong downregulation on the Actin-C5 promoter, but only if both Proteins are overexpressed. If an OSF-2 promoter was cotransfected with those two Proteins, this downregulation was strongly inhibited, likely due to sequestration effects of the OSF-2 promoter, which further identifies it as a potential target for the binding of TWIST heterodimers.

P 244

The homoallelic complementation of the Lys423Glu MYOC mutation is resembeld by a protective effect in heterologous expressing insect cells

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Mutations in the MYOC gene are responsible for about 3.5% of juvenile open angle glaucoma (JOAG) patients. A large glaucoma pedigree, harbouring the Lys423Glu mutation in homo- and heterozygous state was reported. Surprisingly the homozygous carriers do not develop a glaucoma, but the heterozygous ones. Hence it was interesting to investigate possible effects of this mutation compared to others in homo- and heterallelic conditions on a cellular level. The biological function of MYOC remains unknown, but a transmembrane domain and some adhe-

sive properties were predicted. Since Myelin Protein Zero (P0) belongs to the adhesion molecules, the P0 expressing insect cells was consulted as a reference expression system. We have investigated the effects of hetero- and homoallelic expression of different mutant forms of MYOC in insect cell lines. Cultured cells were cotransfected with wildtype MYOC-GFP and mutant forms of MYOC not fused to a reporter gene. An insect cell expression vector (pIB/V5-His, Invitrogen) was modified (pEXIV) to express the mutation Lys423Glu in frame with GFP, while a second one was constructed to determine any unexpected effects of GFP on the expression. A Gln368stop and a Pro370Leu MYOC mutation have been investigated similarly. An adhesion test performed with cells cotransfected with wt-MYOC and the 3 mutated constructs did not lead to any increased cell-cell adhesion compared to cells expressing P0, the peripheral myelin „glue". Using fluorescence microscopy the mutation carrying cells revealed the same intracellular localization in the cytosol and vesicles as the MYOC wildtype. An MYOC accumulation in these vesicles followed by a cell death was observed. Surprisingly the cells expressing the Lys423Glu mutation homoallelic showed a several hours retarded entrance of the cells (trypan blue uptake) compared to the overexpressed wildtype. This indicates a reduced cytotoxicity of this MYOC variation on the cells. Retarded cell death due to Myocilin accumulation in cytosolic vesicles indicates that the mutated protein has indeed a protective effect supporting the hypothesis of homoallelic complementation in glaucoma patients.

P 245

Gene expression in superficial bladder carcinoma

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This study was designed to combine gene expression profiling with analysis of genetic alterations in superficial transitional cell carcinoma (TCC) of the urinary bladder. Ten patients underwent transurethral resection (TUR) of a pTa/pT1 GI-III-tumor. We analyzed gene expression profile and DNA microsatellites both in tumor and mucosa specimens. Each tumor specimen was compared directly with its mucosal counterpart. Specimen DNA and RNA were prepared simultaneously using trifast reagent kit (MRC Inc.). Ten microsatellite markers were analyzed using PCR and fluorescence-based detection system ABI 310 and GeneScan software (Applied Biosystems). Expression data were obtained by using hybridization of radioactively labelled specimen cDNA to ATLAS HumanCancer 1.2Array (Clontech) representing 1176 tumor-related genes. In six out of the ten patients we found at least one microsatellite alteration like loss of heterozygosity (LoH) or allelic imbalance (AI). Combined statistical analysis of the ATLAS-arrays revealed 77 differentially expressed genes, with 48 of them being up-regulated and 29 down regulated in tumor. Among the latter ones there were several transcripts of proteins of extracellular matrix including collagens 6, 8, 11, 16, and 18. Preliminary cluster and tree analysis of the expression data separated two major branches, the first one grouping together all but two of the tumors and the second one containing all mucosa samples in addition to two tumor samples. Interestingly, these two tumor samples were among the four samples without microsatellite alterations. Therefore, we cannot exclude that these two samples were not suitable for molecular analyses due to a reduced proportion of tumor cells in specimen. The clustering of the two other microsatellite-negative tumor samples to the tumor branch suggests that the number of microsatellites should be increased in order to improve the diagnostic power of this method. There were some physically closely neighboring genes down regulated in tumors suggesting the possibility of a loss of at least one allele, respectively due to chromosomal deletions. This could be further investigated by detailed microsatellite analysis of the suspected chromosomal regions. [Supported by DFG, FR 1397/2-1; correspondence: finckh@uke.uni-hamburg.de]

P 246

A new array-based diagnostic tool for detection of uniparental disomy?

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Uniparental disomy (UPD) occurs when an offspring receives both copies of a particular chromosome or part of a chromosome from only one of its parents. Recent studies have revealed that problems arise if the transmitted chromosome or part of a chromosome contains the so-called "imprinted genes". The imprinted allele is the one switched off and the term "genomic imprinting" describes a process underlying differences in expression of a gene according to its parental origin. UPD may lead to an imbalanced expression of these imprinted genes and cause an abnormal phenotype. To date, UPDs with a phenotypic relevance are described for different chromosomes. Among them are Prader-Willi syndrome (maternal UPD15), Angelman syndrome (paternal UPD15) and Silver-Russell syndrome (maternal UPD7) which are classical examples of UPD related to imprinted genes. However, it is likely that additional not yet identified UPD phenotypes exist. A whole genome search for UPDs is time consuming and laborious. We thought to use the oligonucleotide microarray analysis to approach the problem of whole genome UPD search. As a first step, we therefore analyzed single nucleotide polymorphisms (SNPs) in six families (child and mother/father) where the child was already diagnosed of having Prader-Willi syndrome, Angelman syndrome or Silver-Russell syndrome. Oligonucleotide microarray analysis was performed according to manufacturer's instructions by using the Affymetrix HuSNP-Arrays. The HuSNP Mapping Array is designed to interrogate approximately 1500 SNPs distributed throughout the whole human genome. On average, 4-5 SNPs were informative for detection of UPD in the way that the child and the parent under study are differently homozygous for one allele. We also detected on average two false positive SNPs per family, which we could confirm as being false positive signals through direct sequencing. Although the system needs to be re-evaluated this new, array-based tool is extremely fast compared to classical diagnostic methods and data are completed within 3-5 days. Improvement of the number of significant signals will shorten the time required to diagnose a UPD.

P 247

Identification and characterization of candidate genes from chromosomal region 1p22 in mantle cell lymphomas

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Aims: Overexpression of cyclin D1 due to t (11;14) is not sufficient for the development of mantle cell lymphomas (MCL). Thus, secondary genetic alterations are necessary for the malignant transformation. They may also trigger the progression into blastic variants and determine the outcome of the disease. This project focuses on the delineation of deletions on 1p22, frequent secondary changes in MCL, and on the analysis of functionally interesting candidate tumor suppressor genes mapping to this region. Methods: To detect the exact borders of losses in 1p22 we used the technique of quantitative microsatellite analysis (QuMA). The ensembl database (Sanger Institute) was screened for functionally interesting genes, present in the 1p22 region. Genomic DNA from 15 MCL patients (5 with a known deletion of 1p22 and 10 with an unknown karyotypic status) was used to screen for mutations leading to the inactivation of the second allele by direct sequencing. Methylation-specific PCR was performed on bisulfite-modified DNA from these 15 MCL patients to check the methylation status of the promoter region of interesting candidate genes. Results: The commonly deleted region of 1p22 contains a putative tumor suppressor gene, SH3GLB1, functionally interesting due to its interaction with the apoptosis-inducer Bax. In two cell lines GRANTA-519 and NCEB-1 as well as in 15 primary cases of mantle cell lymphomas, no mutations were detected. Methylation of CpG islands as the mode of inactivation could not be excluded since the promotor region could not be amplified, in most likelyhood, due to a very high GC content. QuMA on 58 MCL cases highlights one region of loss affecting locus AC092392 containing LMO4, a gene which is known as a candidate tumor suppressor in neuroblastoma. Conclusions: Loss of only one copy of SH3GLB1, i.e. haplo-insufficiency, may itself play a pathogenic role in MCL and induce tumor progression into blastic variants, in which deletions of 1p22 occur in approximately 40%. Screening of further genes mapping to the delineated region for inactivating mutations, including LMO4, is under investigation.

P 248

On testing causality of genotype data for binary traits in incomplete nuclear families

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In genetic data sets, observations of alleles can be highly correlated on account of high linkage disequilibrium (LD) and therefore hamper the effort to pinpoint causal loci influencing some phenotypic trait. We exploit the interesting fact that conditioning on genotypes the phenotypes of parent-offspring pairs are uncorrelated whenever a causal locus is observed. The dependence structure induced by the genetic mechanism is analyzed in detail. The corresponding likelihood can be generalized to more extensive pedigrees.A likelihood-ratio test is presented to test hypotheses about the conditional correlation as quantified by the LD parameter \delta of an allele at an observed candidate locus and an allele at a linked unobserved causative locus. The statistical properties of the test are studied by simulations. Power turns out to be excellent for many common situations (e.g. allele frequencies 0.3 at both loci, LD: \delta`=0.4, penetrance: f=0.8 result in power of 0.97 with just 80 pairs). Applications of this method include fine mapping efforts and the assessment of contributions of individual polymorphisms to traits. Additionally the method can be used to reconstruct haplotypes in incomplete or ambiguous nuclear families.

P 249

Molecular genetic analysis of autism

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Autism is a complex neurodevelopmental disorder with an onset in early childhood, characterized by impaired social and communication skills and patterns of repetitive and stereotyped behaviours and interests. A geneticetiology is strongly indicated by twin and family studies with a risk to siblings of idiopathic cases which is 50-100 times greater than the general population prevalence of 5/10,000 births. In order to identify autism susceptibility genes we have currently collected 259 patients from 213 families throughout Germany and Austria available for candidate gene screening, while 150 complete trios are available for association studies. In addition, IMGSAC has collected approximately 250 families with more than one child or relative affected. The latest genome-wide linkage screen by IMGSAC identified four autism susceptibility regions including chromosome 2q, 7q, 16p and 17q, three of which have been replicated by other groups. Coding regions of several candidate genes in these regions have been systematically screened with DHPLC (denaturing high performance liquid chromatography), SSCP and sequencing by us and IMGSAC (e.g. WNT2, WNT16, TAC1, RELN, PEG1/MEST, gamma2-COP, CPA1, CPA5, FOXP2, cAMP-GEFII). Most of the variants detected have been polymorphisms or did not co-segregate with the autism phenotype in the families. The low frequency of the remaining number of mutations segregating with autism does not explain the relatively strong linkage results on 7q and 2q. Therefore, analyses of genes will be extended also to intronic and 5' and 3'UTR sequences to search for alternative mechanisms possibly involved in the etiology of autism. Furthermore, we started a proteomic approach by antibody profiling of plasma probes from more than 70 autistic patients to investigate the possible influence of immune reactions in the disease.

P 250

Chromosome 3p and genetic susceptibility to sarcoidosis

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Sarcoidosis is a complex disease of systemic inflammation, affecting a wide variety of organs, primarily the lung and lymph system. It is characterised by enhanced T helper 1 cell activity and non-caseating granulomas. The aetiology of sarcoidosis is unknown, presumably based on the interaction of environmental triggers and an inherited susceptibility of the patient. A major contribution to the genetic predisposition is assumed to be linked to the major histocompatibility complex on chromosome 6p. However, additional susceptibility genes are most likely involved. The short arm of chromosome 3 with non-parametric linkage scores of up to 2.39 (p=0.009) in a previous low density genome scan appears to be a promising candidate chromosomal region. This segment harbours functional candidate genes coding for interleucin 5 receptor, a cluster of chemokine receptors, macrophage stimulating protein and its receptor, and toll like receptor 9. We have genotyped extended panels of patients (complex families, affected sib pairs and single patients plus parents / trios) suffering from sarcoidosis for 34 microsatellites and for intragenic polymorphisms of the named functional candidate genes. Nonparametric linkage calculations and family based association analyses have been conducted by use of Genehunter 2.0. From our results, the D3S3624 to D3S1300 interval of approximately 10 cM appears to be the most interesting target for on-going detailed studies

P 251

Group sequential study designs in genetic-epidemiological case control studies

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In the past years, the focus of genetic-epidemiological studies has shifted to the analysis of complex diseases. Here, a single gene often contributes only little to the manifestation of a trait; hence, many patients have to be included in a study to reliably detect small effects. To reduce the number of required phenotypings and genotypings in a study and thus facilitate the analysis of complex traits, sequential study designs can be applied. In this presentation, several sequential procedures that have been suggested for use in genetic-epidemiological settings are described an compared. Formal group sequential procedures fare best with regard to error rates, efficiency, and practical applicability.

P 252

Evolution of a non-channel vertebrate protein superfamily involved in epilepsy

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Until recently, all genes found to be mutated in idiopathic epilepsies coded for subunits of ion channels. The aetiology of these epilepsies could be directly related to the impaired electric transduction. Two exceptions to this rule were identified last year. The human LGI1 gene is mutated in ADLTE (autosomal dominant lateral temporal lobe epilepsy) and the MASS1 gene is mutated in the Frings mouse model of audiogenic epilepsy. The human orthologue of MASS1 was also found to be mutated in febrile seizures. Neither gene apparently codes for an ion channel, pointing to a formerly unknown mechanism of epilepy aetiology.Sequence analysis revealed a novel sequence motif EPTP common to both LGI1 and MASS1, defining them as a new protein superfamily. LGI2, LGI3 and LGI4, the three paralogues of human LGI1, harbor the same EPTP motif and also belong to this superfamily. We cloned five formerly unknown Danio rerio homologues of the LGI genes (dLGI1- dLGI5) and one homologue of MASS1 gene (dMASS1) via RT-PCR using degenerated primers. The zebra fish genes and their human counterparts share 60-80 % similarity between their predicted protein sequences. The fact that there are five zebra fish LGI genes but only four human counterparts is probabaly another example of the observation that multigene families in teleosts often have more members than their mammalian counterparts. One possible explanation for this phenomenon is that the alternative splicing and differential regulation of gene expression in mammals compensates for the fewer gene copies. In situ hybridization experiments demonstrated the expression patterns of the zebra fish EPTP superfamily genes in different developmental stages. Additional database analysises were performed using the genomic sequence database of Xenopus laevis, Drosophila melanogaster, Caenorhabditis elegans and the Tunicate Ciona interstinalis, the latter being the the most primitive chordate sequenced today. These database analysises clearly demonstrated that the EFTP superfamily is of vertebrate origin.

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Functional analysis of the Cl—channel ß-subunit Barttin

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Renal salt loss in Bartter`s syndrome is caused by impaired transepithelial transport in the loop of Henle. NaCl is taken up apically by the combined activity of NKCC2, Na-K-2Cl cotransporters and ROMK K+ channels. Cl- exits from the cell through basolateral ClC-Kb channels. Mutations in the three corresponding genes were identified in Bartter syndrome types 1-3. The recently identified gene encoding the integral membrane protein Barttin is mutated in a form of Bartter syndrome that is associated with

congenital deafness and renal failure. Barttin acts as a an essential ß-subunit for ClC-Ka and ClC-Kb with which it co-localises in basolateral membranes of renal tubules and of K+-secreting epithelia of the inner ear (Estévez et al., Nature. 2001 Nov 29;414 (6863):558-61). ClC-K/Barttin heteromers are crucial for renal salt reabsorption and inner ear K+ recycling.ClC-K currents can be further stimulated by mutating a tyrosine-residue on Barttin in position 98 to alanine. This region of the protein contains a putative PY-motif and/or a tyrosine-based sorting motif. The analysis of this region provides further insight into the regulation and proper function of ClC-K/Barttin complexes.

P 254

Molecular identification of genes involved in skeletogenesis - potential candidates for osteoarthritis

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Our project aims at the identification of genes and pathways involved in the complex processes of cartilage/bone formation, growth, differentiation and homeostasis. The goal is to isolate a number of candidate genes for inherited monogenic (skeletal dysplasias) as well as for muligenic disorders with specific attention to osteoarthritis (OA). A main aspect of OA seems to be an imbalanced cartilage matrix turnover, and a deeper understanding of molecular events within the chondrocytes - as the cells of this tissue - will provide not only a better understanding of pathogenetic mechanisms but should also help to identify new diagnostic markers and cellular targets for therapeutic intervention.To monitor the entire spectrum of genes expressed during the OA disease process the cDNA array technology is a powerful tool. As member of a BMBF supported project („Molecular medicine as diagnostic and therapeutic approach to study osteoarthritis“) we have access to microarray experiments identifying differentially regulated genes in osteoarthritic cartilage. Besides a number of known and well-characterized genes there are a multitude of genes without cartilage or bone specific functional data. We have focused our interest on this group of genes which now are been characterized by light cycler analysis. Six genes show significant upregulation in murine chondrocytes. To further elucidate their role in skeletogenesis we will determine the cartilage specific expression by in situ hybridization. The analysis of these genes might provide clues to specify pathogenetic processes and pathways essential for the development of OA.

P 255

Molecular diagnostics in hypertrophic cardiomayopathy: development of a rapid screening assay for TNNT2 mutations

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Hypertrophic cardiomyopathy (HCM) is characterized by ventricular hypertrophy, disarray of myocytes and fibrosis. Arrhythmias and sudden cardiac death are common, and HCM often results in heart failure. HCM is transmitted as an autosomal-dominant trait and exhibit substantial inter- and intragenic heterogeneity. Most HCM mutations are missense mutations or short in-frame deletions in at least 11 different genes, almost exclusively sarcomere protein genes. Penetrance and expressivity varies widely and only in part depends on the specific mutation. Some mutations carry a high risk of malignant arrhythmias (so called "malignant" mutations). Mutations in beta-myosin heavy chain (MYH7) or cardiac troponin T (TNNT2) are found in 50-70% of cases. Routine genetic screening of HCM patients for mutations in the mostly involved genes provides important diagnostic and prognostic information.Molecular diagnosis by complete sequencing aiming to identify the causative mutations is time-consuming and labour-intensive. In this study we report the successful use of DHPLC to analyse rapidly the complete coding sequence of the TNNT2 gene. In our screening protocol all TNNT2 amplicons were adjusted to one common PCR condition. Amplicons of HCM patients together with controls were analysed in one run with suitable DHPLC protocols followed by direct sequencing of aberrantly migrating fragments. This genotyping platform was tested on 56 samples of a pre-determined genotype, which had been previously identified by direct sequencing. By this analysis it was proved that this genotyping technique is a sensitive and fast method for the detection of TNNT2 mutations.Finally, we report novel mutations in the TNNT2 gene identified in patients with HCM.

P 256

Balanced chromosomal rearrangements and late-onset diseases - a survey among carriers

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Adult carriers of balanced chromosomal rearrangements (BCRs) are usually healthy. There are, however, numerous reports of families in which BCRs co-segregate with late-onset diseases, e.g. schizophrenia or maturity-onset diabetes. An increased frequency of BCRs has recently been described among patients with Amyotrophic Lateral Sclerosis (Meyer et al., Neurology 2003, and abstract by Dullinger et al.). Therefore, a survey among adult carriers of BCRs to investigate possible associations between chromosomal breakpoints and late-onset diseases was initiated. We send out questionnaires to carriers of balanced chromosomal rearrangements above the age of 18 years who were healthy at the time of chromosome analysis. In these questionnaires we inquire about all aspects concerning health and disease, particularly focusing on late-onset diseases such as neurodegenerative, cardiovascular, and metabolic diseases, as well as cancer and fertility problems.We thus aim to find associations between chromosomal loci and susceptibility for late-onset diseases. Such an association is likely either if a breakpoint co-segregates with a disease in a family; or if it lies in a region mapped by linkage and/or association studies; or if there is a significant association of a breakpoint with the same disease in many unrelated individuals. Probands will then be asked to give blood for a molecular cytogenetic breakpoint analysis which might reveal genes with disturbed expression at the breakpoints.This survey has been successfully undertaken in Denmark (I. Bache and N. Tommerup). In a pilot study in Berlin, 60% of contacted translocation carriers have so far returned the questionnaires. They reported several common disorders such as hypo- and hyperthyroidism, depression, allergy, cardiac arrhythmia, and others. For example, in one patient with psoriasis and a t (2;4) translocation, the breakpoint on chromosome 4 (4q31.1) is located in a region where a psoriasis susceptibility locus has been mapped (Zhang et al, J Invest Dermatol 2002). To increase the number of participating carriers of BCRs, we intend to collaborate with many human genetics institutes and laboratories.

P 257

Construction of a linkage disequilibrium map in a schizophrenia locus on 15q with microsatellite markers

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We earlier reported significant evidence for linkage on chromosome 15q15 in periodic catatonia (PECA), a sub-phenotype of schizophrenic psychoses. Recently we were able to replicate this initial finding in a second independent set of families. Linkage and haplotype analysis in a set of exceptionally large multiplex families each showing linkage to this locus revealed an 11 cM critical region. In order to aid the positional cloning effort, we set out to construct a linkage disequilibrium (LD) map of this region using 57 microsatellite markers.We genotyped these markers in 179 individuals from 44 families, 27 trios and 17 extended families with PECA. Next we determined phase using Simwalk2 and extracted the non-transmitted haplotypes. We assessed LD using HaploXT from the GOLD software package. We detected significant LD (D'>0.5; p<0.05) for 22 marker pairs. Six of them had significant values of p<0.0001 and chisq>68 for distances up to 830 kb. Three marker pairs showed D'>0.6 and strongest LD (D'=0.71) was seen for one marker pair 87kb apart. As expected, LD tended to clearly decline with intermarker distance. LD was significantly higher in the cytogenetic light band 15q15.1 as compared to the adjacent darker bands and correlates with the distributaion of recombination frequencies.A block-like pattern of LD over longer distances interrupted by short stretches without any detectable LD was observed. Although this LD pattern resembles those generally seen for regions with high density SNP genotyping, resolution of LD block structure was imprecise.More dense typing of markers including SNPs is in progress for use in disease oriented LD studies.

P 258

No support for association of the DTNBP1 (dysbindin) gene with schizophrenia in large samples from Germany, Poland, and Sweden

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Chromosomal region 6p24-21 is one of the most consistently replicated susceptibility regions in schizophrenia. Recently, Straub et al. (2002) identified significant associations between single nucleotide polymorphisms (SNPs) within the positional candidate gene DTNBP1 (dystrobrevin-binding protein 1, or dysbindin) on 6p22.3 and schizophrenia in the Irish population. Since then, two replication studies have been published: Schwab et al. (2003) tested six SNPs in two independent parent-offspring trio samples from Germany and obtained confirmatory evidence for association. However, different susceptibility alleles appeared to be present in their sample. The second study by Morris et al. (2003) tried to replicate the finding by Straub et al. in an independent case-control sample of schizophrenia from the Irish population. No evidence was found to suggest an association between the dysbindin gene and schizophrenia in their sample. This was surprising because inter-marker LD of the tested SNPs suggested that the samples of Straub et al. and Morris et al. had been drawn from the same homogeneous population. The major difference between their study and the reports by Straub et al. (2002) and Schwab et al. (2003) was that the case-control sample used by Morris et al. (2003) was not selected for familiality. We have performed an independent replication study in a large sample of 418 schizophrenic cases from Germany that were not selected for familiality and 284 controls. We genotyped five SNPs: P1635, P1325, P1320, P1757, and P1578. According to the odds ratios given by Schwab et al. (2003) this sample had a good power to replicate their finding. Two other, smaller case-control samples were tested, one from Sweden (158 cases/292 controls) and one from Poland (296 cases/115 controls). In all three samples, no significant p-values were observed in single-marker and haplotype analyses. Likewise, no significant p-values were observed in a seperate analysis of the subsamples with a positive family history of schizophrenia and other psychiatric disorders.Our results are in line with the finding by Morris et al. (2003) and might reflect different genetic mechanisms underlying individuals derived from high-density families (as in the study by Straub et al. and Schwab et al.) and cases coming from families less heavily loaded with schizophrenia.

P 259

Linkage disequilibrium analysis in a susceptibility region for bipolar affective disorder on 8q24

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A recent genome-wide screen for linkage to bipolar affective disorder (BPAD) has suggested a new susceptibility locus on 8q24 (Cichon et al., 2001). In our sample comprising 66 German, 8 Israeli, and 1 Italian family with BPAD (n=445 individuals), chromosomal region 8q24 provided the best evidence for linkage: marker D8S514 gave a two-point LOD score of 3.62 and a GENEHUNTER-NPL score of 3.56 (p=0.00029). The positive linkage region is large and extends over approximately 30 cM. We have been performing linkage disequilibrium studies to narrow down the candidate region in two samples: 1) 120 independent trios, partially derived from the linked families; 2) 300 cases and 300 controls. In a first step, eight microsatellite markers (A-H) located in the highest linked region (about 5 Mb) were tested for LD and revealed a region of interest covered by one particular two-marker haplotype (F-G) which was significantly undertransmitted in the trio-sample (p=0.017). The same haplotype was also significantly associated with BPAD in the 300 cases and 300 controls (p=0.026).Database searches indicate that there are only three known genes located in the region covered by the two-marker haplotype F-G. We re-sequenced the coding regions of these genes in a representative, European population-based sample of 80 healthy individuals to determine the naturally occurring variability. The identified SNPs plus non-coding SNPs derived from the public databases were genotyped in the case-control sample. In one of the genes, which covers a genomic region of 30 kb, we identified three SNPs that are significantly associated with BPAD in both single-marker and haplotype analyses. Replication of these findings in the independent trio sample as well as genotyping of additional SNPs in this particular gene is underway.

P 260

Periodic catatonia: systematic mutation scan of candidate genes at the major disease locus on chromosome 15q15

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In genome-wide linkage analyses on 12 multiplex pedigrees segregating for periodic catatonia (MIM 605419) we identified a major disease locus on chromosome 15q15, and recently replicated the chromosomal locus in an independent set of four pedigrees. Linkage and haplotype analysis in three exceptionally large pedigrees linked to chromosome 15q15 disclosed an 11 cM critical region between marker D15S1042 and D15S659. Periodic catatonia is characterized by qualitative hyperkinetic and akinetic psychomotor disturbances through acute psychotic episodes, and debilitating symptoms in the long term with psychomotor weakness, grimacing facial movements, and apathy. In our efforts in revealing the disease gene we perform linkage disequilibrium mapping and haplotype analyses in multiplex pedigrees and parent-offspring trios in new sets of microsatellite markers. Concurrently, we complete systematic mutation scans of a series of candidate genes annotated in that region by automated sequencing of DNA fragments of individuals from the linked pedigrees and controls. We systematically analyze the coding region including the exon-intron boundaries as well as large fragments of the 5'-UTR and 3'-UTR regions. The mutation scan of KIAA0252, TYRO3, and SNAP23 revealed a highly polymorphic structure with a total of 27 SNPs, and these SNPs are included in ongoing linkage-disequilibrium mapping and family-based and case-control association studies.

P 261

ASSOCIATION OF A COMT HAPLOTYPE WITH BIPOLAR AFFECTIVE DISORDER – EVIDENCE FOR A GENDER-SPECIFIC EFFECT

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Catechol-O-methyltransferase (COMT) catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. This O-methylation results in one of the major degradative pathways of the catecholamine neurotransmitters, pointing to a putative involvement in the aetiology of psychiatric disorders. In addition, COMT is located in an interesting candidate region for psychiatric disorders, the VCFS region on 22q11. A recent study by Shifman et al. (2002; Am J Hum Genet 71:1296-1302) reported a significant association between a COMT haplotype and schizophrenia. In the present study, we aimed at investigating the COMT gene in bipolar disorder. We genotypied seven single nucleotide polymorphisms (SNPs) in the COMT gene in two case-control samples with bipolar affective disorder (BPAD), of German (300 patients, 837 controls) and Polish (301 patients, 301 controls) origin, respec-

tively. Analysis of the German and Polish sample failed to show any association in the single-locus and haplotype-analysis. Since the findings of Shifman et al. (2002) suggested a sex-specific effect in schizophrenia, we analyzed our BPAD samples with respect to gender. We observed a five-locus haplotype in the German sample that was more common in males with BPAD (n=137) compared to healthy males (n=417) (haplotype frequency: 56,3% vs. 44,2%, p=0.00078, OR=1.63). In the Polish sample, a two-locus haplotype (located within the five-locus haplotype associated in the German sample) was associated with BPAD males (n=130) compared to control males (n=116) (haplotype frequency: 70.9% vs. 62.3%, p=0.036, OR=1.47). Our findings suggest COMT as a risk factor in males with BPAD. Regarding this gender specific effect, it is interesting to know that the identified haplotypes are close to the promoter region, which contains an estrogen sensitive element. Further studies of genetic variation in the COMT, in particular in the promoter region, will be presented.

P 262

A GENOME-WIDE SCAN FOR LINKAGE IN 42 ANDALUSIAN MULTIPLEX FAMILIES WITH BIPOLAR AFFECTIVE DISORDER

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Bipolar affective disorder (BPAD), also known as manic depression, is a severe psychiatric illness characterized by episodes of mania and depression. It has a lifetime prevalence of approximately 1% in all human populations. In order to identify chromosomal regions containing genes that might play a role in determining susceptibility to this psychiatric condition, we have performed a genome-wide screen for linkage with 413 STR markers in a sample of 42 families consisting of 357 individuals recruited in Andalusia, Spain. Parametric and non-parametric affecteds-only linkage analyses were carried out under three affection status models: a narrow model which included only individuals with bipolar I (103 affecteds), an intermediate model which included also individuals with schizoaffective disorder and bipolar II (144 affecteds) and a broad modell which also comprised persons with recurrent unipolar depression (209 affecteds). Best results under the non parametric analysis were obtained on chromosome 1p36, 2p22 4q31, 6q23-q24, 11q13, and 13p13. The highest findings in the parametric two-point-analysis were on chromosomes 1p36, 4q31, 8p22, 13q13, and 19p13. Multipoint-analysis provided evidence for linkage of BPAD to chromosome 19p13. We are currently performing fine mapping with dense sets of STR markers at chromosomal regions 1, 2, 4, 6 and 13. These regions are very promising because linkage findings in these regions have previously been reported in independent genome screens of BPAD.

P 263

Strong association of a haplotype block in SLC12A8 to PSORS3 suggests a role in the etiology of psoriasis arthritis rather than of psoriasis vulgaris

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Recently SLC12A8 has been proposed as a candidate gene for psoriasis susceptibility at locus PSORS3 on chromosome 3q. Association of various intronic SNPs was detected in a Swedish cohort. We attempted to replicate this finding in a group of 200 trios with psoriasis originating from Germany but failed to identify significant association using TDT statistics. Since in a previous study linkage to this locus was stronger when families were stratified according to joint involvement we hypothesized SLC12A8 might be a susceptibility locus for psoriasis arthritis rather than psoriasis vulgaris. We therefore recruited an independent cohort of patients with a verified diagnosis of psoriasis arthritis and investigated previously reported SNPs for association in a case control design. We detected significant association to one intronic SNP 15S1533 (chi-square = 21.46; $p < 0.0001$) supporting this hypothesis. Next we systematically screened the entire coding region for sequence variations in 24 psoriasis patients and determined the haplotype structure of SLC12A8. We detected a total of 24 SNPs which we grouped into five LD-blocks based of 30-50 kb size each. We used these haplotypes to test for association in both cohorts and again failed to detect association in the psoriasis cohort. In contrast, we detected strong association in the psoriasis arthritis cohort to the haplotype encompassing B1551S3 (chi-square = 46.05; $p << 0.0001$) with an OR of 3.56. This haplotype spans more than 30 kb and includes the 5' end of an alternative spliced transcript starting in exon 5. The other haplotypes showed only weak association. Our findings indicate that SLC12 A8 is rather a susceptibility locus for psoriasis arthritis than for psoriasis vulgaris and confirms the different nature of these two conditions. We conclude that a variant within this haplotype is involved in the aetiology of this disorder and further functional analyses are required to identify it's exact nature.

P 264

Single nucleotide polymorphisms in PPARG are associated

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Introduction: PPARG (OMIM *601487) encodes PPAR-gamma, a nuclear receptor that induces transcription of genes involved in insulin sensitivity, adipocyte differen-tiation and inflammation. Therefore, PPARG was thought to be a promising candidate gene for several disorders including type 2 diabetes, obesity and dyslipoproteine-mia. Amino acid position 12 of this transcription factor is a polymorphic site (OMIM *601487.0002) showing either a more common proline allele (Pro12) or an alanine allel (Ala12) due to a single nucleotide polymorphism (SNP). Pro12 was associated with higher body mass index (BMI) in Caucasians, suggesting a contribution of this mutation to genetic susceptibility of obesity. In Danish subjects the homozygous state of Pro12 was associated with an increased risk of insulin resistance syndrome. In Japanese and Scandinavians, Pro12 was shown to be significantly higher in type 2 diabetic patients than in controls. In contrast to that, Ala12 - the less frequent allele - was strongly associated with type 2 diabetes in Oji-Cree women, but not in men. The objective of our study was to investigate whether the well char-acterised coding Pro12Ala polymorphism and one non-coding intronic SNP of PPARG were associated with type 2 diabetes in patients of German origin.Material and Methods: 489 type 2 diabetes patients and 454 controls were recruited in the Diabetes Hospital Bad Mergentheim and the Center of Blood Transfusion Tuebingen, Germany. Individual genotyping of Pro12Ala and an intronic G->A SNP located 1.715 base-pairs downstream of Pro12Ala was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) after PCR and primer extension reactions.Results: The mean age at diagnosis of type 2 diabetes was 47.9 years for male and 49.3 for female patients suffering from type 2 diabetes. For male vs. female controls the mean age was 50.6 vs. 49.0 years. Pro12 was found to be significantly higher in German type 2 diabetes patients than in the control group (89.7% vs. 86.7%; delta 0.03; p=0.044). The more frequent G-allele of the SNP 1.715 base-pairs downstream of Pro12Ala showed a strong association with type 2 diabetes (89.6% in patients vs. 85.9% in controls; delta 0.037; p=0.0015). These two SNP loci were in near-complete linkage disequilibrium. Discussion: Two polymorphic sites of PPARG were associated with type 2 diabetes in patients of German origin. Being in near-complete linkage disequilibrium these two polymorphic loci form part of a common risk haplotype. In the majority of populations tested so far, the more common allele Pro12 of PPARG was more frequent in the type 2 diabetes groups. Our data confirm this observation for the German population. The

functional significance of this finding remains to be investigated. We conclude, that PPARG may play an important role among genetic factors contributing to the development of type 2 diabetes.

P 265

Polymorphisms of genes involved in bone metabolism and their association with osteoporosis in Poland

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Osteoporosis is a common disease characterized by decrease in bone mineral density (BMD) and microarchitectural deterioration of the bone structure leading to higher susceptibility to fractures. Development of osteoporosis is multifactorial process in which environmental and genetic factors play an important role. Recent studies have indicated that the majority (up to 80%) of the variability in bone mass and density is genetically determined. Molecular genetic basis of osteoporosis remains difficult to define because the bone mass, a major determinant of osteoporosis fracture risk, is quantitative trait, influenced by interaction between many genes and environmental factors. To date several candidate genes have been analyzed in relation to osteoporosis in many populations. We studied polymorphisms of osteoprotegerin gene (OPG), transforming growth factor b1 gene (TGF-b1), vitamin D receptor gene (VDR), estrogen receptor 1 gene (ESR1), collagen type 1a1 gene (COLIA1) and ANKH gene (craniometaphyseal dysplasia gene) in group of over 150 Polish patients with BMD determined in lumbar spine and femoral neck. Statistical analysis of all single polymorphisms and analysis of interaction between these polymorphisms were performed.

P 266

Quantitative trait linkage analysis of longitudinal change in body weight

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One of the great strengths of the Framingham Heart Study data, provided for the Genetic Analysis Workshop 13, is the long-term survey of phenotypic data. Participating in GAW13 we used this special kind of data to create new phenotypes representing the pattern of longitudinal change of the provided phenotypes, especially body weight. We peformed a linear regression of body weight on age and took the slope as a new phenotype for quantitative trait linkage analysis using the SOLAR package. Heritability was estimated as 0.15 for adult life "body weight change", measured as the regression slope, and "body weight gain" (including only individuals with a positive regression slope), and of 0.22 for body weight "change up to 50" (regression slope of weight on age up to an age of 50). With multipoint analysis two regions on the long arm of chromosome 8 showed the highest LOD scores of 1.6 at 152 cM for "body weight change" and of >1.9 around location 102 cM for "body weight gain" and "change up to 50". The latter two LOD scores reach the threshold for suggestive linkage. We conclude that the chromosome 8 region may harbour a gene acting on long term body weight regulation, and thereby contributing to the development of metabolic syndrome.

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EXAMINATION OF G72 AND D-AMINO ACID OXIDASE AS GENETIC RISK FACTORS FOR SCHIZOPHRENIA AND BIPOLAR AFFECTIVE DISORDER

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Recent studies had suggested that the brain-expressed genes for G72 and D-amino acid oxidase (DAAO) exert an influence on susceptibility to schizophrenia. Our aim was to replicate this finding in German schizophrenia patients and to assess whether G72 and DAAO might also contribute to the development of bipolar affective disorder (BPAD). We genotyped 7 single nucleotide polymorphisms (SNPs) in the G72 gene and 3 in the DAAO gene in 599 patients (299 schizophrenic, 300 bipolar (BPAD)) and 300 controls, all of German origin. We noted for both diseases significant associations with genetic variation at G72. In schizophrenia 4 SNPs showed association, the strongest evidence in the single-locus analysis was observed for M23 (53% vs. 46%; p=0.033; OR 1.28). The same SNP was significantly associated with BPAD (53% vs. 46%; p=0.013; OR 1.33). Haplotype analysis strengthened the statistical significance for schizophrenia and – to a lesser extent – also for BPAD. We tested the two three-locus haplotypes M12-M15-M23 and M15-M23-M24 and found that M12-M15-M23 was associated in schizophrenic patients (global p=0.006) as well as in patients with BPAD (global p=0.029). Haplotype M15-M23-M24 was associated with schizophrenia (global p=0.002), but was not significant in BPAD (global p=0.083). The 3 SNPs in the DAAO gene were associated with schizophrenia, the strongest evidence in the single-locus analysis was observed for DAAO5 (78% vs. 72%; p=0.019; OR 1.37). In BPAD single-locus analysis failed to reach statistical significance. At the haplotype level, the three-locus haplotype did not give significant global p-values in both schizophrenia (global p=0.06) and BPAD (global p=0.149). Association of variation at G72 with schizophrenia as well as BPAD provides molecular support for the hypothesis that these two major psychiatric disorders share some of their etiologic background.

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MUTATION SCREEN OF THE BRAIN DERIVED NEUROTROPHIC FACTOR GENE (BDNF): IDENTIFICATION OF SEVERAL GENETIC VARIANTS IN PATIENTS WITH OBESITY, EATING DISORDERS AND ATTENTION DEFICIT HYPERKINETIC DISORDER

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Several lines of evidence indicate an involvement of brain derived neurotrophic factor (BDNF) in body weight regulation and activity: Heterozygous BDNF knockout mice (BDNF +/-) are obese, hyperphagic and hyperactive; furthermore, central infusion of BDNF leads to severe, dose- dependent appetite supression and weight loss in rats. We investigated the role of genetic variants of BDNF in obesity, eating disorders and hyperactivity. We performed a mutation screen (SSCP & DHPLC) of the single translated exon of BDNF in 183 extremely obese children and adolescents and 187 underweight students. Additionally we genotyped two common polymorphisms (V66M & 270C>T) in 118 patients with anorexia nervosa, 80 patients with bulimia nervosa, 77 patients with attention deficit hyperkinetic disorder and 96 normalweight controls. We identified three rare (273G>A, I2T, 247+140A>G) and one common variant (V66M). We found no association between V66M or 270C>T and weight regulation, hyperkinetic syndrome or anorexia nervosa, respectively. Exploratory, we detected a trend towards a difference between allele- and genotype frequencies for 270C>T in 80 patients with bulimia nervosa compared to 82 controls (nominal p<0.06). We found no evidence for an involvement of variants in the BDNF and human body weight regulation, attention deficit hyperkinetic disorder or eating disorders. Grants: BMBF 01KW0006, 01GS0118 EU QLK1-CT-1999-00916

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GALANIN SNPs IN BODY WEIGHT REGULATION AND FAT INTAKE

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roduction: The neuropeptide galanin (GAL) was first isolated from porcine intestine (1). In most

species GAL consists of 29 amino acids and is amidated at the carboxy-terminal end. However, in humans GAL consists of 30 amino acids and is not amidated. The 15 N-terminal amino acids are identical in birds, reptiles, amphibia and mammals; the remaining sequence is also highly conserved throughout vertebrate evolution (2, 3). The injection of GAL into the hypothalamic paraventricular nuclei (PVN) or in the third ventricle led to an increased fat intake in rats (4, 5, 6). The effect of GAL on body weight regulation and fat consumption in rats as well as in humans has been analysed in different studies with conflicting results (7-11). We screened the coding region of GAL in 93 extremely obese children and adolescents with either high-fat or low-fat intake. To detect a possible role of variants in GAL in body weight regulation an additional sample of 180 extremely obese and 183 healthy lean controls was genotyped. Methods: Mutation screen in GAL was carried out in 88 (37 male) extremely obese children and adolescents (mean age 13.6 ± 2.6 years, mean BMI 31.7 ± 5.4 kg/m²) and 94 (60 male) healthy underweight students (mean age 25.5 ± 4.0 years; mean BMI 18.5 ± 1.2 kg/m2,) by SSCP and DHPLC. We detected two new SNPs in the untranslated first exon: g.-264T>C and g.-244G>A. These two new SNPs together with two known SNPs rs1042577 (intron five; 16+5C>T) and rs3136540 (3' UTR of exon six; g.4019C>T) were analysed by PCR-RFLP. Of the young extremely obese patients 45 were classified as low-fat (lowest quartile) and 48 as high-fat (highest quartile) consumers according to a food frequency questionnaire (Leeds-FFQ). All four GAL SNPs were genotyped in the 45 obese low-fat consumers and 48 obese high-fat consumers and in 180 (90 male) independent extremely obese patients (mean BMI 33.5 ± 7.1 kg/m2, mean age 14.2 ± 2.7 years) and 183 healthy lean controls, comprising 86 (46 male) normal weight (mean age 24.7 ± 2.6 years, mean BMI 21.9 ± 1.1 kg/m2) and 95 (43 male) underweight individuals (mean age 25.7 ± 3.9 years, mean BMI 18.3 ± 1.0 kg/m2). Results: There were no significant differences in allele- or genotype frequencies for the SNPs of GAL for high-fat compared to low-fat consumers or between extremely obese children and adolescents and controls. Discussion: We found no evidence for an involvement of any of the analysed SNPs of GAL in percent energy consumed as fat or in body weight regulation.

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GHRELIN RECEPTOR GENE: IDENTIFICATION OF SEVERAL SEQUENCE VARIANTS IN EXTREMELY OBESE CHILDREN AND ADOLESCENTS, HEALTHY NORMALWEIGHT AND UNDERWEIGHT STUDENTS AND CHILDREN WITH SHORT STATURE

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Introduction Growth hormone secretagogue receptor (GHSR), the receptor of endogenous ghrelin and synthetic growth hormone secretagogues (GHSs), is involved in regulation of body weight and growth hormone (GH) secretion. Intracerebroventricular and peripheral administration of ghrelin or GHSs leads to increased food intake and decreased fat utilisation and stimulates GH secretion in rodents. Previously, the human ghrelin gene has been studied in several association studies pertaining to obesity with controversial results. GHSR mRNA is expressed at high levels in the hypothalamus and pituitary. Transgenic rats with impaired GHSR function in the arcuate nucleus of hypothalamus had lower body weight and reduced GH secretion. Subjects and methods At first we performed high throughput genotyping of two single nucleotide polymorphisms (SNPs) of the GHSR in 182 extremely obese children and adolescents and 183 healthy underweight students. For SNP rs495225, additional association studies in 270 obese, 49 underweight and 95 normal weight individuals and a transmission disequilibrium test in 332 obesity-trios were performed. Additionally, the entire coding region of GHSR 1a and 1b was systematically screened in 93 extremely obese children and adolescents, 96 normal weight students, 94 underweight students and 43 children with body height 5th percentile without detected pathological cause (subsequently referred to as short stature). Results We found that the frequency of the 171T allele at SNP rs495225 is higher in the obese (75.0%) than in the underweights (70.2%, nominal p=0.14). Additional association studies showed the same trend. The TDT revealed that the 171T allele was transmitted only slightly more frequently to obese index patients (52%, two sided p=0.46), indicating a lack of involvement of this SNP in obesity. By mutation screen, we identified seven sequence variants in the coding region of GHSR in different weight groups or children with short stature. Five SNPs were found in similar genotype frequencies among obese, nomal weight and underweight individuals and in children with short stature (nominal p>0.05). The two novel variants detected only in an obese carrier and a child with short stature need to be functionally characterized. Conclusion We did not obtain conclusive evidence for an involvement of the ghrelin receptor gene in body weight regulation or short stature.

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MAJOR CONTRIBUTION OF A HAPLOTYPE ENCOMPASSING A NON-CONSERVATIVE MISSENSE SNP IN THE MCHR1-GENE TO HUMAN OBESITY IN GERMAN CHILDREN AND ADOLESCENTS

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The lean phenotypes of the melanin-concentrating hormone (Pmch-/-) and melanin-concentrating-hormone receptor 1 (Mchr1-/-) knockout mice and the anorectic effects of MCHR1-antagonists underline the importance of the MCH-system in regulation of energy homeostasis and body weight. We screened 13,379 bp of the MCHR1 locus in obese and lean individuals using single-stranded conformation polymorphism analysis and genomic sequencing. Within the coding sequence 11 variants (allele frequencies 1%) and the SNPs rs133072 (G/A) and rs133073 (T/C) were detected. In the 5'- and 3'-regions 18 additional SNPs were detected, 8 of them being novel. Both association and transmission disequilibrium to obesity were detected for several of these SNPs in large and independent study groups of German obese children, overweight, normal weight and underweight young adults, respectively. The MCHR1-haplotype associated with obesity includes the A-allele of the SNP rs133072 which leads to the exchange of Asp32 to Asn32 within the N-terminus. The measurement of receptor-induced inositol phosphate production revealed that MCH is a more potent agonist in COS-7 cells transiently expressing Asn32-MCHR1 (EC50: 9.4nM±4.9) when compared to Asp32-MCHR1 (EC50: 242nM±2.4). Gain-of-function of the MCHR1-haplotype associated with obesity was also detected in a promoter luciferase assay. The relative risks for a BMI ³ 90th percentile were estimated as 1.51 for heterozygous and 1.95 for homozygous carriers of the A-allele (Asn32). The attributable risk of the A-allele for obesity was 24%. Two German samples, including an epidemiological sample encompassing 4,056 probands (KORA), genotyped to confirm our original findings showed a trend towards association.
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Megalocornea-mental retardation syndrome: a suspicious case

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Megalocornea-mental retardation syndrome (MMR-syndrome) is a recessively inherited clinical entity which was first descriebed by Neuhauser et. al in 1975. The two minimal diagnostic criteria are megalocornea and mental retardation which can be accompanied by additional non-obligatory manifestations like short stature, neurological symptoms, micro- or macrocephaly and further minor anomalies. We present a today 23,5 year old woman who was first seen in our institute at the age of 14 years. She was born as second girl of non-consanguinous parents in 42nd week after uneventful pregnancy (body weight 3530 g [50. percentile], body length 52 cm [> 50. percentile], head circumference 34 cm [10. percentile], APGAR 8/9/10). The older sister of our propositus is healthy. Family history is apart from a miscarriage and twin-stillbirth, respectively, in second degree relationship uneventfull. Our propositus presented after birth and for the first nine month of life healthy. Than symptoms concerning motoric and speech development delay became more and more recognizable. The following physical examinations revealed neurological symptoms like muscular hypotonia, tremor and

ataxia. A pathological EEG was without clinical appearent seizures and CCT was evaluated normal. Metabolic defects could be excluded with high probability. Ophthalmologic examinations showed a nystagmus, strabismus and a suspicious big cornea-diameter for a one year old girl. The exact cornea-diameter could not be measured because of incompliance of the little girl during examination. Because of the remarkable speech delay a hearing impairment was excluded. Summarizing all the above mentioned symptoms there was at the age of 14 years suspicion for a MMR-syndrome. The cytogenetic analysis for the propositus was normal as well as an examination for the FMR1-gene. Our case report describes the symptoms of a young woman suspicious of suffering from the rare megalocornea-mental retardation syndrome and reviews the literature of the so far less than thirty known MMR-cases. The small number of published cases may be due to the fact that the association of the two minimal diagnostic signs (megalocornea combined with mental retardation) has not given enough attention to.

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Decrease of mature dendritic spines in the Arhgef6-deficient mice, a model for non-specific X-linked mental retardation

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Mutations in ARHGEF6, the gene encoding a guanine nucleotide exchange factor for the Rho GTPases Rac1 and Cdc42, are associated with X-linked non specific mental retardation. We generated an Arhgef6 knock-out mouse that will help to elucidate and understand the pathophysiological mechanisms underlying the phenotype in patients. By western blot analysis, we showed that the Arhgef6 protein was expressed in thymus, spleen, and brain in wild-type mice whereas in the Arhgef6-deficient mice, the protein was absent in these tissues. Remarkably, a smaller Arhgef6 isoform of ~83 kD was present in brain of wild-type mice compared to the protein expressed in thymus and spleen which has a molecular weight of ~87 kD. To characterize the novel, brain-specific Arhgef6 isoform, we performed RT-PCR using total RNA extracted from wild-type brain and primers flanking different regions of the Arhgef6 cDNA. For one primer combination, we obtained two PCR products of different sizes that were cloned and sequenced. In addition to the normal Arhgef6 transcript, two RNA variants were identified lacking either exon 16 alone or in combination with exon 17. These transcripts were most likely generated by alternative splicing and could give rise to the smaller Arhgef6 isoform present in brain. Mental retardation in patients was found to be associated with decreased dendritic complexity and dendritic spine density. Moreover, spine morphology often appears immature. This suggests that mental retardation results from abnormal neuronal connectivity causing deficient information processing. Thus, we investigated the spine density and morphology in the Arhgef6-deficient mice. Spines were visualized by Golgi staining, followed by quantitation of their density along the dendrites of pyramidal neurons in the visual cortex. In Arhgef6 knock-out mice, the density of mushroom spines (the mature type of spines) along basal dendrites was decreased by 25 %, relative to that of wild-type mice, whereas the density of filiform spines (an immature spine morphology) was not altered. These data suggest that the mental retardation phenotype in patients with ARHGEF6 mutations may be caused by altered synaptic connectivity in the cerebral cortex. In the near future, Arhgef6-deficient mice will be tested for behavioural and cognitive deficits.

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Identification of a missense mutation in the putative HP1 interacting domain of the XNP/ATR-X gene in severe X-linked mental retardation syndrome

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Mutations in the XNP/ATR-X gene have been identified in patients with alpha thalassaemia / mental retardation syndrome (ATR-X) as well as those with other severe X-linked mental retardation syndromes. The XNP/ATR-X protein is a putative global transcription factor of chromatin-mediated transcriptional regulation. It contains a zinc-finger domain with striking similarity to PHD finger domains followed by a putative heterochromatin protein 1 (HP1) interacting domain in the N-terminal region and a DNA helicase domain in the C-terminal region. Most mutations in the XNP/ATR-X gene have been found within the PHD zinc finger and the helicase domain.We identified a novel XNP/ATR-X missense mutation located in the putative HP1 interacting domain in a 14 year old boy with X-linked severe mental retardation and facial dysmorphy. Linkage analysis of the family has previously included the XNP/ATR-X gene as a candidate gene. In addition, obligate-carrier females had a skewed pattern of X inactivation that is associated with XNP/ATR-X gene mutations. This novel missense mutation TTG to TCG (Leu to Ser) in codon 409 of the XNP/ATR-X gene was not detected in 130 X chromosomes of normal control individuals (50 females, 30 males) excluding the possibility of a common single nucleotide polymorphism (SNP). Comparison of the human, mouse and rat XNP/ATR-X amino acid sequences showed evolutionary conservation of Leu[409] in all three species suggesting structural or functional importance of Leu[409]. When analysing the wild-type XNP/ATR-X amino acid sequence by the computer program Plotstructure (GCG version; DKFZ) amino acids 390 - 440 fold into an alpha-helical structure. In the mutated protein containing Ser[409] this alpha-helical structure is disrupted suggesting some impact on the overall structure of the protein. In conclusion, these results suggest that Leu409 to Ser in the putative HP1 interacting domain of XNP/ATR-X interferes with the chromatin-mediated transcriptional regulation leading to the observed disease phenotype.

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A comparative expression analysis of four MRX genes regulating intracellular signalling via small GTPases

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The X chromosomal mental retardation genes have attained high interest in the past. A rough classification distinguishes syndromal mental retardation, MRXS, and non-syndromic mental retardation, MRX, conditions. The latter are suggested to be responsible for human specific development of cognitive abilities. These genes have been shown to be engaged in chromatin remodelling or in intracellular signalling. During this analysis we have compared the expression pattern in the mouse of four genes from the latter class of MRX genes: Ophn1, Arhgef6 (also called αPix), Pak3, and Gdi1. Ophn1, Pak3, and Gdi1 show a specific neuronal expression pattern with certain overlap which allows to assign these signalling molecules to the same functional context. We noticed highest expression of these genes in the dentate gyrus and cornu ammonis of the hippocampus, in structures engaged in learning and memory. A completely different expression pattern was observed for Arhgef6. It is expressed at sites where neuronal cells originate during development, but is found also in other non-neuronal tissues. Our analysis provides evidence that these signalling molecules are involved in spatio-temporal specific expression domains of common signalling cascades and that considerable functional redundancy of Rho mediated signalling pathways must exist in the brain.

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Identification of interaction partners of serine-threonine kinase 9 (STK9), which was functionally absent in two female patients with severe infantile spasms

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Recently we have shown that the X-chromosomal serine-threonine kinase 9 (STK9) gene was disrupted in two mentally retarded female patients with infantile spasms and a de novo balanced X;autosome translocation (Kalscheuer, Tao et al, AJHG, in press). Functional absence of STK9 in two unrelated patients with almost identical phenotype suggests its causal role in this disorder. To learn more about the function of the STK9 protein, its signaling pathway and the biological mechanism underlying the pathogenesis of the disease, we searched for STK9 interaction partners by a yeast two-hybrid screen. In addition to the wild type STK9, a kinase deficient mutant which carries a lysine to arginine mutation at position 42 in the ATP binding domain has been used as a bait, assuming that this site mutation might lead to the stabilization of STK9 - substrate interactions. From approximately 8.8x10E6 human fetal brain cDNA clones screened, 170 positive clones were isolated. Sequencing has revealed that several of these cDNA clones overlap and correspond to 3 different genes. One of these has been implicated previously in non-syndromic X-linked mental retardation. To confirm these findings, protein-protein interactions are currently being studied in a mammalian system.

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Monosomy 1p36 - a mental retardation-metabolic disorder?

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Monosomy 1p36 is the most common terminal deletion syndrome, presumably caused by haploinsufficiency of a number of genes and is associated with a distinct and complex phenotype, including physical, developmental, and neurobehavioral features. The frequency of monosomy 1p36 has recently been estimated to be 1 in 5000 birth since recent advances in cytogenetic technology have greatly improved the ability to identify 1p36 deletions. Clinical characteristics include moderate to severe psychomotor retardation, seizures, growth abnormalities (growth retardation, microcephaly, obesity), and dysmorphic features (large anterior fontanel, prominent forehead, deep set eyes, flat nasal bridge and midface hypoplasia, ear asymmetry, a pointed chin). Additional clinical characteristics include seizures, cardiomyopathy, developmental delay, and hearing impairment. Similarity between patients with 1p deletions and those with Prader-Willi syndrome has been noted. Here, we report on a 31-year-old man with monosomy 1p36. Cytogenetic analysis of peripheral blood cells showed mosaicism for the deletion being the second observation of 1p36 deletion in mosaic form. Parental karyotypes were normal. The development of a rather distinctive phenotype in the patient was personally observed from H.K-P over a period of 30 years. He has characteristic facial dysmorphic features with flat midface, small mouth with high arched palate, deep set and malformed ears, and a pointed chin. Measurements at birth were normal, but reduced growth was detected during the first year of life. No malformations of the internal organs are known. In II. decennium he developed severe obesity and seizures. Behavioural problems including temper outbursts became apparent. In recent years metabolic studies showed hypercholesterolemia and severe hyperferritinemia. Marked mental deficiency and speech delay is apparent. In spite of many attempts, a diagnosis had not been made until the deletion was detected by molecular-cytogenetic techniques at the age of 31 years during a follow up visit. Further characterization of this phenotype and long follow ups from further patients should assist in the clinical diagnosis of this chromosome abnormality.

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Study of disease-associated balanced chromosome rearrangements in four patients with mental retardation and congenital abnormalities

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Studying patients with disease-associated balanced chromosome rearrangements is a powerful strategy for the isolation of disease genes and for linking phenotype and genotype. We investigated the breakpoints in four patients with different degrees of mental retardation, dysmorphisms and congenital malformations, who carry de novo balanced translocations.The first patient presenting with mild mental retardation, short stature and mild dysmorphism has a t (1;10)(p22;p11.2) translocation. Iterative fluorescent in situ hybridisation (FISH) of YAC clones to metaphase chromosomes of the patient identified a breakpoint-spanning clone for chromosome 1 and narrowed the breakpoint region on chromosome 10 to an interval of 7 cM.The second patient presenting with severe mental retardation and epilepsy carries a t (1;11)(p34.1;p13) translocation. A breakpoint-spanning YAC clone has been identified for chromosome 1. On chromosome 11, FISH hybridisation signals indicated that one PAC clone overlapped the breakpoint. This clone contains part of the excitatory amino acid transporter 2 (EAAT2) gene (MIM # 600300) which may be disrupted by the rearrangement.The third patient presenting with mild mental retardation, mild hearing impairment, obesity and eunuchoid proportions carries a t (7;8)(p13;q22.3). FISH with YAC clones narrowed the breakpoint region to 6 and 3 cM, respectively.In the fourth patient presenting with mild mental retardation and ectrodactyly with apparently balanced t (11;16)(p13;q13) translocation, systematic FISH analysis revealed a more complex chromosome rearrangement, which also involves chromosome 8.Ongoing breakpoint mapping studies and in silico sequence analyses should soon shed more light on underlying gene defects.

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Am Institut für Humangenetik, Universitätsklinikum Essen (Direktor: Prof. Dr. rer.nat. B. Horsthemke, Ltd. Ärztin: Prof. Dr.med. G. Gillessen-Kaesbach)

ist die Stelle einer/eines

Ärztin/Arztes im Praktikum bzw. Ärztin/Arztes

ab sofort halbtags zu besetzen. (Erlaubnis für eine Tätigkeit als AiP wird für 18 Monate erteilt).

Aufgabengebiet:
Diagnostik und genetische Beratung bei Patienten mit einem breiten Spektrum genetisch bedingter Erkrankungen, ungeklärten Entwicklungsstörungen, chronischen Erkrankungen des Erwachsenenalters, Beurteilung von Risiken für Embryo und Fetus während der Schwangerschaft, Pränataldiagnostik, Bewertung von genetischen Laboruntersuchungen für die ärztliche Praxis. Die Tätigkeit ist klinisch und hat eine Reihe von allgemeinmedizinischen Aspekten. Teilnahme an konsiliarischer Tätigkeit bei stationären Patienten vor allem in der Kinderklinik und der Neugeborenenstation. Eine volle Weiterbildungsermächtigung ist vorhanden.

Anforderungen: Erlaubnis für eine Tätigkeit als AiP bzw. Erlaubnis für eine Tätigkeit als Arzt.

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Ansprechpartnerin: *Tel.: 030 577 987 18*
Frau Dr. Britta Belitz *Belitz@pdmg.de*

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Tätigkeitsbereich: prä- und postnatale zytogenetische Diagnostik, Befundung, genetische Beratung

Tätigkeitsbeginn:
nach Vereinbarung

Bewerbungen bitte an:
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Institut für Medizinische Biologie und Humangenetik
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