18. Jahrestagung der Deutschen Gesellschaft für Humangenetik (GfH)
07. – 10. März 2007, Bonn, Beethovenhalle

Wissenschaftliches Programm / Scientific Program

Mittwoch, 7. März 2007

Veranstaltung für Oberstufenschüler

Unterstützt durch die Deutsche Telekom Stiftung

Großer Saal

09:30-14:00	**Der Mensch und seine Gene** Führende Wissenschaftler referieren für Bonner Oberstufenschüler
09:30-09:45	**Einführung** Propping, Peter (Bonn)
09:45-10:30	**Das Humangenomprojekt** Sperling, Karl (Berlin)
10:30-11:15	**Wie wird ein Krankheits-Gen identifiziert?** Rieß, Olaf (Tübingen)
11:15-12:00	**Genetik und Krebs** Bartram, Claus R. (Heidelberg)
12:00-12:30	**Pause**
12:30-13:15	**Ist unser Leben genetisch determiniert?** Nöthen, Markus M. (Bonn)
13:15-14:00	**Das Schicksal Behinderter – Ein historischer Rückblick** Bergdolt, Klaus (Köln)

Satelliten-Symposium mit der Deutschen Huntington Hilfe e.V.

Seminarraum I

14:30-16:00	**Genetische Diagnostik bei der Huntingtonschen Krankheit – Erfahrungen aus Sicht der Betroffenen** Moderation: Henn, Wolfram (Homburg/Saar) Rieß, Olaf (Tübingen)
14:30-14:40	**Für und Wider die prädiktive Diagnostik** Pfeiffer, Lutz (Berlin)
14:40-14:50	**Genetische Diagnostik aus Sicht der Selbsthilfegruppe: Ethische Grundfragen anhand von Beispielen** Obst, Volker (Berlin)
14:50-15:00	**Genetische Diagnostik aus Sicht der Selbsthilfegruppe: Begleitung nach der Diagnostik** Bambach, Martin (Werl)
15:00-15:10	**Inanspruchnahme der prädiktiven Diagnostik** Kreuz, Friedmar (Berlin)
15:10-16:00	Diskussion mit den Teilnehmern

Wednesday, 7 March 2007

Qualitätssicherung QW 1-2

14:30-16:00 **QW1: Zytogenetik**
Held, Karsten (Hamburg)
Bartsch, Oliver (Mainz)
Eiben, Bernd (Essen)
Studio

14:30-16:00 **QW2: Molekulargenetik**
Müller-Reible, Clemens (Würzburg)
Seminarraum II+III

Fort- und Weiterbildung EDU 1-2

14:30-16:00 **EDU1: Interdisziplinäre Patientenversorgung bei chronischen erblichen Krankheiten**
Koordination:
Mangold, Elisabeth (Bonn)
Zschocke, Johannes (Heidelberg)
Kammermusiksaal

14:30-15:00 Mit Haut und Haar – Interdisziplinäre Diagnose und Therapie erblicher ektodermaler Krankheiten
Happle, Rudolph (Marburg)

15:00-15:30 Differenzialdiagnostisches Vorgehen bei erblichen Stoffwechselkrankheiten
Zschocke, Johannes (Heidelberg)

15:30-16:00 Interdisziplinäre Diagnostik und Versorgung bei Lynch-Syndrom (HNPCC)
Mangold, Elisabeth (Bonn)

14:30-16:00 **EDU2: Risikoberechnung in Familien**
Koordination:
Fischer, Christine (Heidelberg)
Vortragssaal

14:30-15:00 Einführung in die Methodik und Anwendung bei autosomalen monogenen Krankheiten
Fischer, Christine (Heidelberg)

15:00-15:30 Risikoberechnung bei der Duchenneschen Muskeldystrophie
Grimm, Tiemo (Würzburg)

15:30-16:00 Risikoberechnung bei erblichen Mamma- und Ovarialkarzinomen
Fischer, Christine (Heidelberg)

Qualitätssicherung QW 3-4

16:15-17:45 **QW3: Tumorzytogenetik**
Koordination:
Siebert, Reiner (Kiel)
Rieder, Harald (Düsseldorf)
Kammermusiksaal

16:15-17:45 **QW4: Genetische Beratung**
Koordination:
Wolff, Gerhard (Freiburg)
Vortragssaal

Fort- und Weiterbildung EDU 3-4

16:15-17:45 **EDU3: Diagnostische Strategien bei mentaler Retardierung**
(mit Möglichkeit zur Vorstellung unklarer Fälle)
Koordination:
Gillessen-Kaesbach, Gabriele (Lübeck)
Rauch, Anita (Erlangen)
Seminarraum II+III

16:15-17:45 **EDU4: Populationsgenetik / Genomweite Assoziations-untersuchungen**
Koordination:
Krawczak, Michael (Kiel)
Wienker, Thomas F. (Bonn)
Studio

16:15-17:00 Populationsgenetische Konzepte und Methoden in der Genetischen Epidemiologie
Krawczak, Michael (Kiel)

17:00-17:45 Genomweite Assoziationsstudien: Probleme und Lösungswege
Wienker, Thomas F. (Bonn)

18:00-20:00 **Eröffnungsveranstaltung / Opening Ceremony**
Großer Saal

Grußworte / Welcome

Naaß, Horst
Mayor of the City of Bonn

Baur, Max
Prorector of the University of Bonn

Propping, Peter (Bonn)
President of the GfH Meeting

Festakt zur Verleihung der Ehrenmedaille der GfH / Award of the GfH Medal of Honor to Jürg Ott (Beijing/New York)

Laudation by Markus M. Nöthen (Bonn)

Genome-wide association studies
Ott, Jürg (Beijing/New York)

Begleitet vom / Accompanied by the **Bonner Jugendsinfonieorchester** unter / under Andreas Winnen

20:00 **Get Together**
Foyer

Thursday, 8 March 2007

08:30-10:00 **Key Note Lectures I und II**
Chairs:
Propping, Peter (Bonn)
Reis, André (Erlangen)
Großer Saal

08:30-09:15 Genetics of neurodevelopmental disorders
Monaco, Tony (Oxford, UK)

09:15-10:00 Chronic inflammatory bowel diseases – from discovery of disease genes to genomic pathophysiology
Schreiber, Stefan (Kiel)

10:00-10:30 **Coffee Break – Industrial Exhibition – Self-Support Groups**

Symposia S 1-3

10:30-12:00 **S1: Disease Mechanisms in Cancer**
Chairs:
Schlegelberger, Brigitte (Hannover)
Speicher, Michael (Graz, Austria)
Studio

10:30-11:00 Cell polarity during asymetric division. Lessions for stem cells?
Horge, Carsten (Dresden)

11:00-11:30 Myc function in stem cell self-renewal and niche dependent differentiation
Trumpp, Andreas (Lausanne, Switzerland)

11:30-12:00 A bioinformatics approach leads to the discovery of recurrent gene fusions in prostate cancer
Rubin, Mark A. (Boston, USA)

10:30-12:00 **S2: Genetics of Neuropsychiatric Diseases**
Chairs:
Nöthen, Markus M. (Bonn)
Rieß, Olaf (Tübingen)
Seminarraum II+III

10:30-11:00 Genetics of Parkinson's disease
Krüger, Rejko (Tübingen)

11:00-11:30 Genetics of affective disorder
Craddock, Nick (Cardiff, UK)

11:30-12:00 Genetics of drug addiction
Zimmer, Andreas (Bonn)

10:30-12:00 **S3: Genetics in Reproductive Medicine**
Chairs:
Haaf, Thomas (Mainz)
Wieacker, Peter (Magdeburg)
Kammermusiksaal

10:30-11:00 Genetics of hydatiform moles
Slim, Rima (Montreal, Canada)

11:00-11:30 Genetics of premature ovarian failure
Persani, Luca (Milan, Italy)

11:30-12:00 Epigenetics and reproductive technology
Horsthemke, Bernhard (Essen)

12:00-13:00 **Lunch – Industrial Exhibition – Self-Support Groups**

12:00-13:00 **Meeting of the Working Group „Reproductive Medicine"**
(by invitation)
Wieacker, Peter (Magdeburg)
Seminarraum I

Workshops W 1-4

13:00-14:30 **W1: Cancer Genetics**
Chairs:
Jauch, Anna (Heidelberg)
Wieser, Rotraud (Vienna, Austria)
Kammermusiksaal

13:00-13:15 W1 01
Functional effect of KUB3 amplification and overexpression in human glioblastoma
Rheinheimer S. (Homburg)

13:15-13:30 W1 02
Germ cell nuclear factor is a repressor of CRIPTO-1 and CRIPTO-3
Hübner C.A. (Hamburg)

13:30-13:45 W1 03
Cooperative mutations in acute myeloid leukemia: a study on 3789 patients
Schnittger S. (Munich)

13:45-14:00 W1 04
Functional oncogenomics in mantle cell lymphoma: a model cell system for complex diseases
Pscherer A. (Heidelberg)

14:00-14:15 W1 05
Microarray-based genome-wide analyses of single nucleotide polymorphisms (SNPs) revealed regions of partial uniparental disomy (pUPD) and small homozygous deletions in mantle cell lymphoma (MCL)
Nieländer I. (Kiel)

Thursday, 8 March 2007

14:15-14:30 W1 06
DNA methylation profiling in Hodgkin lymphoma cell lines using bead arrays reveals a large number of hypermethylated promoter regions
Richter J. (Kiel)

13:00-14:30 W2: Clinical Genetics: Genotype and Phenotype
Chairs:
Rudnik-Schöneborn, Sabine (Aachen)
Wieczorek, Dagmar (Essen)
Studio

13:00-13:15 W2 01
Comprehensive mutation and phenotype analysis in patients with CFC syndrome
Schulz A.L. (Hamburg)

13:15-13:30 W2 02
Genetic heterogeneity and analysis of genotype-phenotype correlations in Cornelia de Lange syndrome
Borck G. (Paris, France)

13:30-13:45 W2 03
Variable functional impairment of RMRP mutations explain genotype – phenotype correlation in Cartilage hair hypoplasia and Anauxetic dysplasia
Thiel C. (Erlangen)

13:45-14:00 W2 04
A new subtype of brachydactyly type B is caused by specific mutations in NOGGIN
Lehmann K. (Berlin)

14:00-14:15 W2 05
Extraintestinal tumour spectrum in MUTYH-associated polyposis (MAP)
Aretz S. (Bonn)

14:15-14:30 W2 06
Mapping of genetic modulators for cystic fibrosis disease severity on 12p13, 16p12 and 19q13
Stanke F. (Hannover)

13:00-14:30 W3: Therapy of Genetic Disease
Chairs:
Rappold, Gudrun (Heidelberg)
Stuhrmann-Spangenberg, Manfred (Hannover)
Vortragssaal

13:00-13:15 W3 01
Targeting TGF-ß mediated glioma cell migration and brain invasion by the PPAR-gamma agonist troglitazone
Seufert S. (Cologne)

13:15-13:30 W3 02
Early-onset epileptic encephalopathy caused by autosomal recessive PNPO deficiency is treatable with pyridoxal 5-phosphate
Zschocke J. (Heidelberg)

13:30-13:45 W3 03
Growth hormone is effective in treatment of short stature associated with SHOX deficiency: two-year results of a randomized, controlled, multi-center trial
Rappold G. (Heidelberg)

13:45-14:00 W3 04
Mice made with ES-cell derived sperm display growth abnormalities and aberrant imprinted gene methylation
Zechner U. (Mainz)

14:00-14:15 W3 05
The 1139del21 and g1743c variants of BARD1 increase cellular radiosensitivity
Surowy H. (Ulm)

14:15-14:30 W3 06
Humans and chimpanzees differ in their cellular response to DNA damage
Weis E. (Mainz)

13:00-14:30 W4: Complex Disorders
Chairs:
Cichon, Sven (Bonn)
Ziegler, Andreas (Lübeck)
Seminarraum II+III

13:00-13:15 W4 01
Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham heart study
Kronenberg F. (Innsbruck, Austria)

13:15-13:30 W4 02
Polymorphism near the insulin induced gene 2 (INSIG2) is associated with obesity
Hinney A. (Essen)

13:30-13:45 W4 03
The common SNP KCNH2-K897T is significantly associated with atrial fibrillation: Results from a large linkage-disequilibrium based study
Sinner M.F. (Neuherberg)

13:45-14:00 W4 04
Association and linkage of allelic variants of the dopamine transporter gene in ADHD
Friedel S. (Essen)

14:00-14:15 W4 05
Genomewide 100k and 500k association studies identify quantitative trait loci for QT-interval at the NOS1AP and other genes
Pfeufer A. (Munich)

14:15-14:30 W4 06
250K SNP genome scan in age-related macular degeneration (AMD)
Fritsche L.G. (Regensburg)

14:30-15:00 Coffee Break – Industrial Exhibition – Self-Support Groups

14:30-15:30 Aussteller Meeting
Kleines Raucherfoyer

Thursday, 8 March 2007

Technical Workshop TW 1

15:00-16:30 **TW1**
New Tools for Human Genetics Research
Roche Diagnostics GmbH
Vortragssaal

New applications in real-time PCR: High resolution melting analysis on the LightCycler® 480 System.
Geulen, Oliver, Roche Diagnostics GmbH (Penzberg)

Genome Sequencer FLX – The next generation high throughput sequencing system from Roche Applied Science perfectly suited to address a broad range of applications such as identification of genetic variations in disease associated regions.
Matthiesen, Peter, Roche Diagnostics GmbH (Penzberg)

15:00-17:00 **Poster Session I**
(Posters with uneven numbers)

15:00-17:00 **Industrial Exhibition – Self-Support Groups**

15:30-17:00 **Treffen der Naturwissenschaftler in der Humangenetik**
Koordination:
Heidemann, Weber, Zuehlke (GfH)
Haas-Andela, Neitzel, Stumm, Kunz (BVDH)
Seminarraum I

17:00-19:30 **BVDH Mitgliederversammlung Berufsverband Deutscher Humangenetiker e. V.**
Studio

17:00-19:30 **ÖGH Mitgliederversammlung Österreichische Gesellschaft für Humangenetik**
Kammermusiksaal

19:45-20:45 **Öffentlicher Abendvortrag / Public Lecture** (in German)
Großer Saal

Die Humangenetik in den Schlagzeilen – Öffentliche Kontroversen um das neue Wissen
Weingart, Peter (Bielefeld)

Friday, 9 March 2007

Symposia S 4-6

08:30-10:00 **S4: Evolution and the Human Genome**
Chairs:
Hameister, Horst (Ulm)
Wienker, Thomas F. (Bonn)
Studio

08:30-09:00 Evidence for recent selection in the human genome
Wang, Eric (Santa Clara, USA)

09:00-09:30 A scan for positively selected genes in the genomes of humans and chimpanzees
Nielsen, Rasmus (Copenhagen, Denmark)

09:30-10:00 Natural selection in human non-coding sequence
Ponting, Chris P. (Oxford, UK)

08:30-10:00 **S5: Public Health Genetics**
Chairs:
Nippert, Irmgard (Münster)
Schmidtke, Jörg (Hannover)
Seminarraum II+III

08:30-09:00 Evidence-based advice to clinicians about genetic tests: EGAPP Model Project
Berg, Alfred (Washington, USA)

09:00-09:30 Public health genomics
Zimmern, Ron (Cambridge, UK)

09:30-10:00 Public health genetics in a global perspective
Christianson, Arnold (Johannesburg, South Africa)

08:30-10:00 **S6: Genetics of Limb Malformations**
Chairs:
Mundlos, Stefan (Berlin)
Rappold, Gudrun (Heidelberg)
Kammermusiksaal

08:30-09:00 New insights into limb malformations from studies on chick embryos
Tickle, Cheryll (Dundee, UK)

09:00-09:30 Malformations of the limbs – a developmental perspective
Mundlos, Stefan (Berlin)

09:30-10:00 Preaxial polydactyly and long-range regulation
Hill, Robert (Edinburgh, UK)

10:00-10:30 **Coffee Break – Industrial Exhibition – Self-Support Groups**

Friday, 9 March 2007

Fort- und Weiterbildung EDU 5

10:30-12:00 **EDU5: Diagnostische und prognostische Marker bei Leukämien**
Koordination:
Rieder, Harald (Düsseldorf)
Schlegelberger, Brigitte (Hannover)
Vortragssaal

10:30-11:00 Kindliche akute lymphatische Leukämie (ALL)
Harbott, Jochen (Giessen)

11:00-11:30 Chronisch lymphatische Leukämie (CLL) / Multiples Myelom
Stilgenbauer, Stephan (Ulm)

11:30-12:00 Chronische myeloproliferative Syndrome
Reiter, Andreas (Mannheim)

Workshops W 5-7

10:30-12:00 **W5: Molecular Mechanisms**
Chairs:
Bolz, Hanno (Köln)
Dufke, Andreas (Tübingen)
Studio

10:30-10:45 W5 01
LADD syndrome is caused by mutations that reduce the tyrosine kinase activity of FGFR2
Rohmann E. (Cologne)

10:45-11:00 W5 02
The PP2Ac-specific ubiquitin ligase MID1/alpha4 assembles an mRNP that regulates microtubule-associated protein production
Aranda-Orgilles B. (Berlin)

11:00-11:15 W5 03
The cohesion protein NIPBL is associated with chromatin-remodeling enzymes
Jahnke P. (Lübeck)

11:15-11:30 W5 04
The murine Dnali1 gene encodes an axonemal dynein that is essential for embryonic development
Neesen J. (Vienna, Austria)

11:30-11:45 W5 05
Multiple roles for neurofibromin 1 in skeletal development and growth
Kolanczyk M. (Berlin)

11:45-12:00 W5 06
Constitutional KRAS mutations are associated with a broad phenotypic spectrum and predicted to cause diverse gain-of-function mechanisms
Kutsche K. (Hamburg)

10:30-12:00 **W6: Population Genetics and Evolution**
Chairs:
Bauer, Peter (Tübingen)
Illig, Thomas (Munich)
Kammermusiksaal

10:30-10:45 W6 01
The rare nonsynonymous SCN5A-S1103Y variant in Caucasians is due to recent African admixture as revealed by 100k SNP genotyping
Pfeufer A. (Munich)

10:45-11:00 W6 02
The Affymetrix 500K control project and beyond: Chip-based assessment of European genetic diversity
Krawczak M. (Kiel)

11:00-11:15 W6 03
Population stratification across Europe
Wjst M. (Munich)

11:15-11:30 W6 04
Neolithic origin of the Mediterranean p.Thr93Met mutation in the DHCR7 gene causing Smith-Lemli-Opitz syndrome
Witsch-Baumgartner M. (Innsbruck, Austria)

11:30-11:45 W6 05
Fanconi anemia genes in vertebrates: evolutionary conservation, sex-linkage, and embryonic expression of FANCC and FANCG in avian cells
Nanda I. (Würzburg)

11:45-12:00 W6 06
A systematic HAPMAP-based survey of schizophrenia candidate genes in the German population
Cichon S. (Bonn)

10:30-12:00 **W7: Copy Number Variation**
Chairs:
König, Rainer (Frankfurt/M.)
Weber, Ruthild (Bonn)
Seminarraum II+III

10:30-10:45 W7 01
Identification and characterization of subtelomeric aberrations by FISH and array CGH in 11 of 189 patients with unexplained mental retardation
Engels H. (Bonn)

10:45-11:00 W7 02
Molecular karyotyping with 100 K SNP arrays detects de novo submicroscopic chromosomal aberrations in 10% of 104 patients with unexplained mental retardation
Hoyer J. (Erlangen)

11:00-11:15 W7 03
Genome-wide analysis of copy-number variations in patients with mental retardation by single nucleotide polymorphism arrays
Wagenstaller J. (Munich)

11:15-11:30 W7 04
Submegabase-resolution array CGH reveals high rate of DNA copy number changes in Attention Deficit / Hyperactivity Disorder (ADHD)
Ullmann R. (Berlin)

Friday, 9 March 2007

11:30-11:45 W7 05
Loci of segmental aneuploidy in the human genome detected by Array-CGH
Poot M. (Utrecht, Netherlands)

11:45-12:00 W7 06
Interdependency of DNA copy number changes, methylation patterns and gene expression
Muradyan A. (Berlin)

12:00-13:00 Meeting of the Working Group „Public Health Genetics“
(by invitation)
Schmidtke, Jörg (Hannover)
Kleines Raucherfoyer

12:00-13:00 Forschungsförderung durch die DFG
(mit besonderem Schwerpunkt Nachwuchsförderung)
Golla, Astrid, (Bonn) Seminarraum 1

12:00-13:00 Lunch – Industrial Exhibition – Self-Support Groups

Fort- und Weiterbildung EDU6

13:00-14:30 EDU6: Genetische Testung und klinische Relevanz der Thrombophilie
Koordination: Oldenburg, Johannes (Bonn) Vortragssaal

13:00-13:30 Monogene Ursachen der Thrombophilie
Oldenburg, Johannes (Bonn)

13:30-14:00 Faktor-V-Leiden und Prothrombin-Mutation – Genetische Testung und Konsequenzen für die Behandlung
Pötzsch, Bernd (Bonn)

14:00-14:30 Klinische Relevanz weiterer häufig untersuchter Polymorphismen für venöse und arterielle Thrombosen
Harbrecht, Ursula (Bonn)

Workshops W 8-10

13:00-14:30 W8: Cytogenetics and Prenatal Diagnostics
Chairs: Petek, Erwin (Graz, Austria)
Tönnies, Holger (Berlin)
Seminarraum II+III

13:00-13:15 W8 01
Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics
Liehr T. (Jena)

13:15-13:30 W8 02
Microdeletion on 2q11.2 detected in a case with Nievergelt-like mesomelic dysplasia
Klopocki E. (Berlin)

13:30-13:45 W8 03
Effects of MCPH1 mutations on chromosome assembly
Trimborn M. (Berlin)

13:45-14:00 W8 04
3-D multicolor banding reveals the orientation of chromosomes in human sperm – a pilot study
Hunstig F. (Jena)

14:00-14:15 W8 05
Aneuploidy screening in polar bodies of IVF oocytes by molecular copy number counting
Daser A. (Mainz)

14:15-14:30 W8 06
A rapid comparative genomic hybridization protocol for prenatal diagnostics and its application to aneuploidy screening of human polar bodies
Landwehr C. (Bonn)

13:00-14:30 W9: Disease Gene Identification
Chairs: Eggermann, Thomas (Aachen)
Strom, Tim (Munich) Studio

13:00-13:15 W9 01
Mutations in the Wnt signaling component RSPO4 cause autosomal recessive anonychia
Bergmann C. (Aachen)

13:15-13:30 W9 02
SOS1 is the second major gene for Noonan syndrome
Zenker M. (Erlangen)

13:30-13:45 W9 03
Autosomal recessive bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature sensitive phenotype
Hennies H.C. (Cologne)

13:45-14:00 W9 04
A novel gene for Usher syndrome type 2 (USH2D): Mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss
Bolz H.J. (Cologne)

14:00-14:15 W9 05
Mutations of the mitochondrial holocytochrome c-type synthase in X-linked dominant microphthalmia with linear skin defects (MLS) syndrome
Kutsche K. (Hamburg)

14:15-14:30 W9 06
Complex inheritance pattern involving a micro-deletion in thrombocytopenia-absent radius (TAR) syndrome
Klopocki E. (Berlin)

13:00-14:30 W10: Neurogenetics
Chairs: Laccone, Franco (Vienna, Austria)
Rautenstrauß, Bernd (Erlangen)
Kammermusiksaal

13:00-13:15 W10 01
Array CGH identifies reciprocal 16p13.1 duplications and deletions which predispose to autism and/or mental retardation
Ullmann R. (Berlin)

13:15-13:30 W10 02
Mutations in autism susceptibility candidate 2 (AUTS2) in patients with mental retardation
Kalscheuer V. (Berlin)

13:30-13:45 W10 03
Evaluation of an X-linked mental retardation resequencing array
Jensen L. (Berlin)

Friday, 9 March 2007

13:45-14:00 W10 04
The agenesis of the corpus callosum: Clinical-genetic study with systematic classification of different types including molecular-cytogenetic methods
Schell-Apacik C. (Munich)

14:00-14:15 W10 05
The genetics of alcoholism: A critical role of tryptophan hydroxylase 1
Walther D. (Berlin)

14:15-14:30 W10 06
SCA17 transgenic mice show a severe neurodegenerative phenotype
Bauer P. (Tübingen)

14:30-15:00 Coffee Break – Industrial Exhibition – Self-Support Groups

Technical Workshop TW 2

15:00-16:30 TW2
Applications for Biomedical Research: Mutation Detection, SNP-Genotyping, Methylation
Applied Biosystems (Darmstadt)

Finkelnburg, Britta, Applied Biosystems (Darmstadt)
Vortragssaal

15:00-17:00 Poster Session II
(Posters with even numbers)

15:00-17:00 Industrial Exhibition – Self-Support Groups

17:00-19:30 GfH Mitgliederversammlung Deutsche Gesellschaft für Humangenetik e. V.
Studio

20:00-23:00 Social Event / Gesellschaftsabend im Rheinischen Landesmuseum Bonn
Colmantstr. 14-18, Bonn

Saturday, 10 March 2007

09:00-09:45 Key Note Lecture III
Chair:
Propping, Peter (Bonn)
Studio

09:00-09:45 Spermatogonial stem cells and their perspectives
Engel, Wolfgang (Göttingen)

09:45-11:25 Selected Presentations and Last Minute Reports
Chair:
Propping, Peter (Bonn)
Reis, André (Erlangen)
Studio

09:45-10:00 SEL 1
Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia and mental retardation
Pasutto F. (Erlangen)

10:00-10:15 SEL 2
Genetic variation in the gene for brain-expressed tryptophan hydroxylase 2 (TPH2) is associated with bipolar affective disorder in independent samples from Germany and Russia
Cichon S. (Bonn)

10:15-10:30 SEL 3
DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis
Lorenz-Depiereux B. (Neuherberg)

10:30-10:45 SEL 4
The BRCA2-binding protein PALB2 is defective in the tumor prone Fanconi anemia subtype FA-N
Neveling K. (Würzburg)

10:45-11:00 Last Minute Report 1

11:00-11:15 Last Minute Report 2

11:25-11:40 Awards

11:40-12:00 Closing Ceremony

12:00 End of the Meeting

18. Jahrestagung der Deutschen Gesellschaft für Humangenetik (GfH)
07. – 10. März 2007, Bonn, Beethovenhalle

Abstracts

The names of the speakers are underlined.

Symposia

Selected Presentations

Workshops

W1 Cancer Genetics

W2 Clinical Genetics: Genotype and Phenotype

W3 Therapy of Genetic Disease

W4 Complex Disorders

W5 Molecular Mechanisms

W6 Population Genetics and Evolution

W7 Copy Number Variation

W8 Cytogenetics and Prenatal Diagnostics

W9 Disease Gene Identification

W10 Neurogenetics

Poster

P001 - P059 Clinical Genetics

P060 - P093 Cytogenetics

P094 - P165 Cancer Genetics

P166 - P286 Molecular and Biochemical Basis of Disease, Developmental Genetics, Neurogenetics

P287 - P341 Genetic Analysis, Linkage and Association, Complex Genetics/Diseases

P342 - P350 Normal Variation, Population Genetics, Genetic Epidemiology, Evolutionary Genetics

P351 - P366 Genomics, Technology and Bioinformatics

P367 - P377 Prenatal Diagnosis, Reproductive Medicine

P378 - P385 Genetic Counselling, Education, Genetic Services, Public Policy

P386 - P391 Therapy for Genetic Diseases

Symposia

S2 Genetics of Neuropsychiatric Diseases

S2 03

Genetics of drug addiction

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The genetics of drug addiction has been extremely difficult to study in humans, due to the strong influence of socioeconomical and environmental factors that contribute to this complex disorder. Because environmental factors can be standardized in animals and because the various stages of drug addiction can be accurately modeled in rodents, we are using the strategy of identifying candidate genes in animal models, followed by a genetic analysis of human cohorts. We thus studied the role of the endogenous opioid and cannabinoid systems as modulators of alcohol and nicotine dependence using different knockout mouse models. Our results indicate that opioids and cannabinoids influence distinct aspects of the multistep disease process related to drug reward in relapse. We will also describe unbiased genome-wide approaches for the identification of genetic risk factors. We performed a QTL-analysis in an intercross population of C3H and C57BL6/J mice for alcohol addiction-related phenotypes. Finally, we will present results from expression profiling studies after nicotine self-administration.

Selected Presentations

SEL 1

Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia and mental retardation

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Homozygosity mapping revealed linkage to a common locus on chromosome 15 in two unrelated consanguineous families with bilateral anophthalmia and distinct eye brows as common sign, but differing for alveolar capillary dysplasia or complex congenital heart defect in one and diaphragmatic hernia in the other family. Homozygous mutations were identified in STRA6, a gene of unknown function mapping within the critical region and originally identified as being stimulated by retinoic acid. Subsequently, homozygous STRA6 mutations were also demonstrated in three out of 13 patients chosen on the basis of significant phenotypic overlap to the original cases. The absence of STRA6 protein in cultured fibroblasts was confirmed in one patient with a deletion leading to a premature stop codon (p.G50AfsX22). Structural analysis of three missense mutations (P90L, P293L and

T321P) predicted significant effects on the geometry of the loops connecting the transmembrane helices of STRA6. Two further variations in the C-terminus (T644M and R655C) instead are predicted to alter specific functional sites, an SH2-binding motif and a phosphorylation site, respectively. STRA6 mutations thus define a pleiotropic malformation syndrome with phenotypic overlap with Matthew Wood syndrome. Our failure to identify a STRA6 mutation in one case with definite Matthew Wood syndrome may suggest either locus or clinical heterogeneity.

SEL 2

Genetic variation in the gene for brain-expressed tryptophan hydroxylase 2 (TPH2) is associated with bipolar affective disorder in independent samples from Germany and Russia

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Serotonin is a neurotransmitter that is involved in a wide range of physiological functions including mood control. It regulates sleep, hormonal activity, aggression, sexual drive, appetite, and energy level. Since many of these functions are relevant to the etiology of affective disorders, it has been hypothesized that disturbances of serotonin activity may contribute to their development. The tryptophan hydroxylase 2 (TPH2) gene encodes the rate-limiting enzyme in serotonin biosynthesis in the brain. Recently, genetic variants of the TPH2 gene have been associated with impulsivity, suicidality, depression, and anxiety disorders. We tested whether genetic variants of the TPH2 gene might contribute to the development of bipolar affective disorder. A total of 15 HapMap-based haplotype tagging SNPs and one rare non-synonymous SNP identified by systematic sequencing of the coding region in a population-based sample were genotyped using Sequenom's iPLEX technology. Analysis of these SNPs in a sample of 652 patients with bipolar affective disorder and 1,105 population-based controls, all of German descent, identified 3 significantly associated SNPs in a haplotype block including the 5' regulatory part of TPH2. The non-synonymous SNP, which is not in linkage disequilibrium with the 5' SNPs, also showed significant association. We replicated these findings in an independent Russian sample comprising 250 bipolar patients and 232 controls. Our results provide strong evidence that genetic variation in TPH2 is involved in the development of bipolar disorder. We are currently performing functional studies to elucidate the potential effect of the non-synonymous variant. Preliminary results obtained from E. coli and human embryonic kidney cell lines (HEK293) containing the two variant forms of TPH2 point to differences regarding thermal stability of the enzyme. The rare variant TPH2 form may have a lower serotonin production rate than the common TPH2 form.

SEL 3

DMP1 mutations in autosomal recessive hypophosphatemia implicate a bone matrix protein in the regulation of phosphate homeostasis

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Monogenic phosphate-wasting disorders leading to rickets typically follow an X-linked (XLH) or autosomal dominant mode of inheritance (ADHR). We identified a novel autosomal recessive form of hypophosphatemia (ARHP) in three unrelated families. The affected individuals showed clinical and biochemical parameters that were similar to those observed in XLH and ADHR, which are hypophosphatemia due to renal phosphate loss, osteomalacia and rickets. We performed a genome-wide linkage analysis using SNP array genotyping. Assuming that the disease alleles could be identical by descent in each family because of their rare occurrence, we analyzed the data by homozygosity mapping and identified a 4.6 Mb candidate region on chromosome 4q21 between the SNPs rs340204 and rs722937 (max. LOD score of 3.1, 2.38 and 4.15, respectively, in families 1, 2, and 3). Sequence analysis of candidate genes identified different homozygous loss-of-function mutations in DMP1 (dentin matrix protein 1), which codes for a non-collagenous bone matrix protein expressed in osteocytes along with FGF23 (fibroblast growth factor 23) and PHEX (phosphate regulating endopeptidase on the X chromosome). FGF23 plasma levels were elevated in the two younger of four individuals indicating that DMP1 is involved in regulating FGF23 expression. Our results extend the functions of DMP1, a bone matrix protein involved in mineralization, to phosphate regulation. The link between DMP1 and FGF23 places DMP1 on a growing list of proteins identified by human mutational analysis and by genetic mouse models that result in disorders associated with hypophosphatemia, osteomalacia and rickets.

SEL 4

The BRCA2-binding protein PALB2 is defective in the tumor prone Fanconi anemia subtype FA-N

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The intimate connection between genes that cause the rare disease Fanconi anemia (FA) and genes that predispose to breast cancer became evident in 2001 when biallelic mutations in the breast cancer gene BRCA2 were found to be responsible for a rare subtype of FA previously classified as FA-D1. These patients are severely affected and suffer from malignancies in early childhood such as Wilms tumor, medulloblastoma and AML. They frequently succumb to their aggressive cancers prior to the manifestation of bone marrow failure such that many of these children may never be diagnosed as FA. FA-D1 patients have intact monoubiquitination of FANCD2 but lack nuclear foci formation after induction of DNA damage. A severe early tumor phenotype combined with these cellular features has been noted so far only in subtype FA-D1 patients. Although a number of unclassified patients with the early neoplasia phenotype could be assigned to subtype FA-D1, we identified 7 young patients who did not carry mutations in the FANCD1/BRCA2 gene. Subsequent mutation screening of PALB2, a gene coding for a recently described binding partner and localizer of BRCA2/ FANCD1, revealed these 7 patients as biallelic carriers of mutations in PALB2. This BRCA2-associated gene therefore qualifies as yet another downstream FA gene that has been termed FANCN. Cells from FA-N patients do not express PALB2 protein, but expression could be restored by transduction of wildtype PALB2 cDNA. Thus, in addition to BRCA2/FANCD1 and BRIP1/FANCJ, PALB2/FANCN represents the third FA gene that acts downstream of FANCD2, and the second gene whose biallelic mutations cause a severe FA-like disease with an early and prominent tumor phenotype.

Workshops

W1 Cancer Genetics

W1 01

Functional effect of KUB3 amplification and overexpression in human glioblastoma

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DNA amplifications are frequent genetic alterations in tumour cells but were never found in normal human cells. The downstream effects of various amplified genes on tumour development are still unknown. Here we set out to characterize downstream effects of KUB3 amplification and possible function of this Ku70-binding-protein-3 in double strand break repair by the DNA-PK complex. KUB3 is highly overexpressed upon amplification in many glioblastoma. Here we demonstrate that KUB3 overexpression influences DSB repair after gamma-irradiation of glioblastoma cell cultures. The DSB repair was significantly increased in one glioblastoma cell line with KUB3 amplification compared to one glioblastoma cell line without KUB3 amplification. To further proof this effect we next knocked down the KUB3 overexpression in the glioblastoma cell line using siRNAs specific for KUB3. The number of repaired DSBs was significantly reduced in the glioblastoma cells after knock down of the KUB3 overexpression. These results clearly demonstrate that KUB3 overexpression in glioblastoma cells leads to an improved DSB repair after gamma-irradiation and this is a reasonable advantage for glioblastoma cells in respect to the treatment with gamma-irradiation.

W1 02

Germ cell nuclear factor is a repressor of CRIPTO-1 and CRIPTO-3

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The pluripotency of embryonic stem and embryonic carcinoma cells is maintained by the expression of a set of „stemness" genes. Whereas these genes are down-regulated upon induction of differentiation, the germ cell nuclear factor (GCNF) is transiently up-regulated and represses several pluripotency genes. CRIPTO-1, a co-receptor for the morphogen nodal, is strongly expressed in undifferentiated cells and is rapidly down-regulated during retinoic acid-induced differentiation. Although CRIPTO-1 is expressed at very low levels in adult tissues under normal conditions, it is found highly expressed in a broad range of tumors, where it acts as a potent oncogene. We show that expression of CRIPTO-1 is directly repressed by GCNF during differentiation of the human teratocarcinoma cell line, NT2. GCNF bound to a DR0 element of the CRIPTO-1 promoter in vitro, as shown by electrophoretic mobility shift assays, and in vivo, as demonstrated by chromatin immunoprecipitation. Reporter gene assays demonstrated that GCNF-mediated repression of the CRIPTO-1 promoter is dependent upon the DR0 site. Overexpression of GCNF in NT2 cells resulted in repression of CRIPTO-1 transcription, whereas expression of the transcription-activating fusion construct GCNF-VP16 led to an induction of the CRIPTO-1 gene and prevented its retinoic acid-induced down-regulation. Furthermore, we demonstrated that CRIPTO-3, a processed pseudogene of CRIPTO-1 on the X chromosome, is expressed in undifferentiated NT2 cells and is regulated by GCNF in parallel to CRIPTO-1. Thus, our study supports the hypothesis of GCNF playing a central role during differentiation of stem cells by repression of stem cell-specific genes.

W1 03

Cooperative mutations in acute myeloid leukemia: a study on 3789 patients

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At least two type of genetic alterations are required for the clinical manifestation of leukemia. One type comprizes alteration of transcription factors causing a stop in differentiation. This type of mutations is reflected mainly in the morphologic phenotype and is called type II mutation. In contrast, type I mutations usually lead to enhanced proliferation and typically are activating mutations in genes coding for tyrosine kinases or downstream effectors. These two kind of mutations often are referred to as cooperating mutations. Based on our molecular analyses of 3789 AML we found that the pattern in which these mutations occur is not random and it was shown that certain type I mutations prefer to occur together with certain types of type II mutations. The following preferential combinations of type I mutations with chromosomal aberrations were found: FLT3-LM with t(15;17), t(6;9) and normal karyotype; FLT3-TKD with normal karyotype; NRAS with inv(16)/t(16;16) and inv(3)/t(3;3); KITD816 with t(8;21); KITexon11 with inv(16)(t(16;16); and JAK2 in de novo AML with trisomy 8 and in t-AML with t(8;21). In addition, some of the type I mutations were correlated to normal karyotypes and were associated with other molecular mutations: FLT3-LM is preferentially found in AML with MLL-PTD as well as NPM1-, CEBPA-, and AML1-mutations. For some combinations a prognostic relevance could be shown: FLT3-LM has unfavourable impact on event free survival (EFS) in normal karyotype ($p=0.04$) and on overall survival (OS) in t(15;17) ($p=0.05$) and CEBPA ($p=0.03$) even completely abrogates the favourable impact of NPM1 ($p<0.001$). KITD816 mutations were shown to confer an extreme bad impact on OS in t(8;21) ($p<0.001$) . In contrast, NRAS seems to be associated with an improved outcome in cases in which all unfavourable molecular markers are negative ($p=0.06$). In conclusion this study supports the impact of molecular characterization of AML with respect to diagnosis and prognosis.

W1 04

Functional oncogenomics in mantle cell lymphoma: a model cell system for complex diseases

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A broad range of malignant diseases, such as mantle cell lymphoma (MCL), is associated with complex genomic alterations, demanding multimodal functional testing of candidate genes. Therefore we have developed a bidirectional targeted transgenesis tool, which allows well-controlled modulation of individual gene activities within a cellular MCL system via RMCE. The versatile transgenesis system permits functional analysis of virtually any candidate gene: for tumor suppressor genes by complementation of respective genomic DNA or for oncogenes by inactivation via integrated shRNA coding plasmids. Complementation by genomic DNA ensures wild-type regulated gene expression whereas genomic integration of shRNA coding inserts by an advanced RNAi-strategy mediates specific knock-down of gene expression. Site-specific genomic integration of an unmodified BAC, which contains the CDKN2A/B genes absent in the MCL model system, restored CDKN2A/B expression resulting in the inhibition of cell proliferation. CCND1, strongly overexpressed in the model system, was downregulated via shRNA expression, again inhibiting proliferation. Notably, this site-specific shRNA-strategy circumvents interferon-response induced when using other RNAi gene knock-down methods. (FASEB J. 2006 Jun; 20(8)).
We have used the presented transgenesis system furthermore to compensate MCL cells for deleted or downregulated miRNAs, which where identified as pathogenetically relevant. Using these stable miRNA-expressing cell clones we screen, based on the SILAC-technique, on a proteome level for target genes, which are regulated by the distinct miRNA.
This study is funded by grants of the Fritz Thyssen Stiftung (10.04.1.169), the Deutsche Jose Carreras Leukaemie Stiftung e.V. (DJCLS R 06/13) and Landesstiftung Baden-Wuerttemberg (P-LS-Prot/54).

W1 05

Microarray-based genome-wide analyses of single nucleotide polymorphisms (SNPs) revealed regions of partial uniparental disomy (pUPD) and small homozygous deletions in mantle cell lymphoma (MCL)

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Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL) characterized by the translocation t(11;14)(q13;q32). It is well established that this chromosomal event alone is not sufficient to result in lymphoma and secondary genomic alterations are required for malignant transformation. Recently, genome-wide array CGH analyses revealed a large number of genomic gains and losses which are recurrent in MCL and therefore may be associated with ist pathogenesis. Microarray-based single nucleotide polymorphisms (SNP) analyses cannot only identify copy number alterations but also detect regions of partial uniparental disomy (pUPD). pUPD has been reported to be a frequent mechanism of tumor suppressor inactivation in acute leukemias and solid tumors. Therefore, we have performed genome-wide analyses of single nucleotide polymorphisms (SNPs) using a 100K SNP-array (GeneChip Human Mapping 100K Set, Affymetrix, Santa Clara, CA, USA) in 28 t(11;14)-positive mantle cell lymphoma primary cases and seven MCL cell. In this way we could confirm various previously reported regions of recurrent genomic gains and losses as well as the presence of small homozygous deletions in 2q13 (BIM) and 9p21.3 (P16/INK4A). Moreover, we identified novel homozygously deleted regions whereof six could be validated and delineated by PCR-based methods. In addition, pUPDs have been detected in both MCL cell lines and MCL cases. Regions of pUPD frequently affected loci which are known to be recurrently deleted in MCL like 1p, 8p, 9q, 11q and 17p. Further characterization of these altered chromosomal regions may identify tumor suppressor genes which play important roles in the pathogenesis of MCL.

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W1 06

DNA methylation profiling in Hodgkin lymphoma cell lines using bead arrays reveals a large number of hypermethylated promoter regions

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DNA methylation of CpG islands in promoter regions is a key epigenetic mechanism that regulates gene expression. In cancer, DNA hypermethylation is frequently reported to be associated with tumor suppressor gene inactivation.

DNA methylation changes associated with Hodgkin lymphoma (HL) have been so far poorly studied. Therefore, we have performed a high-throughput DNA methylation profiling of HL cell lines with the bead array technology (Illumina Inc.), which allows the detection of 1536 individual CpG sites from the 5´ regulatory region of over 500 genes involved in tumorigenesis and imprinting disorders. Four classical HL (cHL) and one nodular lymphocyte predominant HL (NLPHL) as well as two lymphoblastoid cell lines from healthy donors (negative controls) were studied in duplicate and analyzed with the BeadStudio software. A methylation coefficient ranging from 0 (completely unmethylated) to 1 (completely methylated) was calculated and differences between entities above 0.5 were considered differentially methylated. In comparison with the controls, cHL showed 160 hypermethylated and 9 hypomethylated gene promoters whereas 101 and 37 promoters were hyper- and hypomethylated in NLPHL, respectively. CHL displayed a high number (n=95) of hypermethylated promoters in comparison to NLPHL. Hypermethylated gene promoters in HL included known tumor suppressor genes (e.g. p16, p73, DAPK). Interestingly, CpG-islands from genes involved in B-cell specific pathways (e.g. BCAM, BLK, MME and SYK) were exclusively hypermethylated in cHL. Such epigenetic silencing of B-cell specific genes can be the cause of loss of the B-cell identity characteristic for cHL, and thus, might play a key role in its pathogenesis. In conclusion, genome-wide methylation profiling revealed a global alteration of the methylome in cHL, being mostly characterized by promoter hypermethylation of a large number of genes.

W2 Clinical Genetics: Genotype and Phenotype

W2 01

Comprehensive mutation and phenotype analysis in patients with CFC syndrome

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Cardio-facio-cutaneous syndrome (CFC) is a developmental disorder characterized by congenital heart defects, distinct craniofacial dysmorphism, ectodermal abnormalities, short stature, and mental retardation. The phenotype of patients with CFC clinically overlaps with Noonan and Costello syndrome. Recent data revealed that these disorders are indeed pathogenetically related as they are caused by constitutional deregulation of the Ras signalling pathway. Specifically, CFC has been found to be caused by heterozygous missense mutations of KRAS, BRAF, MEK1, and MEK2.

We intended to define genotypes and associated phenotypes in a large cohort of CFC patients. Forty patients with a clinical diagnosis of CFC were screened for mutations of BRAF, MEK1, MEK2, and KRAS by direct sequencing of all coding exons. Overall, we discovered heterozygous missense mutations in 27 patients with CFC (68%), a rate comparable with that of previous studies. The majority of mutations were found in BRAF (19/27), while only three, two, and three patients showed mutations of MEK1, MEK2, and KRAS, respectively. Mutation screening of KRAS and related phenotypes will be presented elsewhere (Abstract by Zenker, Lehmann et al.). Six novel mutations were found that cluster within the known mutation hotspots; all of them affect highly conserved amino acid residues. In 17 patients we demonstrated de novo occurrence of the mutation. Phenotype analysis revealed a 'classical' CFC phenotype in patients with a BRAF alteration, while patients with MEK1/MEK2 mutations show a tendency toward a more severe phenotype, reminiscent of Costello syndrome in some individuals. Our data confirm that missense mutations in five hotspot exons of BRAF and two exons of MEK1 and MEK2 are causative for CFC syndrome in the majority of cases. Nonetheless, lack of mutations in KRAS, BRAF, and MEK1/2 in about one third of patients with CFC suggests existence of at least one additional gene responsible for this disorder.

W2 02

Genetic heterogeneity and analysis of genotype-phenotype correlations in Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a developmental disorder characterised by growth and mental retardation, facial dysmorphism, and various malformations including limb defects. Mutations in the NIPBL gene account for ~ 45% of CdLS cases. Recently, mutations in the X-linked gene SMC1L1 have been identified in a small number of affected boys. We report the extensive clinical and molecular investigation of a series of 30 patients, 18 boys and 12 girls.

Our analysis included 1) a search for partial or complete NIPBL deletions using multiplex ligation-dependent probe amplification (MLPA), 2)

sequencing of the 46 NIPBL coding exons and the 5' untranslated region (5'UTR) and promoter, and 3) SMC1L1 analysis in patients with no NIPBL anomaly.
No intragenic NIPBL deletions were detected by MLPA. We identified 13 distinct NIPBL mutations: five missense, four splice-site, one nonsense, one frameshift and one in-frame deletion mutation. The only familial case, a mildly affected father and his affected daughter, had a novel type of NIPBL anomaly, a mutation in the highly conserved 5'UTR. Using real-time PCR, we showed that NIPBL mRNA expression was lowered in patients' lymphocytes. When subcloned into a luciferase vector, the mutation lead to a significant reduction of reporter gene activity. Sequencing of the SMC1L1 gene identified two novel de novo missense mutations in two unrelated boys: p.R196H and p.Y1085C.
Our findings confirm that NIPBL mutations account for ~ 40% of CdLS cases and that a significant proportion of boys (2/18) have a SMC1L1 mutation, thereby confirming X-linked CdLS. Importantly, our results identified a mutation in a non-coding region of the NIPBL gene as a novel cause of CdLS. They also suggest a trend toward a milder phenotype in patients with SMC1L1 mutations with respect to growth retardation and associated malformations. Finally, absence of NIPBL and SMC1L1 mutations in 50% of our patients suggests additional genetic heterogeneity of CdLS.

W2 03

Variable functional impairment of RMRP mutations explain genotype – phenotype correlation in Cartilage hair hypoplasia and Anauxetic dysplasia

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Mutations in the RMRP gene have been demonstrated to cause a wide phenotypic spectrum from Anauxetic dysplasia (AD) and Cartilage hair hypoplasia (CHH) to Metaphyseal dysplasia without hypotrichosis (MDWH). The RMRP gene encodes the RNA subunit of the ribonucleoprotein endoribonuclease, RNase MRP. So far, 5 mutations causing Anauxetic dysplasia, at least 62 mutations causing CCH and MDWH, and 8 SNP's have been identified. We recently demonstrated that RMRP gene mutations affect growth regulation by altering cyclin dependent cell cycle regulation and impaired ribosomal assembly (Thiel et al. 2005 Am J Hum Genet).
Mutations located between the TATA box and the transcription starting site are reducing the transcription level of the RNA subunit. To clarify the influence of different base substitutions within the transcript on the clinical phenotype, 11 CHH/MDWH and 2 novel AD mutations each in different functional domains of the RMRP gene were tested for rRNA and mRNA cleavage activity. Whereas the AD mutations g.14G>A and c.256_265delCAGCGCGGCT revealed a lack of expression, all remaining mutations demonstrated a variable decrease in cleavage activity. The degree of short stature and skeletal dysplasia correlated with the overall functional impairment, but mainly of rRNA cleavage activity (ribosomal assembly). Whereas significantly diminished mRNA cleavage activity (cyclin dependent cell cycle regulation) was a prerequisite for immunodeficiency. Thus, the clinical phenotype emerges in most cases of the combined effect of either a hypomorphic allele, a null allele, or the respective effect on the cleavage activity.

W2 04

A new subtype of brachydactyly type B is caused by specific mutations in NOGGIN

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Brachydactyly type B (BDB) is characterized by terminal deficiency of fingers and toes. Due to its characteristic phenotype, BDB can be easily distinguished from other types of inherited brachydactylies. So far, heterozygous truncating mutations in the receptor tyrosine kinase-like orphan receptor 2 (ROR2) are known to cause BDB (BDB1, MIM #113000) in the majority of affected individuals. The molecular basis of ROR2 negative BDB is unknown. In a subset of these individuals, we identified specific mutations in NOGGIN (NOG) which are located within the highly conserved NOG/BMP binding pocket. The mutations are expected to alter NOG's ability to inhibit bone morphogenetic proteins (BMPs) thus disturbing the intricate balance of signaling. This new subtype of BDB is characterized by the absence of distal phalanges together with proximal symphalangism (SYM1, MIM #185800), a condition known to be associated with haploinsufficiency of NOG or activating mutations in the BMP-analog GDF5. A BDB phenotype is not reported as a typical feature in SYM1. Thus, we describe a new clinical entity with a distinct molecular cause and suggest to call this condition BDB type 2. The phenotypic overlap between BDB1 and BDB2 indicates a functional connection between the BMP and ROR2 pathway.

W2 05

Extraintestinal tumour spectrum in MUTYH-associated polyposis (MAP)

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MUTYH-associated polyposis (MAP) is a recently discovered autosomal-recessive precancerous condition of the colorectum which is caused by germline mutations in the base excision repair (BER) gene MUTYH. MAP is supposed to be associated with a colorectal cancer lifetime risk of up to 100%, comparable to familial adenomatous polyposis. However, there are only sporadic descriptions of extraintestinal manifestations. Here we report on a systematic evaluation of the tumour spectrum in German MAP patients, based on medical records and anamnestic information. To date, 65 biallelic MUTYH mutation carriers were examined (48 index cases, 17 affected relatives). The median age at evaluation was 54 years (range 29-85). 32 patients (49%) had extracolonic tumours (19 patients had one tumour, 8 had two, 5 had three different tumours). The following tumours were observed more than once: breast cancer in 3 cases (10% of females; age at diagnosis 49-60 years); endometrial cancer in 3 cases (10% of females; age at diagnosis 32-54); ovarian cancer in 2 cases (6% of females; age at diagnosis 45-56); skin cancer (melanoma, spinalioma) in 4 cases (6%; age at diagnosis 32-68); bladder carcinoma in 2 cases (3%; age at diagnosis 45-62); teratomas in 2 cases (3%); lipomas in 5 cases (8%); benign skin tumours including sebaceous gland epithelioma and epidermoid cysts in 11 cases (17%); jaw-bone cysts in 3 cases (5%); and hepatic cysts in 4 cases (6%). Compared to the age-related population-based tumour risk these preliminary data indicate a significantly increased incidence of gynaecological cancer (endometrium, ovary) and cutaneous tumours in MAP patients. The risk of breast cancer is close to the age-related female population risk. The results may influence future surveillance recommendations and contribute to the understanding of the underlying pathophysiological mechanisms in MAP.
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W2 06

Mapping of genetic modulators for cystic fibrosis disease severity on 12p13, 16p12 and 19q13

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Candidates for genetic modifiers in CF are elements of host defence such as the TNFalpha receptor and of ion transport such as the amiloride-sensitive epithelial sodium channel ENaC both of which are encoded side by side on 12p13 (TNFRSF1A, SCNN1A) and 16p12 (SCNN1B, SCNN1G). 37 families with F508del-CFTR homozygous siblings exhibiting extreme clinical phenotypes from the European CF Twin

and Sibling Study were genotyped at 12p13 and 16p12. The ENaC was identified as a modulator of CF by transmission disequilibrium at SCNN1G and association with CF intrapair discordance at SCNN1B. Family-based and case-control analyses and sequencing of SCNN1A and TNFRSF1A uncovered an association of the TNFRSF1A intron 1 haplotype with disease severity. Carriers of risk haplotypes were underrepresented suggesting a strong impact of both loci on survival. The finding that TNFRSF1A, SCNN1B and SCNN1G are clinically relevant modulators of CF disease supports current concepts that the depletion of airway surface liquid and inadequate host inflammatory responses trigger pulmonary disease in CF.
TGFβ1 is encoded on 19q13, 4.5 Mbp from the CFM1 locus that confers a risk of meconium ileus. We scanned this 19q13 region whereby neither the two TGFβ1 SNPs (-509 and codon 10) nor D19S112 at CFM1 was associated with disease severity in our cohort. Interrogation of the region between TGFβ1 and CFM1 by four microsatellites revealed a significant transmission disequilibrium in clinically discordant sibling pairs, suggesting a modulation of factors acting in trans. Moreover, we have analysed our genotype data for indicators of transmission-ratio distortion, imprinting, maternal genetic or maternal non-genetic effects mediated by elements on 19q13 to identify reasons for false-positive findings in previous studies.
In conclusion, we were able to discriminate between a genetic modulator and a neighbouring but equally plausible candidate gene on 12p13 and 19q13.

W3 Therapy of Genetic Disease

W3 01

Targeting TGF-ß mediated glioma cell migration and brain invasion by the PPAR-gamma agonist troglitazone
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Gliomas are the most common primary tumors of the central nervous system, with glioblastomas as the most malignant entity. Rapid proliferation and diffuse brain invasion of these tumors are likely to determine the unfavourable prognosis. Considering its pro-migratory properties, the transforming growth factor ß (TGF-ß) signaling pathway has become a major therapeutic target. Analyses of resected glioma tissues revealed an intriguing correlation between tumor grade and the expression of TGF-ß1-3 as well as their receptors I and II. Here, we analyzed the effects of peroxisome proliferator-activated receptor gamma (PPAR-gamma) agonists on glioma proliferation, migration and brain invasion. Using an organotypic glioma invasion model, we show that micromolar doses of the PPAR-gamma activator troglitazone (TRO) blocked glioma progression without neurotoxic damage to the organotypic neuronal environment observed. This intriguing anti-glioma property of TRO appears to be only partially based on its moderate cytostatic effects. We identified TRO as a potent inhibitor of glioma cell migration and brain invasion, which occurs in a PPAR-gamma independent manner. The anti-migratory property of TRO was mediated by transcriptional repression of TGF-ß1-3 and their receptors I and II and associated with reduced TGF-ß1-3 secretion. TRO is the first compound described to counteract TGF-ß signaling and may thus represent a promising drug for adjuvant glioma therapy. Based on our findings, TRO-derivatives which are unable to activate PPAR-gamma but retain their propensity to antagonize TGF-ß signaling may be developed to specifically inhibit TGF-ß dependent motility and invasiveness of glioblastoma and other highly migratory tumor entities.

W3 02

Early-onset epileptic encephalopathy caused by autosomal recessive PNPO deficiency is treatable with pyridoxal 5-phosphate
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Inborn errors of pyridoxine metabolism have been recognised as a new group of disorders causing neonatal epileptic encephalopathy. We report six children with pyridox(am)ine 5´-phosphate oxidase (PNPO) deficiency from three independent families who presented with neonatal epileptic encephalopathy. Mutation analyses showed homozygosity for mutations R95C (c.283C>T) in the PNPO gene in two unrelated families of Lebanese and Lebanese/Iraqi origin, and compound heterozygous for a missense mutation, D33V (c.98A>T), and a single base pair deletion, c.246delT, in the PNPO gene, in a Swiss family. Two children were treated with pyridoxal 5'-phosphate (PLP) within the first month of life and showed normal development or moderate psychomotor retardation thereafter. Four children with late or no treatment died or showed severe mental handicap. All of the children showed atypical biochemical findings; in particular, biochemical features of reduced aromatic L-amino acid decarboxylase activity such as a decrease of serotonin in plasma or a decrease of homovanillic and 5-hydroxyindoleacetic acids in CSF, thought to be the most suggestive and characteristic findings may only be transiently present or absent altogether. Quick treatment with PLP in all neonates and infants with epileptic encephalopathy should become mandatory and may permit normal development in those affected with PNPO deficiency. Prenatal treatment may be feasible in families with a known risk for affected children.

W3 03

Growth hormone is effective in treatment of short stature associated with SHOX deficiency: two-year results of a randomized, controlled, multi-center trial
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The SHOX gene encodes a homeodomain transcription factor responsible for a significant proportion of long-bone growth. Patients with mutations or deletions of SHOX, including those with Turner syndrome [TS] who are haploinsufficient for SHOX, have variable degrees of growth impairment, with or without a spectrum of skeletal anomalies consistent with dyschondrosteosis. 52 prepubertal subjects (24 male, 28 female; age 3.0-12.3 years) with a SHOX gene defect and height below the 3rd percentile for age + gender (or height below the 10th percentile and height velocity below the 25th percentile) were randomized to either a GH-treatment group (n=27) or an untreated control group (n=25) for 2 years. To compare the GH treatment effect between subjects with SHOX-D and those with TS, a 3rd study group, comprised 26 pts with TS aged 4.5-11.8 years, who also received GH. Between-group comparisons of 1st year and 2nd year height velocity, height standard deviation score (SDS) and height gain (cm) were performed using analysis of covariance accounting for diagnosis, sex and baseline age. The GH-treated SHOX-D group had a significantly greater 1st year height velocity than the untreated control group (mean ± SE: 8.7±0.3 vs. 5.2±0.2 cm/y, $p<0.001$) + similar 1st year height velocity to GH-treated subjects with TS (8.9±0.4 cm/y, $p=0.592$). GH-treated subjects also had significantly greater 2nd year height velocity (7.3±0.2 vs. 5.4±0.2 cm/y, $p<0.001$), 2nd year height SDS (-2.1±0.2 vs. -3.0±0.2, $p<0.001$) and 2nd year height gain (16.4±0.4 vs. 10.5±0.4 cm, $p<0.001$) than untreated subjects. This large scale, randomized, multicenter clinical trial in subjects with SHOX-D demonstrates marked, highly significant, GH-stimulated increases in height velocity and height SDS during the 2-year study period. The efficacy of GH treatment in

subjects with SHOX-D was equivalent to that seen in subjects with TS.
Conclusion: GH is effective in improving the linear growth of patients with various forms of SHOX-deficiency.

W3 04

Mice made with ES-cell derived sperm display growth abnormalities and aberrant imprinted gene methylation
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Transplantation of ES-cell derived spermatogonial stem cells into the testes of germ cell depleted mice can restore complete spermatogenesis. ICSI of ES cell-derived sperm into unfertilized eggs produced embryos and induced full term development. However, most newborns made with ES cell-derived sperm were either too small or too big and died between five days and five months after birth. Since the observed growth abnormalities are likely to be caused by dysregulation of epigenetic components, we analyzed the methylation status of three representative imprinted genes, H19, Snrpn and Igf2r in spleen, kidney and testes of three offspring with overgrowth, two offspring with growth retardation and one control mouse. The majority of analyzed tissues in mice made with ES-cell derived sperm showed aberrant methylation patterns of the differentially methylated regions (DMRs), which regulate imprinted expression of our three study genes. In contrast, the control mouse displayed normal methylation of all three analyzed DMRs. The most striking observations were a strong hypomethylation of the H19 and Snrpn DMRs in spleen of two mice with overgrowth and a strong hypermethylation of the Snrpn DMR in spleen of one growth retarded mouse. Strong hypermethylation of the Igf2r DMR was seen in spleen and kidney of a mouse with overgrowth and in spleen of a growth retarded mouse. Thus, similar methylation abnormalities were not always associated with the same phenotype. We assume that the observed growth abnormalities are not directly linked to specific imprinting defects in our study genes, but result from a cumulative and stochastic dysregulation of multiple imprinted and possibly non-imprinted genes due to faulty epigenetic reprogramming during gametogenesis and/or early embryogenesis.

W3 05

The 1139del21 and g1743c variants of BARD1 increase cellular radiosensitivity
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Mutations of the two breast cancer susceptibility genes BRCA1 and BRCA2 increase cellular radiosensitivity, which can be detected with the G0 – micronucleus test. Higher micronucleus frequencies after irradiation have also been detected in the majority of sporadic breast cancer patients, compared to controls. The two genes ATM and BARD1 are both involved in the repair of DNA double strand breaks, and interact with BRCA1 or BRCA2. The aim of the present study was to ascertain if variants of ATM or BARD1 can imply enhanced sensitivity to ionizing radiation. Cellular radiosensitivity was measured with the G0 – micronucleus test after exposure of lymphocytes to ionizing radiation, in 67 breast cancer patients and 73 patients without carcinoma. In ATM and BARD1, four polymorphisms each were genotyped with the ddNTP primer extension method and tested for an association with the MN-frequency. Statistical analysis was conducted with StatView 5.01 and JMP IN 5.1. None of the variants of the ATM gene (gIVS17-58a, tIVS38-8c, g5557a, gIVS62-60a) shows a significant effect on micronucleus rates. There was no evidence that the tested ATM variants do affect cellular radiosensitivity. The micronucleus frequencies of heterozygous carriers of the rare variants of the BARD1 gene, 1139del21 or g1743c, are significantly higher compared to controls. The common c1207g or g1592a variants of BARD1 did not reveal any influence on MN frequencies. These results indicate that BARD1 variants may have an effect on DNA repair capacity that can be detected with the G0 – MNT. This is consistent with the proposed association of the 1139del21 and g1743c variants with increased breast cancer risk.

W3 06

Humans and chimpanzees differ in their cellular response to DNA damage
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Compared to humans, chimpanzees are less susceptible to many types of cancer. Because defects in DNA repair lead to accumulation of mutations in the genome and finally to the development of cancer, species differences in DNA repair are one plausible explanation. To test this hypothesis, we have analyzed the repair kinetics of human and chimpanzee cells after cisplatin treatment and X-ray irradiation. DNA-immunodot-blots for the quantification of single-stranded (ss) DNA repair intermediates revealed a biphasic response of human and chimpanzee lymphoblasts to cisplatin-induced damage. The "fast" phase of DNA repair was identical in both species with a peak of ssDNA intermediates at 1 h after DNA damage. However, the "slow" phase of DNA repair differed: Human cells showed a second peak of ssDNA intermediates at 6 h, chimpanzee cells at 5 h. We have developed ICP MS (inductively coupled plasma mass spectrometry) coupled with a gel electrophoresis to directly measure the cisplatin integrated in DNA fragments. This allows one to quantify DNA damage in human and chimpanzee cells. To test a second DNA repair pathway, human and chimpanzee lymphoblasts were exposed to an X-ray dose of 4 Gy. The number of double-strand breaks (gH2AX foci) was quantified in three independent cell lines of each species, using immunofluorescence staining with an antibody against phosphorylated histone H2A. Although shortly (at 30 min) after irradiation cells of both species displayed comparable numbers of double-strand breaks, chimpanzee cells repaired much faster. At 1 h chimpanzee cells had significantly less foci than human cells, whereas at 2 h the number of unrepaired DNA breaks was similar in both species. Our results suggest an accelerated response of chimpanzee cells to different types of DNA damage. Consistent with species-specific DNA repair kinetics, expression profiling with customized cDNA microarrays showed differential regulation of some DNA repair genes in human and chimpanzee.

W4 Complex Disorders

W4 01

Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham heart study
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Background: Bilirubin is an antioxidant that suppresses lipid oxidation and retards atherosclerosis formation. An inverse association between serum bilirubin and coronary heart disease has been reported. Linkage studies have identified a major locus at the chromosome 2q telomere that affects bilirubin concentrations. A candidate gene in the linkage region encodes hepatic bilirubin uridine diphosphate-glucuronosyltransferase (UGT1A1). The insertion of a TA in the TATAA box of the gene, an allele designated UGT1A1*28, decreases gene transcription. Individuals homozygous for UGT1A1*28 (genotype 7/7) have increased serum bilirubin levels compared with carriers of the 6 allele. To date, no significant association between UGT1A1*28 and cardiovascular disease (CVD) events has been reported. We performed an association study in the Framingham Heart Study population to investigate whether UGT1A1*28 is associated with the risk of CVD events.
Methods and results: The study population included 1780 unrelated individuals from the Offspring cohort (49% males, mean age 36 years at entry) who had been followed up for 24 years. Individuals with genotype 7/7 had significantly higher bilirubin levels (mean±SD 1.14±0.44 mg/dL) than those with genotypes 6/6 and 6/7 (mean±SD 0.69±0.27 mg/dL, $P<0.01$). Using the Cox proportional hazards model, we found sig-

nificant associations between the UGT1A1*28 allele and decreased risk of CVD. Individuals with genotype 7/7 (population frequency of 11%) had approximately one third the risk for CVD and coronary heart disease as carriers of the 6 allele, which resulted in a hazard ratio (95% confidence interval) of 0.36 (0.18 to 0.74) and 0.30 (0.12 to 0.74), respectively.
Conclusions: Homozygote UGT1A1*28 allele carriers with higher serum bilirubin concentrations exhibit a strong association with lower risk of CVD.

W4 02

Polymorphism near the insulin induced gene 2 (INSIG2) is associated with obesity
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A 100k whole-genome SNP scan in families of the Framingham Heart Study revealed association of a SNP (rs7566605) upstream of the insulin induced gene 2 (INSIG2) with obesity in children and adults. Homozygotes for the C allele of rs7566605 were on average 1 BMI-unit heavier than individuals with the other two genotypes. This obesity predisposing genotype was present in about 10% of individuals (Herbert et al., Science 2006). However, apart from four independent confirmations including a sample of ours encompassing 368 trios based on an obese offspring and both parents, the initial study also reported a non-confirmation. Hence, independent confirmations are warranted. We genotyped rs7566605 in 1,381 German adults (990 obese cases: BMI > 30kg/m2; mean BMI 36.02 ± 5.38 and 391 lean controls: BMI < 20 kg/m2, mean BMI 18.17 ± 1.00) and performed an association analysis under a recessive mode of inheritance. The frequency of CC homozygotes was increased in obese (12.32%) compared to controls (7.24%; p=0.005). Additionally, we extended the number of German families to a total of 589 (221 additional independent German obesity families comprising at least two obese sibs and both parents) and calculated an effect size estimate given a recessive mode of inheritance. In light of the potential relevance of the severity of obesity our first analysis was based on only the sib with the higher BMI percentile of each sib pair. With this approach we obtained a genotype relative risk of 1.49 (95%CI 1.09-2.03, p=0.006, Wald test, one-sided within conditional regression model) for CC homozygotes. However, inclusion of all sibs resulted in a genotype relative risk of only 1.11 (95%CI 0.84-1.47 p=0.23). Our findings are a confirmation of the recessive effect of the C allele in obesity in an independent case control study. The results in our families substantiate the notion that the CC genotype might be particularly relevant in extreme obesity

W4 03

The common SNP KCNH2-K897T is significantly associated with atrial fibrillation: Results from a large linkage-disequilibrium based study
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Aims: Atrial Fibrillation (AF) has a prevalence of at least 1% in the general population. While monogenic forms of AF are known, the genetic background of common AF is poorly understood. Cardiac ion channels play a major role in its pathophysiology. We intended to clarify, if common variants of KCNH2, encoding the cardiac rapid delayed rectifier potassium-channel, predispose to AF.
Methods: In stage I we examined 40 haplotype tagging SNPs of a 150 kbp genomic region (chr7: 150030000-150180000) around KCNH2 (NM_000238). Tagging criteria were r2=0.8, MAF=5% and pairwise tagging based on the HapMap release #20 CEU population. 663 patients with AF from the Deutsches Herzzentrum München and 702 controls from the KORA-S4 study in Augsburg were studied. SNPs significantly associated with AF in stage I ($p \leq 0.05$) were replicated in stage II. Here we genotyped 521 participants with AF from the Großhadern University Hospital of Munich and the AFNET-study. Controls were 1796 different individuals from the KORA-S4 study. Genotyping was performed using PCR, iPlex primer extension and MALDI-TOF mass-spectrometry.
Results: Five variants (rs1805123, rs4725984, rs2373962, rs6951150, rs1799983) showed significant associations with AF in stage I. In stage II only KCNH2-K897T (rs1805123) could be replicated (p=0.0164). This association was analyzed in the entire study population. The calculated odds ratio was 1.25 (95% CI 1.11-1.41, p=0.00033) for a log-additive model of inheritance. The common 897K-allele predisposed to AF while the rare 897T-allele was protective.
Discussion: The results of this large, systematic linkage-disequilibrium based study in 1209 cases and 2399 controls suggest that KCNH2-K897T is associated with AF and explains partly the genetic background of AF. Interestingly, the rare allele protects against AF, although it goes along with a shortened QT interval. This raises pathophysiological questions that need to be addressed; a haplotype analysis is ongoing.

W4 04

Association and linkage of allelic variants of the dopamine transporter gene in ADHD
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Introduction: Previously, we had reported a genome wide scan for attention-deficit/hyperactivity disorder (ADHD) in 102 affected sibs of German ancestry; the highest multipoint LOD score of 4.75 was obtained on chromosome 5p13 (parametric HLOD analysis under a dominant model) harbouring the dopamine transporter gene (DAT1).
Methods: We genotyped 30 single nucleotide polymorphisms (SNPs) in this candidate gene and its 5´region in 329 families (including the 102 initial families) with 523 affected offspring.
Results: We found that:
a) SNP rs463379 was significantly associated with ADHD upon correction for multiple testing (p= 0.0046);
b) the global p-value for association of haplotypes was significant for the second block upon correction for all (n=3) tested blocks (p=0.0048);
c) within block two we detected a nominal p = 0.000034 for one specific marker combination. This CGC haplotype showed relative risks of 1.95 and 2.43 for heterozygous and homozygous carriers, respectively;
d) finally, our linkage data and the genotype-IBD sharing test (GIST) indicated that genetic variation at the DAT1 locus explains our linkage peak and that rs463379 (p<0.05) is the only SNP of the above haplotype that contributed to the linkage signal.
Conclusion: In sum, we detected that genetic variation at the DAT1 locus underlies our ADHD linkage peak on chromosome 5; additionally solid association for a single SNP and a haplotype were shown. Future studies are required to assess if variation at this locus also explains other positive linkage results obtained for chromosome 5.
Acknowledgements: The participation of the probands and their families is gratefully acknowledged. This project was supported by the National Genome Research Net (NGFN).

W4 05

Genomewide 100k and 500k association studies identify quantitative trait loci for QT-interval at the NOS1AP and other genes

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Background: The QT-interval from the ECG is a normally distributed quantitative trait in the general population and is associated with sudden cardiac death. Its heritability is between 30% and 50%. In a previously published 100k scan we have identified a QTL for QT-interval at the NOS1AP gene ($p<1e-11$ in n=3,966).
Aim: To map additional modifier genes we now undertook a 500k-genomewide association scan in more than 1,500 general population individuals from KORA.
Method: From the population-based KORA S4 survey n=1.664 individuals were randomly selected and genotyped on Affymetrix 500k arrays.
Results: In the 500k dataset the QTL at the NOS1AP gene gave a clear and unmistakably association signal. Overall n=60 SNPs throughout a 500kb genomic region were associated with p-values between p=1e-4 and p=1e-7. In addition we identified nine more QTLs between 2 and 5 SNPs in size with association signals between p=1e-4 and p=1e-6. Three of these additional QTLs tested positively ($p<0.01$) in a n=1,200 replication sample.
Conclusions: The QTL at the NOS1AP has been identified in a 100k scan and was now identified as clearly the strongest genomewide signal in a 500k scan. Its well identifyable signal is due to its high allele frequency (MAF=0.35), 500kb long LD relationship and relatively strong effect (ca. 2% explained variance). Three other highly suggestive QTLs show significant associations in a replication sample and are currently followed up in even larger population based samples. They have less favourable effect sizes, LD patterns and allele frequencies decreasing their probability of detection or are located in genomic regions not covered by the 100k-array which explaines why they went undetected in the 100k scan.

W4 06

250K SNP genome scan in age-related macular degeneration (AMD)

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Introduction: AMD is the leading cause of blindness in industrialized countries. Frequent single nucleotide polymorphisms (SNPs) at two gene loci namely complement factor H (CFH) at 1q31 and LOC387715/HTRA1 at 10q26 have been shown to contribute to AMD risk. Together, risk alleles at CFH and LOC387715/HTRA1 account for up to 60% of AMD cases. Other as yet unidentified genes likely contribute to disease risk although eventually with minor contributions to the overall disease load.
Methods: A genome-wide association study was performed using the Affymetrix 250K array. AMD patients (initial group n=339, confirmation study n=739) were recruited at the Eye Hospital Wuerzburg. SNP data from a total of 1179 gender-matched, population-based controls were included from the KORA study group. The case/control association design provides enough power to detect common risk alleles with frequencies over 0.1 assuming a threshold for relative risk equalling 1.6. Quality threshold parameters were set as follows: array call rate $\geq$ 0.9, HWE p-value $\geq$ 2.5x10-7 (Bonferroni adjusted significance), individual SNP call rate $\geq$ 0.925.
Results: Analyzing the dataset under five genetic models revealed 94 loci with p-values ranging from 1.5x10-26 to 1.0x10-4. Seven SNP markers were located either within the CFH or the 10q26 loci. In addition, we identified 62 different candidate regions with single (n=45; p-values between 1.5x10-6 and 1.0x10-4) or multiple association signals (n=17; p-value between 2.2x10-6 and 8.9x10-5). In a second phase, these SNPs were replicated by iPLEXing an independent AMD (n=294)/control (n=314) group. In cases with reproducible significance an extended panel of neighbouring SNPs was tested in 739 cases/759 controls.
Conclusions: Our analysis of 62 candidate loci is in progress. SNP fine mapping of the respective loci will facilitate the exclusion of false positives but should also allow the identification of novel candidate genes in AMD susceptibility.

W5 Molecular Mechanisms

W5 01

LADD syndrome is caused by mutations that reduce the tyrosine kinase activity of FGFR2

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Lacrimo-auriculo-dento-digital (LADD) syndrome is an autosomal dominant disorder mainly affecting the lacrimal system, the ear-shape and hearing, teeth development, and digit patterning. We recently identified the molecular basis of LADD syndrome and found heterozygous missense mutations in LADD families in FGFR2, and FGFR3, and FGF10, one of FGFR2 ligands. Notably, the mutations in the FGF-receptor molecules were located in regulatory regions of the tyrosine kinase domain of the receptors.
Previous studies have shown that different craniosynostosis syndromes are caused by gain-of-function mutations in FGFR2 which result in elevated tyrosine kinase activity of FGFR2. We have compared the tyrosine kinase activity of three LADD-FGFR2 mutations and demonstrated that their intrinsic tyrosine kinase activity is reduced as compared to the tyrosine kinase activity of wild type FGFR2 or to the constitutively activated tyrosine kinase activity of FGFR2 mutant responsible for Pfeiffer syndrome. In addition, we show for the first time that FGFR2 autophosphorylation is a sequential and precisely ordered mechanism and that LADD mutations dramatically affect this ordered phosphorylation process. Furthermore, stimulation with a LADD-FGF10 mutant protein led to a decreased FGFR2 activation and to compromised MAPK stimulation as compared to stimulation of these responses induced by wild type FGF10. We conclude that FGFR2- and FGF10-LADD mutations lead to reduced FGFR2 autophosphorylation and diminished cell signaling via FGFR2 and that the reduced tyrosine kinase activity of FGFR2 is a hallmark underlying the molecular basis of LADD syndrome.

W5 02

The PP2Ac-specific ubiquitin ligase MID1/alpha4 assembles an mRNP that regulates microtubule-associated protein production

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How protein gradients are established as a basis for cell migration and polarization is unclear, although there is general agreement that the process involves tightly regulated protein translation localized at the cytoskeleton. Here, we show that the midline regulatory E3 ubiquitin ligase MID1 and the protein phosphatase 2A subunit alpha4 associate with active polyribosomes, mRNA and several proteins involved in mRNA transport and translation, including RACK1, Annexin A2 and Nucleophosmin, thus forming a microtubule-associated ribonucleoprotein complex (mRNP). We further show that this cytoskeleton-

bound mRNP regulates the amount of protein produced from mRNAs that are specifically associated with the complex via G-rich RNA-protein motifs, known as G-quartets. Destruction of the G-core of the G-quartet structure by a single point mutation destroys the binding of the mRNA to the mRNP and its influence on expression of the protein. Similarly, mutations in the MID1 protein, as found in Opitz BBB/G syndrome (OS) patients with abnormal ventral midline development, disrupt this regulatory mechanism. Therefore, our findings not only provide a novel mechanism for the compartmentalized control of protein production but also offer a molecular basis for the development of OS and related disorders.

W5 03

The cohesion protein NIPBL is associated with chromatin-remodeling enzymes

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Cornelia de Lange Syndrome (CdLS) is a rare malformation disorder with multiple congenital anomalies, a characteristic face, growth and mental retardation as well as gastrointestinal and limb abnormalities. About 50% of the patients with CdLS carry mutations in the NIPBL gene. NIPBL encodes a protein called delangin, a homologue of fungal Scc2-type sister chromatid cohesion protein and the Drosophila Nipped-B developmental regulator. Delangins are known to be involved in sister chromatid cohesion, probably as part of a protein complex recruiting cohesion to particular sites of the chromatin. Recent papers show that chromatin-remodeling complexes also play a role in sister-chromatid cohesion, either in the recruitment of cohesion to particular sequences along chromosome arms or in the establishment of cohesion. In yeast-two-hybrid assays, with NIPBL as bait, we could identify the chromatin remodeling factors histone deacetylases 1 and 3 (HDAC1 and 3) as potential NIPBL-interacting proteins. Using of a diversity of fragments encoding parts of NIPBL in liquid β-galactosidase assays, we could narrow down the interacting region for HDAC1 and 3 to a stretch of 162 amino acids (aa) within a highly conserved region of NIPBL. These 162 aa perfectly overlap with a region which was predicted to be a HDAC-interacting domain by in silico studies. To confirm whether this domain is able to recruit the chromatin remodeling factors HDAC1 and HDAC3 to DNA we fused it to the GAL4 DNA-binding domain. This fusion construct and a GAL4-TATA luciferase reporter were used for cotransfections of different mammalian cell lines. Interestingly, the NIPBL construct reduces the luciferase reporter gene expression down to 50%. Cotransfection with HDAC1 and HDAC3 respectively, even enhances this effect significantly. Whereas cotransfection with HDAC6 as a control does not alter the activity. Our data show for the first time a specific association of NIPBL with chromatin-remodeling enzymes.

W5 04

The murine Dnali1 gene encodes an axonemal dynein that is essential for embryonic development

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Axonemal dyneins are large motor protein complexes generating the force for the movement of eukaryotic cilia and flagella. Disruption of axonemal dynein function can result in male infertility or lateralization defects. We have cloned the murine gene encoding the dynein axonemal light intermediate chain Dnali1. The Dnali1 gene is predominantly expressed within the testis but at a lower level Dnali1 transcripts were also observed in different murine tissues. Antibodies were raised against a GST-Dnali1 fusion protein and used to localize Dnali1 in cilia of the trachea as well as in flagella of mature sperm supporting its function as an axonemal dynein. To identify putative Dnali1 interacting polypeptides a yeast two hybrid approach was performed. By this assay the C-terminal part of the cytoplasmic dynein heavy chain 1 was identify as a putative interacting polypeptide of Dnali1. The interaction between the axonemal and the cytoplasmic dynein fragments was proven by co-immuno and co-localization experiments. To analyse the function of the Dnali1 gene a targeted disruption was carried out by homologous recombination. Heterozygous animals were found to be fertile and had no apparent abnormalities. However, no Dnali1-/- offspring were obtained, indicating that Dnali1 gene deficiency leads to embryonic lethality. The Dnali1 deficient embryos die shortly after implantation, due to a putative defect in the differentiation of the embryonic inner cell mass. Interestingly, Dnali1 is associated with the Golgi apparatus in early embryos. Thus, it can be speculated that Dnali1 function is necessary for the organization of the Golgi apparatus and is required for the proper function of this organelle presumably as a putative component of the cytoplasmic dynein complex machinery. In summary, our findings suggest that components of the axonemal dynein complex are essential in mammalian embryonic development.

W5 05

Multiple roles for neurofibromin 1 in skeletal development and growth

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Neurofibromatosis type I (NF1) is an autosomal, multisystem disease with an incidence of 1:3500 live births. The skeleton is frequently affected in NF1, and bone abnormalities are present in approx. 50% of patients. Major skeletal symptoms associated with this disease are severe and progressive scoliosis, congenital tibia bowing, thinning and pseudarthrosis of long bones, focal bone lesions, sphenoid wing dysplasia, and focal bone gigantism. In order to address the hypothesis that bone abnormalities in NF1 are directly related to a yet unknown function of /NF1/ in skeletal development and/or remodeling we inactivated NF1 gene in the limb-mesenchyme using Prx1-Cre mice. Limb specific Nf1 knockout recapitulates key aspects of Von Recklinhausen's disease associated bone dysplasia, including long bone shortening and tibia bowing. Our results indicate tibial bowing is caused by decreased stability of the cortical bone due to a high degree of porosity, decreased stiffness and reduction in the mineral content as well as hyperosteoidosis. Accordingly, osteoblasts show an increase in proliferation and a decreased ability to differentiate and mineralize in vitro. The reduction in growth is due to lower proliferation rates and a differentiation defect of chondrocytes. Abnormal vascularization of skeletal tissues is likely to contribute to this pathology as it exerts a negative effect on cortical bone stability. Furthermore, Nf1 has an important role in the development of joints, as shown by joint fusions and other abnormalities. Thus, Nf1 has multiple essential roles in skeletal development and growth.

W5 06

Constitutional KRAS mutations are associated with a broad phenotypic spectrum and predicted to cause diverse gain-of-function mechanisms

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Noonan syndrome (NS), cardio-facio-cutaneous syndrome (CFC), and Costello syndrome (CS) constitute a group of developmental disorders with a pattern of overlapping congenital anomalies. Each of these conditions is caused by germline mutations in key components of the highly conserved Ras-MAPK pathway, possibly reflecting a similar pathogenesis underlying the three disorders. Germline mutations in KRAS have recently been found in a small number of patients with NS and CFC. We screened a total of 276 patients and identified heterozygous mutations in 12 patients, including 3 known and 8 novel sequence KRAS alterations. All mutations are predicted to cause single amino acid substitutions. Remarkably, our cohort of individuals with KRAS mutations showed a high clinical variability, ranging from NS to CFC, and also included two patients who met the clinical criteria of CS. Our findings refine the picture of a clustered distribution of disease-associated KRAS germline alterations. We further delineate the phenotypic spectrum associated with KRAS missense mutations and provide first evidence for clinical differences in patients with KRAS mutations compared with NS-affected individuals with heterozygous PTPN11 mutations and CFC patients carrying a BRAF, MEK1, or MEK1 alteration, respectively. Structural analysis of the known and newly identified amino acid substitutions in K-Ras in accordance with available functional data indicate the existence of various gain-of-function mechanisms of the altered K-Ras proteins, including spontaneous GTP-GDP exchange (fast cycling), resistance to GTPase-activating proteins (GAPs), and sterical stabilization of the active, GTP-bound state. The central role of K-Ras in a number of different sinaling pathways together with a great functional diversity of altered K-Ras proteins probably explain the high phenotypic variability in patients with KRAS mutations.

W6 Population Genetics and Evolution

W6 01

The rare nonsynonymous SCN5A-S1103Y variant in Caucasians is due to recent African admixture as revealed by 100k SNP genotyping

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The SCN5A-S1103Y variant is a confirmed risk factor conferring an odds ratio up to 8.5 for cardiac ventricular arrhythmias and sudden cardiac death (Splawski et al, Science, 2002, Burke et al., Circulation, 2005, Plant et al., J. Clin. Invest. 2006). In Africans it is a common nonsynonymous SNP (MAF=8%), but it is rarely observed in Caucasians (Chen et al, J. Med. Genet. 2002). In a Bavarian family of Caucasian descent and affected with long-QT syndrome we have detected this variant in a heterozygote state as the only nonsynonymous variation upon diagnostic ion channel resequencing. To resolve the question, whether in the family the variant was
(a) of ancient African descent,
(b) due to recent African admixture or
(c) a de novo mutation, we analyzed the genetic segment it resided on.
Dense SNP genotyping in admixed individuals allows to infer the ethnicity of chromosomal regions if allele frequencies are known in the original populations. Ethnicity inference for any given locus can be carried out by applying the product rule to a sliding window of neighboring SNPs or via modeling ancestry by hidden Markov Chain Monte Carlo Methods (Tang et al. Am. J. Hum. Genet, 2006). By 100k SNP genotyping of the Bavarian family, we demonstrate that the S1103 variant is due to recent African admixture (b) and could rule out possibilities (a) and (c). This application demonstrates that inferring ethnicity of chromosomal regions by high density SNP genotyping is a powerful approach with prospects also to admixture mapping of disease loci and population stratification correction of genomewide association mapping of complex disease loci.

W6 02

The Affymetrix 500K control project and beyond: Chip-based assessment of European genetic diversity

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In order to assess the degree of variation in the genetic background of white European populations, academic institutions including Erasmus University, Rotterdam, and Christian-Albrechts-University, Kiel, as well as industrial partners Affymetrix and GlaxoSmithKline have formed a collaboration to facilitate 500K chip-based SNP genotyping of samples of approximately 50 unrelated individuals, each systematically drawn from one of 20-30 different geographic locations in Europe. The main purpose of the study is to assess the impact of genetic variability upon the power and the validity of 500K genome-wide association studies, and to develop a subset of ancestry-sensitive SNPs that will allow the "calibration" of samples of unknown ethnic origin (e.g. in a clinical trial) against known populations. Parts of the data are being provided by the Affymetrix 500K control program; others are generated anew. We will report on the project status and present first results on the ascertained 500K SNP diversity of European populations.

W6 03

Population stratification across Europe

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Genetic disease association studies across Europe require a detailed knowledge of population structure and genetic ancestry. So far, SNP allele differences have been mainly tested between Europe and other continents while there is less known about European population stratification.
The European Community Respiratory Health Survey was performed in a representative population sample of 20- to 44-year-old men and women. 5,350 individuals in 19 centers ranging from Umea in the North to Huelva in the South of Europe could be analyzed. 22 anonymous telomeric SNPs from nearly all chromosomes were tested as well as 4 SNPs in 2 genes known to be under selective pressure.
4 SNPs were not in Hardy Weinberg equilibrium (2 in LCT, 1 in OCA, 1 anonymous SNP) while allele frequencies differed significantly from averaged European estimates. For example Galdakao, a Basque population, showed unique properties that were found also by genetic distance measures in the Parisian samples. Many of the anonymous SNPs showed a strong correlation with geographical latitude; LCT SNPs indicated additional micro-stratification as being different between rural and city regions. 9 SNPs showed a spurious association with body weight, a trait varying widely across Europe. Mixed effects models including city of origin, multiple regression including principal components as well as a genomic control approach all retained significant associations.
We conclude that there is clear indication of genetic stratification across Europe that is only partially corrected by current methods. Probably more SNP marker are needed to control for population stratification, where true ancestry informative SNPs would be of considerable value.

W6 04

Neolithic origin of the Mediterranean p.Thr93Met mutation in the DHCR7 gene causing Smith-Lemli-Opitz syndrome

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The Smith-Lemli-Opitz Syndrome (SLOS, MIM 270400) is a mental retardation and malformation syndrome with variable clinical severity. SLOS is caused by mutations in the delta7sterol-reductase gene (DHCR7, E.C. 1.3.1.21), which impairs the cholesterol biosynthesis.
The prevalence of the SLOS has been estimated to range from approximately 1:20.000 to 60.000 in European populations. Mutational spectra analysed in SLOS patients were significantly different across populations with frequency maximum of common mutations in East-Europe (p.W151X, p.V326L), North-West-Europe (g.IVS8-1G>C), and South-Europe (p.T93M).
In order to understand the relatively high frequency of SLOS mutations in the European population today and their geographically different distribution an age estimation for several common mutations was performed, SNP haplotypes were constructed based on cSNPs in the DHCR7 gene and mutation frequencies in SLOS patients of different origins were compared. The p.T93M mutation was found associated with 4 intragenic SNP haplotypes. Hence this mutation originated recurrently and has at least two founders, one in the north of Europe (associated with the A haplotype), and the other in the Southern part of Europe (J haplotype). SLOS patients from Spain (44 alleles), Italy (36 alleles), Greece (8 alleles), and Turkey (9 alleles) showed allele frequencies of the p.T93M mutation of 18%, 31%, 3/8 (circa 38%), and 4/9 (circa 44%) respectively. This might indicate a propagation of this mutation from the East of the Mediterranean Sea to the West. Interlocus extended haplotype variation was calculated in trio families with the p.T93M mutation, which resulted for D11S4139 and D11S1314 in 195 and 206 generations respectively (average 200), corresponding to about 6000 years since appearance (5800–6200). Hence a neolithic origin of this mutation associated to J haplotype which originated in the Middle-East and spread from east-to-west along with the farming culture seems likely.

W6 05

Fanconi anemia genes in vertebrates: evolutionary conservation, sex-linkage, and embryonic expression of FANCC and FANCG in avian cells

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Orthologues of the human Fanconi anemia (FANC) genes have been identified in several vertebrate and invertebrate model organisms, indicating variously conserved functions of the FANC protein complex. In particular, the analysis of chicken DT40 cells has made important contributions to the functional characterization of FANC genes. Orthologs for most human FANC genes have been found in the chicken genome which is considerably smaller than the human genome. Indeed, we found specific FISH signals for both FANCC and FANCG on the short arm of the chicken Z sex chromosome with FANCC close to the centromere and FANCG at a telomeric position of Zp. We also show that the chicken FANCC and FANCG genes are highly expressed in multiple avian embryonic tissues, whereas in adult birds strong expression was only observed in gonads which underlines the putative function of these genes during premeiotic DNA replication and meiotic recombination. Even though a Fanconi anemia-like phenotype has not been described in avian species, FANC-deficient chicken cells were found to display the same sensitivity to DNA cross-linking agents as mammalian cells. Given the assignement of two of the FA gene orthologs to the chicken Z chromosome and the presumed absence of dosage compensation for Z-linked genes in birds, we tested the sensitivity of chicken embryonic fibroblasts from males (ZZ) and females (ZW) towards the bifunctional alkylating agent mitomycin C (MMC). We observed a striking gender difference at high doses of MMC, with female (ZW) fibroblasts being significantly more sensitive towards MMC than male (ZZ) fibroblasts. This observation is consistent with the absence of Z chromosome dosage compensation in birds, emphasizes the essential and conserved role of FANCC and FANCG in the cellular defence against DNA crosslinking agents, and may in part explain the greater longevity of male as opposed to female birds.

W6 06

A systematic HAPMAP-based survey of schizophrenia candidate genes in the German population

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Recent association studies have identified a number of potential schizophrenia susceptibility genes that currently await replication in independent, large samples of schizophrenia. In our present study, we systematically covered 6 promising genes with haplotype tagging SNPs capturing all haplotypes with a frequency >1% using HAPMAP phase I and II data and tested these SNPs for association in a total of 2,230 individuals, comprising a family-based sample of 210 parent-offspring trios with schizophrenia and in an independent sample of 715 schizophrenia patients and 738 controls, all of German origin. All diagnoses were made based on DSMIV criteria. Using Illumina's GoldenGate assays, we genotyped a total of 580 SNPs in these samples, covering the genes RGS4 in chromosomal region 1q23.3 (15 SNPs), the DISC1/DISC2/TSNAX locus on 1q42.1 (125 SNPs), ENTH on 5q33 (26 SNPs), DTNBP1 (Dysbindin) on 6p22.3 (39 SNPs), NRG1 on 8p21 (359 SNPs), and COMT on 22q11.2 (16 SNPs). We have completed genotyping of all individuals and data are currently under statistical analysis. Preliminary analyses show that genetic effect sizes seen in some of the genes are small and that there is evidence for gender-specific effects.

W7 Copy Number Variation

W7 01

Identification and characterization of subtelomeric aberrations by FISH and array CGH in 11 of 189 patients with unexplained mental retardation

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Subtelomeric chromosome aberrations are known to play an important role in the etiology of mental retardation (MR), especially in patients with multiple congenital anomalies (MCA). However, genotype-phenotype correlations are emerging slowly and only for the most frequent aberrations. Here, we analyzed 189 probands with MR, mostly combined with MCA, and normal karyotypes. Known causes of MR (e.g. microdeletion syndromes, fraX syndrome, metabolic disorders) had been excluded where appropriate. Screening was performed by two FISH

methods (Subtelomere COBRA FISH; Vysis ToTelVysion). All imbalances are being characterized precisely by array CGH or BAC/PAC-FISH.
Eleven causal subtelomere aberrations were detected (diagnostic yield: 5.8%). Five of the aberrations were de novo deletions [2q (size: 3.7Mb), 14q (5.7Mb), 16p (0.8Mb), 20p (~0.8Mb), 22q (0.6Mb)]. Six aberrations were unbalanced translocations [der(1)t(1;10)(pter-(4.3Mb); qter+(8.0Mb)), der(2)t(2;5)(qter-(2.8Mb); pter+ (17.7Mb)) de novo, der(2)t(2;18)(qter-(7.9Mb);pter+(0.5Mb)), der(10)t(10;18)(pter-(~6.2Mb);qter+(~3.1Mb)) de novo, der(11)t(11;16)(qter-(9.4Mb);qter+ (5.1Mb)) mat, der(22)t(7;22)(qter+(0.4Mb);qter-(2.0Mb)) de novo]. We also detected a new euchromatic variant (trisomy 16qter (<0.3Mb), der(18)t(16;18)(qter+;pter+)mat).
In addition to these molecular karyotypes, clinical phenotypes and examples for the resulting genotype-phenotype correlations will be given: The familial unbalanced t(11q;16q) further delineates the phenotype map for terminal deletion 11q syndrome (Jacobsen syndrome) and together with the 16q+ variant also for partial trisomies of 16q24. The three 2qter- cases contribute to the mapping of terminal deletion 2q syndrome traits. Several rare terminal deletion syndromes (e. g. 14q, 20p) can be characterized further.

W7 02

Molecular karyotyping with 100 K SNP arrays detects de novo submicroscopic chromosomal aberrations in 10% of 104 patients with unexplained mental retardation

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The establishment of an etiologic diagnosis in mental retardation is usually a challenge. We have recently shown that the etiology remains unknown in about 66% of cases, despite extensive clinical and laboratory work up. About 10% of these patients with unknown diagnosis harbor a submicroscopic chromosomal aberration as detected with high-resolution array-CGH or with molecular karyotyping on a 100 K SNP array using the CNAG and dChip programs. As these programs seemingly do not utilize the full capacity of the 100 K arrays, we developed a novel algorithm for aberration calling based on the gsap- and copy-number-values from the Affymetrix copy number tool and implemented it in a user friendly program Copy Number Variation Finder (CNVF). We now performed molecular karyotyping on 100 K SNP-arrays in 104 patients with mental retardation of unknown etiology using CNVF with two different sensitivity thresholds. CNV detected in array experiments was confirmed by q-PCR and/ or FISH. Using lower thresholds, we identified a deletion in 19 of 104 patients ranging from 0.15 Mb to 14 Mb (10 inherited, 9 de novo). 6 patients had a duplication (2 de novo). All in all, we found a de novo aberration in 10 patients (9.6%). 8 of the inherited and 1 de novo aberration were not detected by using the more stringent thresholds. Using the more sensitive parameters a total of 31 (average 1/3 samples) false positive aberration calls were observed, 11 of which occurred in the same patient. Data of 14 patients were also analyzed with the CNAG program. At least 4 aberrations detected with the CNVF were not detected with CNAG. Among the aberrations detected was a case of the only recently described 17q21.31 microdeletion syndrome showing the typical phenotype of mental retardation, hypotonia and dysmorphism. We conclude that molecular karyotyping using our CNVF algorithm with the 100 K SNP array detects cryptic chromosomal aberrations with high sensitivity and specificity.

W7 03

Genome-wide analysis of copy-number variations in patients with mental retardation by single nucleotide polymorphism arrays

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Whole genome analysis using high-density SNP oligonucleotide arrays allows identification of yet unknown microdeletions, microduplications and uniparental disomies. We collected a cohort of 70 children with unexplained mental retardation with and without additional symptoms. High resolution banding analysis and metabolic investigations were inconspicuous. The DNAs were analyzed using the Affymetrix GeneChip 100K arrays. Data analysis was performed with median normalization and genotype-specific dosage calculation using R-scripts and revealed 13 de novo copy number variations (CNVs), 10 deletions and 3 duplications. These CNVs varied in size from 400 kb to 10 Mb. All de novo CNVs were confirmed by quantitative PCR. A single patient showed a 1.4 Mb duplication in Xp22.3 including the STS gene. In 2 patients we found de novo deletions containing only 1 gene. Four of the CNVs were flanked by low-copy number repeats. Two of them were known syndromes (8p21.3 deletion syndrome, 17p11.2 duplication syndrome). A 1 Mb deletion in 17q21.3 has also been found deleted in other studies and encompasses a known inversion polymorphism of 900 kb. This deletion defines a new microdeletion syndrome. The fourth CNV flanked by low-copy number repeats is a deletion on chromosome 15q25.2. We compared the 100K Affymetrix platform with platforms that promise a higher resolution. The signal-to-noise ratio (SNR) of the 500K Affymetrix arrays was considerable lower than the SNR of the 100K Affymetrix arrays and did not significantly increase the overall resolution. In summary, the overall resolution of CNV detection with 100K Affymetrix arrays is comparable with the resolution of BAC-array CGH whereas the resolution is higher in regions containing cluster of SNPs. The SNR of the 300K Illumina arrays was somewhere in between. The increase of the number of features and the new design of feature localization should allow detection of single-gene deletions and known microdeletion syndromes.

W7 04

Submegabase-resolution array CGH reveals high rate of DNA copy number changes in Attention Deficit / Hyperactivity Disorder (ADHD)

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Attention Deficit / Hyperactivity Disorder (ADHD) is one of the most frequent childhood-onset, chronic and lifelong neurodevelopmental disease and affects 5 – 10 % of school – aged children and adolescents. Daily life of patients is impaired by learning problems, relationship crises and unemployment, but also co – morbidities such as sleep – and eating problems, substance abuse, mood – and anxiety disorders are frequently observed. Although several twin and family studies have suggested heritability of ADHD, the likely involvement of multiple genes and environmental factors has hampered the elucidation of its etiology and pathogenesis. Recently, DNA copy number changes have been implicated in the development of a number of neurodevelopmental diseases and the analysis of chromosomal gains and losses by array comparative genomic hybridization (CGH) has turned out a successful strategy to identify disease associated genes. Here we present the first systematic screen for chromosomal imbalances in ADHD using submegabase resolution array CGH. The ADHD cohort comprised 89 males and 21 females affected with ADHD as well as some of their parents. Hybridizations were performed in a sex-matched manner, using a reference DNA pool of 50 males and females, respectively. Up to now, two duplications and one deletion were found to be de novo. In the familial cases, seven duplications and one deletion have been identified, which cosegregate with disease. Our results emphasize the impact of DNA copy number changes on the development of ADHD and highlight potential disease associated candidate genes.

W7 05

Loci of segmental aneuploidy in the human genome detected by Array-CGH

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During genome-wide segmental aneuploidy profiling of 48 unrelated healthy subjects with a 3,783 BAC array we found on average 38 BAC

probes with signals significantly outside the 95% confidence interval. These represented 333 autosomal loci of segmental aneuploidy (SA). The 273 independent, consecutive and unrelated patients with multiple congenital abnormalities and mental retardation patients and healthy subjects making up our study population shared a total of 1350 distinct loci of SA. Plots of the cumulative number loci of SA indicate that their total number may be finite. Loci of SA occur as deletions, duplications, or both, in frequencies up to 45% within our study population. Out of those loci of SA occurring at < 1% in our study population 32.0% were found only as deletions, 34.5% only as duplications, and the remainder as both deletions and duplications. This distribution is not significantly different from what would have been expected for randomly occurring events. Loci of SA occurring at > 1% showed significantly less than expected rates for deletion only (15.6%) and duplication only (15.5%). This suggests that these loci of SA have been subjected to selective pressures. Loci of SA were distributed in the euchromatin with equal densities among Giemsa-light and Giemsa-dark bands. Loci of SA showed highly significant association with segmental duplications. Our data are consistent with the hypothesis that most loci of SA have been generated through non-allelic homologous recombination mediated by intrachromosomal sites of homology. Out of 23 independently confirmed genome aberrations 12 contained at least one locus of SA. Since loci of SA occur frequently in the general population, and appear to overlap with clinically relevant genome aberrations they should be taken into account before conclusions are to be drawn upon detection of SA in patients with congenital abnormalities or mental retardation.

W7 06

Interdependency of DNA copy number changes, methylation patterns and gene expression

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Genetic and epigenetic alterations are a hallmark of tumorigenesis, but the interdependency of DNA copy number changes and methylation is hardly understood. Here we present a comprehensive analysis of a newly established Small Cell Lung Carcinoma (SCLC) cell line by means of submegabase resolution array CGH, oligobased gene-expression arrays, MeDIP (methylated DNA immunoprecipitation) and MCA (methylated CpG island amplification); both techniques dedicated to the investigation of methylation changes at the genomic scale. Analysis of global methylation was complemented by the investigation of various tissues of healthy probands, including brain, muscle, liver and lung, which were used to define an in silico reference that enabled the discrimination of tumor specific and tissue specific effects. Our analysis revealed hypo-methylation of chromosomal regions, which coincided with the location of well known oncogenes and regions involved in chromosomal rearrangements in this, but also other tumors, suggesting a specific chromosomal architecture at these sites.

W8 Cytogenetics and Prenatal Diagnostics

W8 01

Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics

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We collected the tremendous and in parts contradictionary data available in the literature concerning frequency of small supernumerary marker chromosomes (sSMC) in human population in general, and in special sub-populations. 132 studies on sSMC were reviewed, in summary 1.288.693 cytogenetically studied cases detecting 980 sSMC were put together. In all included international surveys there were no ethnic effects in sSMC-frequency detectable. sSMC are present in 0.075% of unselected prenatal cases but only in 0.044% of consecutively studied postnatal ones. In people who are unintentionally childless 0.125% are sSMC-carriers; distinguishing male from female there is a 7.5:1 difference in sSMC-frequency for this special group. In (develop)mentally retarded patients the sSMC-rate is elevated to 0.288%, similar as in prenatal cases with ultrasound abnormalities (0.204%). No increased risk for sSMC-presence was detected in ICSI-induced pregnancies. There are worldwide ~2.7x106 living sSMC carriers - 1.8x106 of them have a de novo sSMC. ~70% of them are clinically normal. Strikingly, 30-50% of pregnancies with the diagnosis 'child with an sSMC' are terminated, which means in at least 50% of those pregnancies a healthy child is aborted. Thus, there is a strong need for a better genotype-phenotype-correlation enabling better genetic counseling.

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W8 02

Microdeletion on 2q11.2 detected in a case with Nievergelt-like mesomelic dysplasia

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Nievergelt syndrome (NS, OMIM 163400) is a rare autosomal dominant disorder characterized by mesomelic dysplasia of the lower legs and in some cases of the forearm. Besides radioulnar synostosis very specific X-ray findings, i.e. typical rhomboid shaped tibiae and fibulae are present in this syndrome. The genetic defect underlying this disorder is currently unknown. We present a case with NS-like mesomelic dysplasia and delayed psychomotor development. The girl died suddenly from apnoeic spells at the age of 4 months. Radiographs of the lower limbs showed a severe mesomelic dysplasia with absence of the fibulae, severely hypoplastic triangular shaped tibiae, and absent toes. The upper limbs were only moderately short and there was dislocation of the radial head. The condition differs from NS by the absence of the fibulae and missing metatarsal and tarsal synostoses. We investigated the genomic DNA from this patient for cryptic genomic imbalances using submegabase resolution BAC array CGH and identified an interstitial microdeletion of 500kb on chromosome 2q11.2. Genetic testing of the parents confirmed the de novo occurrence of the aberration. The deletion encompasses a single gene, LAF4/AFF3 (lymphoid nuclear protein related to AF4), which shows high sequence similarity with AF4 and encodes a nuclear factor with the potential to regulate transcription. We detected limb-specific Laf4 expression in mice embryos at stage day 11. At later stages the highest level of Laf4 expression is detectable in the zeugopod, i.e. radius/ulna and tibia/fibula, and the brain, sites that correlate well with the observed phenotype. Our data indicate that LAF4 plays a major role in development and haploinsufficiency of this gene is associated with a specific form of mesomelic dysplasia.

W8 03

Effects of MCPH1 mutations on chromosome assembly

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Mutations in MCPH1 (microcephalin) cause autosomal recessive primary microcephaly with misregulated chromosome condensation. The hallmarks of the disorder are a pronounced microcephaly and an elevated proportion of prophase-like cells (PLCs) in routine cytogenetic preparations (up to 20 %). The premature chromosome condensation is independent of the formation of the central chromatid axes by the scaffold proteins condensin I, condensin II, and topoisomerase IIa. Chromosomes in the patient cells are profoundly condensed and sister chromatids resolve before the translocation of the proteins to the chromatid axes and nuclear envelope breakdown. These observations contradict the classical protein scaffold/radial loop model of chromosome condensation: scaffold formation is not necessary for significant chromosome condensation in MCPH1. However,

RNA interference against condensin II subunits in MCPH1 patient cell cultures results in a significant decrease of the number of prophase-like cells, while depletion of condensin I subunits remains without consequence. Thus, the condensation defect in MCPH1 is mediated by the condensin II complex, but is independent of its scaffold binding. The presented data provide additional evidence that the classical model of chromosome condensation requires revision. In addition, these findings associate another SMC(structural maintenance of chromosomes)-complex with a developmental disorder linked to a cellular phenotype detectable by conventional cytogenetic methods. Recently, it was demonstrated that Cornelia de Lange syndrome and Roberts syndrome are caused by mutations in NIPBL and ESCO2, respectively. Both genes encode proteins interacting with the SMC-complex cohesin and mutations result in sister chromatid or centromere cohesion defects in patient cells.

W8 04

3-D multicolor banding reveals the orientation of chromosomes in human sperm – a pilot study

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Human cytogenetic preparations obtained by the air drying procedure of chromosome preparations are good targets for molecular cytogenetics, in general. However, this standard procedure leads to a flattening of the originally spherical interphase and metaphase nuclei. Thus, when interphase or metaphase architecture shall be studied this flattening leads to questionable results. Recently we reported an approach called suspension-FISH (S-FISH) where the whole FISH-procedure is performed on cell suspension. We proved that it is possible to do 3-D-analyses on totally spherical interphase nuclei or even on three-dimensional metaphases of human lymphocytes (Steinhaeuser et al., J Histochem Cytochem 2002, 50:1697-1698). Here we present the first MCB-results on 3D-preserved interphases of human sperm to study position and orientation of all chromosomes in this unique haploid state of the human genome. Per chromosome 50 sperm nuclei were acquired and at least 30 of them were evaluated in detail. By now results are available for twelve of the twenty-four human chromosomes: X, 6, 7, 13 and 15-22. The chromosomes 7, 18, 20 and 22 were located preferentially in the periphery and the chromosomes X, 19 and 22 more central within the sperm nucleus. No such preferences were observed concerning the positions of chromosomes 6, 13, 15, 16 and 17. It is noteworthy, that the results obtained for chromosomes 18 and 19 are in concordance with data shown for 3D-preserved fibroblasts (see papers of T. Cremer, Munich). The chromosomes 6, 16, 20 and 22 orientated with their p-arm in direction of the head, while p-arm of chromosome 15 orientated versus the tail of the sperm. No similar tendencies were observed for the other yet studied chromosomes. This pilot study will be continued and shall be expanded for all human chromosomes.

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W8 05

Aneuploidy screening in polar bodies of IVF oocytes by molecular copy number counting

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Chromosomal aneuploidies originating from female meiosis contribute significantly to implantation and pregnancy failure in women of advanced age. Because selection of chromosomally normal oocytes and embryos should increase the implantation and reduce the first-trimester abortion rate, robust methods for the detection of aneuploidies in polar bodies of oocytes and blastomeres of preimplantation embryos are urgently needed. A simple method to quantify genomic DNA is Molecular Copy number Counting (MCC), an established multiplex PCR-based technique which allows to directly count specific target sequences (markers) in genomic DNA samples at limiting dilution. Because oocyte and first polar body contain the reciprocal products of meiosis I, MCC of the polar body chromosome content can serve as a count for oocyte ploidy. We have developed a marker set of at least 4 target sequences for each human chromosome and are adapting MCC to single cell analysis. So far we can amplify marker regions of every chromosome thus providing reliable data of presence or absence of chromosomes. We are now in the process of optimising single cell lysis conditions to quantify the amounts of chromatids in the polar bodies. Once single cell MCC is established we will be able to count the number of all chromosomes and chromatids in the first polar body and confirm euploidy after fertilisation in the second polar body. Thus robust quantification of chromosome content in both polar bodies should significantly improve the success rate of assisted reproduction in poor prognosis women of advanced maternal age and/or failed IVF/ICSI cycles. Because all labor intensive steps can be automatized or half-automatized, single cell MCC should become a high-throughput method for aneuploidy testing of oocytes and preimplantation embryos after ART.

W8 06

A rapid comparative genomic hybridization protocol for prenatal diagnostics and its application to aneuploidy screening of human polar bodies

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Comparative genomic hybridization (CGH) is a technique that allows the detection of aneuploidies of all chromosomes in a single experiment. There would be many applications for this method in prenatal diagnostics, if the conventional CGH protocols could be substantially shortened. It was the aim of this study to establish and validate a rapid CGH protocol for single cell analysis. This protocol was then used to screen 32 first polar bodies from 16 patients aged 33 to 44 years undergoing infertility treatment mainly for advanced age or repeated implantation failure (RIF). By evaluating each step, the conventional protocol was shortened from approx. 78 to 12 hours. For single cell analysis, 4 additional hours were required (16 hours, rapid-CGH). By rapid-CGH of single cells with trisomy 18 and 21, gains of chromosomes 18 and 21 could be detected. Extra material contained in a derivative chromosome was identified by using only 5pg of DNA, approx. equivalent to the DNA content of a single cell. In the 32 polar bodies analyzed by rapid-CGH, an average of 1.8 chromosomal aberrations (range: 0-5) was found. Recurrent gains were found of chromosomes 17, 22 (4/32 cases each), 19 (3/32 cases), 21, X (2/32 cases each). Chromosomes showing recurrent losses were 6 (5/32 cases each), 3, 7, 10, 15, 16 (3/32 cases each), 4, 8, 11, 12, 18, 20 (2/32 cases each). Interestingly, a 33-year-old with RIF (7 embryo transfers without pregnancy) had an average of 2.8 aberrations in the six polar bodies analyzed, whereas a 41-year-old treated for advanced age had no aberrations in the two polar bodies investigated. In summary, the rapid-CGH protocol allows a fast screen for aneuploidies in only 12 hours and can be successfully used for single cell analysis (duration: 16 hours). By applying rapid-CGH to polar body analysis in patients with advanced age or repeated implantation failure, 75% of polar bodies were found to contain aneuploidies and almost all chromosomes were involved at least once

W9 Disease Gene Identification

W9 01

Mutations in the Wnt signaling component RSPO4 cause autosomal recessive anonychia

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Anonychia is an autosomal recessive disorder characterized by congenital absence of finger- and toenails. In a large German non-consanguineous family with four affected and five unaffected siblings with isolated total congenital anonychia we performed genome-wide mapping and showed linkage to 20p13. Analysis of the RSPO4 gene within this interval revealed a frameshift and a non-conservative missense mutation in exon 2 affecting the highly conserved first furin-like cysteine-rich domain. Further RSPO4 mutations were identified in individuals with autosomal recessive anonychia from unrelated families of different ethnic origins. All mutations were not present among controls and shown to segregate with the disease phenotype. RSPO4 is a member of the recently described R-spondin family of secreted proteins that play a major role in activating the Wnt/ß-catenin signaling pathway. Wnt signaling is evolutionary conserved and plays a pivotal role in embryonic development, growth regulation of multiple tissues, and cancer development. Our findings add to the increasing body of evidence indicating that mesenchymal-epithelial interactions are crucial in nail development and put anonychia on the growing list of congenital malformation syndromes caused by Wnt signaling pathway defects. To the best of our knowledge, this is the first gene known to be responsible for an isolated, non-syndromic nail disorder.

W9 02

SOS1 is the second major gene for noonan syndrome

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Noonan syndrome (NS) and the clinically related disorders cardio-facio-cutaneous syndrome (CFC) and Costello syndrome (CS) are concordantly caused by constitutional deregulation of the Ras signalling pathway. While mutations of PTPN11 and – rarely – KRAS have been detected in NS, CFC has been found to be caused by alterations in KRAS, BRAF, MEK1, and MEK2, and CS is consistently associated with specific HRAS mutations. Nonetheless, no genetic lesion has yet been found in ~50% of patients with NS and ~30% of individuals with CFC, suggesting further genetic heterogeneity.

To identify novel genes for NS and CFC, we screened functional candidates encoding proteins implicated in the Ras-MAPK-ERK signalling pathway. Patients fulfilling clinical criteria for NS and CFC, respectively, and with exclusion of mutations in either of the aforementioned genes were included in the study. We analyzed SOS1 encoding an essential Ras guanine nucleotide exchange factor that accelerates the production of active GTP-bound Ras. Heterozygous missense mutations of SOS1 were discovered in ~15% of PTPN11- and KRAS-negative NS patients, but none in patients with CFC. Mutations were found to cluster within exons 10, 12, and 16 and affect highly conserved amino acids. Patients carrying a SOS1 mutation displayed the typical NS phenotype, with pulmonic stenosis as the most common heart defect. Structural analysis of the amino acid substitutions suggests that these changes most likely lead to gain-of-function effects.

We conclude that SOS1 is the second most common gene mutated in NS. This finding reinforces the concept that gain-of-function mutations leading to constitutively activated proteins upstream of Ras cause NS, whereas those in downstream effectors of the MAPK-ERK pathway are responsible for CFC syndrome.

Note added: While this work was in progress, two other groups independently published activating SOS1 mutations in NS (Tartaglia et al. & Roberts et al.; Nat Genet online, Dec 3, 2006).

W9 03

Autosomal recessive bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature sensitive phenotype

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Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous group of keratinization disorders. Mutations in the major gene for ARCI, TGM1, encoding transglutaminase 1 primarily found in suprabasal epidermal keratinocytes, are responsible for almost 40% of ARCI cases. We and others have demonstrated that mutations in TGM1 can be associated with largely different phenotypes of ichthyosis, including the self-healing collodion baby. Here we have shown that bathing suit ichthyosis (BSI), a striking and unique clinical form of ARCI, is caused by transglutaminase-1 deficiency using genetic, ultrastructural, and biochemical investigations. BSI is characterized by pronounced scaling on the bathing suit areas but sparing of the extremities and the central face. Altogether we identified 13 mutations in TGM1 in a series of ten patients, including seven novel missense mutations and one novel nonsense mutation. Structural modelling for the mutation Tyr276Asn revealed that the residue is buried in the hydrophobic interior of the enzyme and that the hydroxyl side chain of Tyr276 is exposed to solvent in a cavity of the enzyme. Cryosections of affected BSI skin demonstrated a cytoplasmic and clearly reduced transglutaminase-1 activity in contrast to healthy skin areas. Digital thermography validated an obvious correlation between warmer body areas and presence of scaling in patients suggesting a decisive influence of the skin temperature. In situ transglutaminase-1 testing in skin of BSI patients demonstrated a marked decrease of enzyme activity when the temperature was increased from 25°C to 37°C. We conclude that BSI is caused by transglutaminase-1 deficiency, yet further increasing the range of phenotypes caused by mutations in TGM1, and suggest that it is a temperature sensitive ichthyosis phenotype.

W9 04

A novel gene for Usher syndrome type 2 (USH2D): Mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss

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Usher syndrome is an autosomal recessive condition characterized by sensorineural hearing loss, variable vestibular dysfunction, and visual impairment due to retinitis pigmentosa (RP). The seven proteins that have been identified for Usher syndrome type 1 (USH1) and type 2 (USH2) may interact in a large protein complex. In order to identify novel USH genes, we followed a candidate strategy, assuming that mutations in proteins interacting with this "USH network" may cause Usher syndrome as well. The DFNB31 gene encodes whirlin, a PDZ scaffold protein with expression in both hair cell stereocilia and retinal photoreceptor cells. Whirlin represents an excellent candidate for USH2 because it binds to Usherin (USH2A) and VLGR1b (USH2C). Genotyping of microsatellite markers specific for the DFNB31 gene locus on chromosome 9q32 was performed in a German USH2 family that had been excluded for all known USH loci. Patients showed common haplotypes. Sequence analysis of DFNB31 revealed compound heterozygosity for a nonsense mutation, p.Q103X, in exon 1, and a mutation in the splice donor site of exon 2, c.837+1G>A. DFNB31 mutations appear to be a rare cause of Usher syndrome, since no mutations were identified in an additional 96 USH2 patients. While mutations in the C-terminal half of whirlin have previously been reported in non-syndromic deafness (DFNB31), both alterations identified in our USH2 family affect the long protein isoform. We propose that mutations causing Usher syndrome are probably restricted to exons 1 – 6 that are specific for the long isoform and probably crucial for retinal function. We describe a novel genetic subtype for Usher syndrome, which we named USH2D and which is caused by mutations in whirlin. Moreover, this is the first case of USH2 that is allelic to non-syndromic deafness.

W9 05

Mutations of the mitochondrial holocytochrome c-type synthase in X-linked dominant microphthalmia with linear skin defects (MLS) syndrome

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The microphthalmia with linear skin defects syndrome (MLS/MIDAS) is an X-linked dominant male-lethal disorder mostly associated with segmental monosomy of the Xp22 region. The 570-kb critical MLS region encompasses the genes MID1, HCCS, and ARHGAP6. We identified heterozygous de novo point mutations, p.R217C and p.R197X, in the HCCS gene in two females with MLS and normal karyotype. In a large family, an asymptomatic mother and two of her daughters, displaying typical signs and symptoms of MLS, were found to carry an 8.6-kb deletion encompassing part of HCCS. HCCS encodes the mitochondrial holocytochrome c-type synthase that functions as heme lyase by covalently adding the prosthetic heme group to both apocytochrome c and c1. Functional analysis demonstrated that in contrast to wild-type HCCS either of the mutant proteins (R217C and delta197-268) was unable to complement a Saccharomyces cerevisiae mutant deficient for the HCCS orthologue Cyc3p. Ectopically expressed wild-type and R217C mutant HCCS proteins are targeted to mitochondria in CHO-K1 cells, whereas the C-terminal-truncated delta197–268 mutant failed to be sorted to mitochondria. Recently, we have detected the novel heterozygous p.E159K mutation in a female patient with bilateral microphthalmia and sclerocornea. Glutamic acid at position 159 is located in the mitochondrial targeting motif of HCCS and evolutionarily conserved. HCCS activity is required for synthesis of the mature form of cytochrome c, implicated in both oxidative phosphorylation (OXPHOS) and apoptosis. We hypothesize that inability of HCCS-deficient cells to undergo cytochrome c-mediated apoptosis may push cell death toward necrosis causing severe deterioration of the affected tissues that results in typical symptoms of MLS. Disturbance of both OXPHOS and the balance between apoptosis and necrosis, as well as the individual X-inactivation pattern may contribute to the variable phenotype observed in patients with MLS.

W9 06

Complex inheritance pattern involving a microdeletion in thrombocytopenia-absent radius (TAR) syndrome

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Thrombocytopenia-absent radius (TAR) syndrome is characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia in the presence of both thumbs. Other frequent associations are congenital heart disease and a high incidence of cow's milk intolerance. Evidence for autosomal recessive inheritance comes from families with several affected individuals born to unaffected parents but several other observations argue for a more complex pattern of inheritance. In this study we describe a common interstitial microdeletion of 200kb on chromosome 1q21.1 in all of the investigated 30 TAR syndrome patients detected by microarray-based comparative genomic hybridization (array CGH) and quantitative PCR. Analysis of the parents revealed that this deletion occurred de novo in 25% of affected individuals. Intriguingly, inheritance of the deletion along the maternal as well as the paternal line was observed. The absence of this deletion in a cohort of control individuals argues for a specific role of the microdeletion in the pathogenesis of TAR syndrome. We hypothesize that TAR syndrome is associated with a deletion on chromosome 1q21.1 but the phenotype develops only in the presence of an additional as yet unknown modifier (mTAR).

W10 Neurogenetics

W10 01

Array CGH identifies reciprocal 16p13.1 duplications and deletions which predispose to autism and/or mental retardation

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Autism and mental retardation (MR) are often associated, suggesting that these conditions are etiologically related. Recently, array-based comparative genomic hybridization (array CGH) has identified submicroscopic deletions and duplications as a common cause of MR. This prompted us to search for such genomic imbalances in autism and related disorders. Here we describe a 1.5 Mb duplication on chromosome 16p13.1, found in four autistic male patients from three families and several variably affected and unaffected relatives. A deletion of the same interval was identified in three unrelated patients with MR and other clinical abnormalities. Duplications and deletions of this interval have not been described before, neither as copy number variants

in the Database of Genomic Variants nor in >600 individuals from other cohorts examined by high resolution array CGH in our laboratory. Thus, this is the first description of a recurrent genomic imbalance predisposing to autism and/or MR.

W10 02

Mutations in Autism Susceptibility Candidate 2 (AUTS2) in patients with mental retardation

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We have identified three unrelated mentally disabled patients with de novo translocation breakpoints in chromosome 7q11.2. Unexpectedly, breakpoint mapping by fluorescence in situ hybridisations with genomic clones and array painting using flow sorted chromosomes indicated that in all three patients the autism susceptibility candidate 2 (AUTS2) gene was truncated. One of the patients shows relatively mild mental retardation; the other two display more profound disorders. One patient is also physically disabled, exhibiting urogenital and limb malformations in addition to severe mental retardation. The function of AUTS2 is presently unknown, but it has been shown to be disrupted in monozygotic twins with autism and mental retardation. Given the overlap of this autism/MR phenotype and the MR-associated disorders in our patients, we ascertain with this study that AUTS2 mutations are clearly linked to autosomal dominant mental retardation. Remarkably, this is one of the very few examples of several de novo translocation breakpoints disrupting the same gene.

W10 03

Evaluation of an X-linked mental retardation resequencing array

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Moderate to severe forms of X-linked mental retardation (XLMR) affect 0.05% of the male population in developed countries. The majority of these patients show non-syndromic forms of XLMR (NS-XLMR) with mental retardation as the only clinically consistent manifestation. NS-XLMR is genetically highly heterogeneous and mutations have so far been found in >23 different genes encoding proteins with very diverse functions in the brain. Mutations in each gene account for only a few percent of the cases, which impedes fast and cost-efficient diagnosis of this common disorder, especially in MR families that are too small for performing linkage analysis. In order to evaluate array based resequencing as a diagnostic tool for detecting mutations in patients with NS-XLMR we have designed a resequencing array based on the Affymetrix 50k platform containing the coding and splice site regions of 17 XLMR genes (ACSL4, ARX, ATRX, DLG3, FTSJ1, GDI1, IL1RAPL1, JARID1C, MECP2, NLGN4, PAK3, PHF6, PHF8, PQBP1, SLC6A8, TM4SF2 and ZNF41). Using a combination of automated and manual PCR, these genes are amplified in 146 separate fragments. Until now, we have performed hybridisations for 46 patients and obtained call rates between 92 and 96% using the GSEQ software (Affymetrix). In this data set a total of 182 sequence changes have been identified including 9 known SNPs. However, the majority of sequence changes are located in regions of poor sequence quality. Only 13 changes are located in high sequence quality regions where the 10 neighbouring nucleotides are called. Of these, 7 non-synonymous and 2 synonymous changes were not present in dbSNP and occurred only once in the patient cohort whereas 4 other changes were found several times in the cohort, though also not in dbSNP. We are currently verifying our findings by conventional sequencing methods.
These results will be important for estimating the potential of array based resequencing in clinical settings.

W10 04

The agenesis of the corpus callosum: Clinical-genetic study with systematic classification of different types including molecular-cytogenetic methods

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The agenesis of the corpus callosum is one of the most frequent brain malformations (incidence 0.5 to 70 in 10000). It is a heterogeneous condition, where genetic causes are known (e.g. chromosomal changes, syndromes). The study's objective has been to evaluate the data of patients, seen in the Children's Center Munich, on a systematical basis: MR imaging of all patients with corpus callosum anomalies (n=179) were re-evaluated according to a standardized protocol. We have identified a number of 50 patients with complete absence (agenesis of the corpus callosum – ACC) or partial absence (dysgenesis of the corpus callosum – DCC), who were clinically described in detail, including chromosome and subtelomeric analyses as well as molecular-genetic testing if necessary, in order to elaborate a genetic diagnosis.
From the 50 patients, 31 had ACC, 15 had DCC, and in 4 patients the corpus callosum was missing due to holoprosencephaly (HPE).
In 9 of the 31 patients with ACC, the following diagnoses could be established: Mowat-Wilson syndrome (n=2), Walker-Warburg syndrome (n=1), ARX-opathy (n=1), and unbalanced chromosomal rearrangements (n=5), including a patient with an apparently balanced reciprocal translocation, which led to the disruption and a predicted loss of function of a gene (FOXG1B-gene) not described previously. The cause of the ACC in 22 patients remained unclear.
In 2 of the 15 patients with DCC, unbalanced chromosomal rearrangements could be detected (n=2), while the cause of DCC in the 13 patients remained unclear.
A causative mutation in ZIC2 was found in one of the 4 children with HPE.
We present the obtained clinical findings of the patients with ACC, DCC and HPE as well as the laboratory findings in detail, comparing our results with the available literature data.

W10 05

The genetics of alcoholism: A critical role of tryptophan hydroxylase 1

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Ethanol abuse is one of the severest socioeconomic problems worldwide. The inheritable genetic predisposition has been recognized for long, and the so far identified genes have narrowed down the etiology to deficiencies in the neuronal serotonergic, dopaminergic, GABAergic, and glutamatergic systems. It is also known that frequent gene variations in the Asian population of either monoamine oxidase or aldehyde dehydrogenase 2 significantly reduce the ethanol preference and, more importantly, the risk of pathological alcoholism (1).
However, the neuronal component of physical ethanol addiction has possibly always been over-esteemed. Some years ago, a second tryptophan hydroxylase was identified, which synthesizes the neurotransmitter serotonin (5-HT) specifically in neurons (2, 3). The formerly known tryptophan hydroxylase (TPH1) is mainly expressed in extraneuronal tissues and thus inves-

tigations into its possible involvement in the etiology of alcoholism were abandoned.
However, some few studies have now again pointed to a correlation of pathological alcoholism with polymorphisms in the TPH1 gene (4, 5). Thus, we decided to verify this possibility in mice lacking TPH1. These animals exhibit significantly higher voluntary ingestion of ethanol and a high percentual preference in a two-bottle paradigm, in which the mice are allowed to choose between ethanolic solutions and tap water. Moreover, we have recently found peripheral 5-HT involved in liver regeneration (6). Hence, our findings disclose a critical role of TPH1 dysfunction and extraneuronal 5-HT metabolism in the etiology of alcoholism and the accompanying organ damage.

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W10 06

SCA17 transgenic mice show a severe neurodegenerative phenotype

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SCA17 is a progressive neurodegenerative disease leading to cerebellar ataxia and dementia. Several accessorial symptoms such as Parkinsonism, dystonia, and psychiatric disturbances commonly aggravate the disease course. Genetically, a CAG/CAA expansion in the TATA binding protein (TBP) is expanded in SCA17 patients, leading to an expanded polyglutamine chain in this ubiquitously expressed transcription factor. We have generated transgenic mice overexpressing a 64 CAG/CAA repeats containing human TBP (Q64TBP) gene under the control of the truncated human prion protein promoter (PrP). Transgene protein expression throughout different brain regions (cortex, basal ganglia, cerebellum, and brain stem) was clearly demonstrable. Onset of motor dysfunction (Accellerod) started by 20 weeks and the life span of transgenic animals was reduced.
We present detailed morphological and phenotypical data for this first rodent model of SCA17, which enables us to further study the pathogenesis of this progressive neurodegenerative disease.

Poster Clinical Genetics

P001

Mutation anaylsis of the NIPBL and the SMC1L1 gene in German patients with suspected Cornelia de Lange syndrome.

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Cornelia-de-Lange-syndrome (CdLS) is a heterogeneous autosomal-dominant disorder characterized by typical facial features, growth retardation, developmental delay, upper-extremity malformations and a variety of other abnormalities. The prevalence of CdLS is estimated to be around 1/10,000. Most cases are sporadic, although several familial cases are described.
Since 2004 it is known that up to 50% of CdLS cases are caused by mutations in the NIPBL gene on chromosome 5p13, the human homolog of the Drosophila Nipped-B gene. This gene consists of 47 exons and mutations were found in the entire coding region.
Recently, Musio et al. described an X-linked form of mild CdLS caused by mutations in the gene SMC1L1.
Both genes code for proteins, which are part of the cohesin complex involved in chromosome cohesion.
We investigated the prevalence of NIPBL gene mutations in 108 German patients with suspected CdLS by DGGE and/or direct sequencing. Additionally, mutation analysis of the SMC1L1 gene was performed in all patients tested negative for mutations in the NIPBL gene. We detected NIPBL gene mutations in 29 (=27%) of our patients, although previous studies reported a higher detection rate (ca. 50%). Our lower detection rate is probably due to patient selection. Furthermore, the prevalence of SMC1L1 mutations in our cohort will be discussed.

P002

Microdeletions and microduplications affecting the STS gene at Xp22.31 are associated with a distinct phenotypic spectrum

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Array-based comparative genomic hybridization (array CGH) allows the detection of submicroscopic alterations in about 10 % of individuals with mental retardation (MR) of unknown origin. Using a submegabase resolution BAC array, we identified a 1.7 MB microdeletion at Xp22.31 in two unrelated cases with a complex phenotype including features of X-linked chondrodysplasia punctata and a corresponding microduplication [arr cgh Xp22.31(RP11-359O20→RP11-93J09)x3] in one patient. A second unrelated patient with a Xp22.31 microduplication was diagnosed using the Affymetrix GeneChip100K Array. Non-allelic homologous recombination between flanking low copy repeats most likely leads to these genomic aberrations at Xp22.31. FISH studies using two BAC probes demonstrated the involvement of the steroid sulfatase (STS) gene in these microdeletions/microduplications. Patient 1 had a maternally inherited microdeletion Xp22.31 and presented with mild ichthyosis, flat midface with nasal hypoplasia, brachytelephalangy, MR and seizures.
Patient 2, a male fetus, also had a maternally inherited microdeletion Xp22.31 and showed nasal hypoplasia and irregularities of femoral metaphyses. Despite clinical signs of chondrodysplasia punctata the arylsulfatase E (ARSE) gene was present in both cases. This phenomenon might be explained by an exerting position effect due to the proximity of the microdeletion.
Patient 3 carried a de novo microduplication Xp22.31 and showed MR, muscular hypotonia, autoagressive behaviour, postnatal overgrowth and craniofacial anomalies including hypertelorism, epicanthus inversus, a bifid nasal tip and relatively long philtrum. In patient 4 who presented with MR, autistic and aggressive behaviour, we detected a maternally inherited microduplication Xp22.31. In summary, we report the previously unknown phenotypic spectrum defined by an Xp22.31 microduplication that encompasses the STS gene.

P003

Phenotypical spectrum in patients with mutations in the CDKL5 gene - report of three new patients and review of the literature

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The clinical phenotypes associated with cyclin-dependent kinase-like 5 (CDKL5/STK9) gene mutations range from early onset infantile spasms to atypical Rett syndrome and autism. The link between CDKL5 and disease was established on the basis of two female patients with X/autosome translocations (Kalscheuer et al., Am J Hum Genet 2003). Until now a total of 29 patients with mutation in CDKL5 has been reported. We present the phenotypical features in three girls with mutations in this gene and a literature review. All three girls were born at term after an uneventful pregnancy. Patient 1 developed epilepsy at the age of 8 weeks, patient 2

at the age of 3 months and patient 3 at the age of 5-6 months. In all three girls the anticonvulsive treatment was not effective, therefore all three have a vagus-nerve stimulator. Profound mental retardation without development of speech is present in all 3 patients. In addition, patients 1 and 3 are not able to walk. The faces were unremarkable, anthropometric measurements including head circumference were normal in patients 1 and 2, whereas patient 3 who was the most severely retarded has short stature and microcephaly.
Patient 1 has a missense mutation at position 455 (c.455G->T) of the CDKL5 gene in exon 7 (p.C152F) which was reported before (Tao et al., Am J Hum Genet 2004), whereas patients 2 and 3 presented with novel pathogenic mutations. Testing of the parents revealed a de novo mutation in patients 1 and 2, for patient 3 the father was not available. Patients with infantile spasms, mental retardation, atypical signs of Rett syndrome or autism should be tested for mutations in the CDKL5 gene.

P004

Mutation analysis in the MKS1-Gene in 22 fetuses affected by Meckel-Gruber syndrome

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The Meckel-Gruber syndrome (MKS) is an autosomal recessive disorder causing severe defects in the developing central nervous system, kidney, limbs and other organs. Mutations in the MKS1 gene have been recently identified in individuals of Finnish origin.
To test the frequency of mutations in the MKS1 gene in 22 aborted fetuses with a clinical and pathological diagnosis of MKS and to obtain a genotype-phenotype correlation, DNA sequence analyses and quantitative real time PCR studies were performed.
We could identify the same type of mutation, i.e. a deletion of 29 base pairs in intron 15 of the MKS1 gene, in 8 out of 22 cases studied. Six out of these 8 cases with such a mutation displayed the campomelic variant of MKS. No further mutations were found. Only one of the remaining 14 cases which lacked mutations in the MKS1 gene showed the campomelic MKS variant.
The deletion of 29 base pairs in intron 15 of MKS1 is highly associated with a distinct subtype of the Meckel-Gruber syndrome, namely the campomelic variant. In individuals of European origin suffering from the campomelic MKS variant, the described deletion is highly likely to be causative. In families with a mutation in the MKS1 gene, it is now possible to offer early prenatal testing, e.g. with chorionic villus sampling and mutation analysis.

P005

A variable combination of features of Noonan syndrome and neurofibromatosis type I is caused by mutations in the NF1 gene

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Signs of neurofibromatosis type 1 (NF1) and Noonan syndrome (NS), two distinct autosomal dominant disorders, occur together in patients reported as Watson syndrome (WS), neurofibromatosis-Noonan syndrome (NFNS), partial LEOPARD syndrome, Noonan syndrome with features of NF1, and NF1 with Noonan-like features. The molecular basis of these combined phenotypes was poorly understood and controversially discussed over several decades. Only recently, there is increasing evidence for Watson syndrome and NFNS being allelic to NF1 in the majority of patients. In this study we describe seven novel patients from five unrelated families with variable phenotypes of the NF1-Noonan syndrome spectrum which were systematically analyzed for mutations in the disease-causing genes NF1 for NF1 and PTPN11 for NS. Heterozygous mutations or deletions of NF1 were identified in all patients, while no PTPN11 mutation was found. The NF1 mutation segregated with the phenotype in both familial cases. These results support the hypothesis that variable phenotypes of the NF1-Noonan syndrome spectrum represent variants of NF1 in the majority of cases. Constitutive deregulation of the Ras pathway either through activating mutations of PTPN11 or through haploinsufficiency of neurofibromin, which acts as a Ras-inactivating GTP-ase, is probably the common pathogenetic mechanism explaining the phenotypic overlap of NS and NF1.

P006

Diagnosis of Wolf-Hirschhorn syndrome and Mowat-Wilson syndrome in two patients with clinical features of Opitz syndrome

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Opitz syndrome (OS; MIM145410 and MIM300000) is a midline malformation syndrome characterized by hypertelorism, hypospadias, cleft lip/palate, laryngotracheoesophageal anomalies, imperforate anus, developmental delay (DD), and cardiac defects (CD). Here, we describe two cases originally referred to us with OS, but later molecularly proven to be different entities, thus demonstrating the broad spectrum of differential diagnoses of ventral midline disorders. We first report a 15-year-old male patient with typical manifestations of OS, including hypertelorism, hypospadias, dysphagia and DD. Unexpectedly, on sub-megabase resolution array CGH, we identified a 2.35 Mb 4pter deletion, indicating Wolf-Hirschhorn syndrome (WHS; MIM194190), and a duplication of Xp22.31. WHS patients are characterized by a 'Greek warrior helmet' facies, due to hypertelorism and a prominent glabella, as well as hypotonia, growth retardation, microcephaly (MC), dysgenesis/agenesis of the corpus callosum (ACC), epilepsy, skeletal anomalies, speech difficulties and DD. Some WHS patients also present with midline defects.
Secondly, we report a 5-year-old male patient with a phenotype suggestive of OS, including hypertelorism, hypospadias, atrial septal defect and DD, who was diagnosed with Mowat-Wilson syndrome (MWS; MIM235730) with a 582_582insA in the ZFHX1B gene. MWS is a multiple congenital anomaly-mental retardation (MR) syndrome with a characteristic facial phenotype that includes MC, hypertelorism, medially prominent eyebrows, prominent columella and pointed chin. In addition, virtually all patients have MR with impaired/absent speech, and common findings include seizures, ACC, CD, hypospadias, and Hirschsprung disease or constipation. These cases illustrate the wide differential diagnosis for hypertelorism and hypospadias, and the striking overlap of clinical findings in seemingly unrelated syndromes, suggesting common or interacting pathways at the molecular and pathogenetic level.

P007

Impact of geometry and viewing angle on classification accuracy in 2D based analysis of dysmorphic faces

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Computer-based analyses of 2D and 3D representations of faces have been developed to classify syndromic conditions. Although we have shown previously that a sizeable number of syndromes can be accurately classified (Fragile X syndrome, Cornelia de Lange syndrome, Williams-Beuren syndrome, Prader-Willi syndrome, Mucopolysaccharidosis (MPS) type III, Cri-du-chat syndrome, Smith-Lemli-Opitz syndrome, Sotos syndrome, Microdeletion 22q11.2, Noonan syndrome; accuracy > 75%), the inclusion of potentially hundreds of syndromes poses new challenges. In the current study we have therefore included four more syndromes (MPS II, Treacher-Collins syndrome, Progeria, Wolf-Hirschhorn syndrome) and achieved an accuracy of 65% on frontal images. To improve classification rates, we included sideview pictures and the geometry of landmarks into the analysis. A combined analysis achieves an accuracy > 85% and it can be shown that geometry contains important information after proper standardization.
Furthermore, we demonstrate by presenting average images of syndromes based on the probands in the study that computer representation is in accord with clinical expectations.
In conclusion, joint information of geometry and texture information of pictures of faces allows for an accurate classification of 14 syndromes,

which encourages an adaptation of computer-assisted picture analysis into clinical practice.

P008

BBS1 and BBS10 molecular genetic analyses in 80 patients with Bardet-Biedl Syndrome

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The autosomal recessively inherited Bardet-Biedl syndrome (BBS, OMIM 209900), a disorder affecting cilia and basal body function in multiple organ systems, is characterized by the association of postaxial polydactyly, retinopathy, obesity, hypogonadism in males, renal dysfunction and variable mental deficiency. Over the past years it has become obvious that BBS is genetically heterogeneous with several genes (BBS1-BBS11) involved. For clinical manifestation mutations in more than one BBS-locus are needed. Therefore, BBS is now regarded as a disorder with digenic or even oligogenic inheritance. The BBS1 mutation p.M390R is observed in about 20% of European BBS-individuals, whereas it is infrequent in other populations. A second major locus is BBS10 in which the mutation p.C91LfsX5 is found in 46% of mutated alleles in different ethnicities. All other mutations in BBS genes have an occurrence of less than 2%.
We screened a sample of 80 BBS patients for mutations in 10 exons of BBS1 and in exon 2 of BBS10. The BBS1 mutation c.1169T>G (p.M390R) was found in 15% of German but not in any Turkish patients. In addition we detected 5 unique mutations in different families: a 50bp insertion in exon 12, a deletion of exon 12-13, c.1318C>T (p.R440X), c.1339G>A (p.A447T) and c.1660A>T (p.S554C). In BBS10 the mutation c.270_271insT (p.C91LfsX5) was found in 28% of German and in 8% of Turkish patients. Additionally we observed 2 unique 1 bp insertion mutations: c.[253_254insA, 254C>G] (p.T85KfsX11) and c.259_260insG (p.I87MfsX9).
In summary, the mutations p.M390R and p.C91LfsX5 were found in 43% of German patients. Therefore, this limited and low cost molecular test is helpful to support the clinical diagnosis in BBS individuals.

P009

Simple and translocation forms of monosomy 4p16.1⟶pter: quantitative syndrome definition and cytogenetic evaluation by FISH

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Although Wolf-Hirschhorn syndrome is known since early 1960's, the knowledge on correlations between partial monosomy 4p and resulting phenotypes corresponding to different breakpoint positions is fragmentary. Recent studies show that two segments of 4p16 are particularly predisposed to chromosome breaks: one in 4p16.1 and one in 4p16.3. In addition, recent studies show that a proportion of WHS cases is due to complex unbalanced rearrangements, involving both 4p and another chromosome. The aim of this work is a contribution to quantitative syndrome definition of monosomy 4p16.1⟶pter. A group of 11 children with monosomy 4p16.1⟶pter (6 with simple terminal deletion, one with additional trisomy 11q23⟶qter, one with trisomy 11p15⟶qter, and three with trisomy 8p23⟶pter) aged from 1 month to 5 years were examined. Cytogenetic analysis was performed using GTG and RBG techniques and verified by BAC clones chosen from the physical maps of the interest regions (4p16.1, 11q23.3 and 8p23) using the Ensembl Genome Browser and obtained from the Sanger Institute. The imbalance segments were further analysed by fluorescent in-situ hybridisation (FISH). In one case paternal UPD11p was examined. A catalogue of well-defined over 800 clinical / developmental and anthropological features from the Munich Dysmorphology Database according to Stengel-Rutkowski was used. On that basis a quantitative phenotype definition of monosomy 4p16.1 syndrome was obtained and then compared to phenotypes of patients with complex rearrangements (described with the same protocol). Among traits identified in the latter patients, nearly a half corresponded to traits observed solely or predominantly in del(4p16.1⟶pter) cases, and most of the remaining traits were attributable to both WHS and the conditions related to imbalance of the given segment. That domination of WHS phenotype should implicate support of patient's needs required for children with that condition.

P010

Phenotypic and genetic studies in a Stargardt disease family segregating four different ABCA4 mutations

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Stargardt disease is the most frequent cause of juvenile macular dystrophy characterized by decreased visual acuity, atrophic macular lesions and attenuated electroretinographic (ERG) responses. It is caused by recessive mutations in the ABCA4 gene encoding a photoreceptor-specific ATP-binding cassette transporter functioning as an outwardly directed flippase for N-retinylidene-phosphatidylethanolamine. Mutations in ABCA4 are also associated with cone rod dystrophy and inverse retinitis pigmentosa. As established by Maugeri et al (2000) there is an inverse correlation between the ABCA4 residual activity and the severity of the retinal dystrophy with the most severe phenotype, RP, caused by two null/severe ABCA4 mutations. If ABCA4 activity is partially retained on at least one allele, patients will develop either cone rod dystrophy or Stargardt disease.
We have now identified a family affected with Stargardt disease in two generations including two parents and their two children. Molecular testing identified four different mutations in the ABCA4 gene segregating in this family. Two mutations should lead to an amino acid change (His1838Asp and Arg2077Trp), one small insertion is predicted to cause a frame-shift (3211insGT) and one nucleotide change is known to alter the splicing behaviour (768G>T) in RNA maturation. Since the two missense mutations are regarded as mild mutations and the splice / frameshift-mutations represent null-alleles with a likely loss of protein function, at least three different phenotypes are feasible depending on the combination of disease alleles (mild/mild, mild/severe, severe/severe). The variable expressivity of ABCA4-asociated retinopathy should call the counsellor's attention to the possibility that clinically different phenotypes may be caused by separate disease alleles of the same gene. This is most crucial for providing accurate recurrence risks.

P011

Molecular diagnostic in hereditary hemorrhagic telangiectasia: Detection of 5 novel large deletions and screening MAP3K7 as a new candidate gene

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Hereditary hemorrhagic telangiectasia (HHT) or Osler-Rendu-Weber disease, is an autosomal-dominant bleeding disorder, characterized by arteriovenous malformations ranging from cutaneous and mucous membrane telangiectasias, to more severe pulmonary, gastrointestinal, and cerebral arteriovenous malformations (AVMs).Mutations in two genes, Endoglin (ENG) and activin receptor-like kinase 1 (ACVRL1 or ALK1),TGFß signaling components, have been associated with HHT.in 35% of clinically confirmed HHT patients no mutations in both Alk1 and endoglin was found by direct sequencing of the coding regions and exon-intron boundaries . We established a method to detect large arrangements using Quantitative Real Time PCR (QRT-PCR). We detected 5 large novel deletions in Alk1 and ENG in patients with clinically confirmedHHT, in two of them whole Alk1 gene was deleted.
Recently, the gene MAP3K7 was suggested also as possible candidate of HHT. Joy et la have shown The similarities in phenotype between HHT mouse model and Tak1(mouse homolog of human MAP3K7) knock-out mice .To check this hypothesis We analyzed this gene in 10 patients with no mutations in either ENG or ALK1, by sequencing the whole coding regions and exon-intron boundaries of MAP3K7 and screening for deletions – duplications using QRT-PCR. No mutations- deletions in MAP3K7 were found.

P012

Wrinkly skin syndrome with and without signs of a congenital defect of glycosylation (CDG)

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We report 12 Omani patients from four consanguineous families with a cutis laxa phenotype which can either be classified as autosomal recessive cutis laxa type II (ARCL; OMIM 219200) or Wrinkly Skin Syndrome (WSS; OMIM 278250). The phenotype seen in our patients evolves during early childhood and includes excessive skin folds, herniae, diverticulae, infantile emphysema, malposition of the feet, hip dislocations, growth retardation and persistence of the anterior fontanel beyond three years of age. Additional features were excessive caries and broken dental crowns, infantile hypotonia and a mild developmental delay. A congenital defect of glycosylation (CDG) type II pattern was evident in four out of five WSS cases tested by capillary zone electrophoresis of serum transferrin. Apolipoprotein CIII (a marker for O-glycosylation) was normal suggesting that WSS is related to a N-glycosylation defect, probably at the level of processing (CDG-IIx). WSS closely overlaps with geroderma osteodysplastica (GO; OMIM 231070), which is characterized by cutis laxa and infantile osteoporosis, but lacks delayed fontanel closure and an abnormal liability to caries. DXA measurement of bone mineral density in a 20 year old WSS case revealed osteoporosis at the lumbar spine. Hence, WSS is a multisystemic disorder that also can involve bone and teeth. A general glycosylation defect might be present at least in a subset of cases.

P013

Translocation and deletion in a patient with acampomelic campomelic dysplasia

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Here, we report on a patient who became obvious soon after birth at term with hypotonia, craniofacial dysmorphy, cleft palate, brachydaktyly, malformation of thoracic spine, and gonadal dysgenesis with female external genitalia and Müllerian duct derivatives. By X-ray examination no signs for campomelia were seen. Nevertheless, because of a suspicion of acampomelic campomelic dysplasia sequencing of SOX9 was carried out but did not reveal any alteration. By subsequent array CGH analysis a deletion of approximately 4,2 Mbp being located about 0,5 Mbp upstream of SOX9 on chromosome 17q was detected. STS-analysis confirmed the deletion, which has occurred de novo on the paternal chromosome. In order to determine the deletion breakpoints an approach of genomic walking was performed. Surprisingly, it turned out that a translocation between chromosomes 7 and 17 must have occurred. The deletion comprises of 4,242,512 bp of chromosome 17 while there is no loss of material of chromosome 7.Cytogenetic analysis revealed the karyotype 46,XY,t(7;17)(q33;q24).ish t(7;17)(wcp7,wcp17+;wcp7+wcp17+) could be determined. To our knowledge this is the first report of a patient with acampomelic campomelic dysplasia carrying both a deletion and a translocation.

P014

Chromosome instability in a patient with VACTERL association

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The VACTERL association is characterized as a non-random pattern of malformations occurring in different developmental fields. VACTERL association denotes a combination of vertebral anomalies, anal atresia, cardiac anomalies, tracheoesophageal fistula or esophageal atresia, renal and limb anomalies, and is a very heterogeneous disorder. Common convention is that three of these criteria at least are needed for the diagnosis. The same defects can also be seen as part of the Fanconi anemia (FA) spectrum. Because VACTERL with hydrocephaly and radial anomalies has been associated in estimated 5 to 10% with FA, chromosome breakage analyses should be performed in these cases.
We report on a fetus affected by complex malformations. Sonography in the 22th week of a 24-year-old woman revealed hydrocephaly, hydrothorax, unilateral kidney cyst, growth retardation and anhydramnia.
Pathological examination showed unilateral aplasia of the kidney, kidney cyst, esophageal atresia, hydrothorax, hydrocephaly and radius aplasia. Chromosomal analysis revealed a high rate of spontaneous chromosome instability including chromosome and chromatid breaks, acentric fragments, triradial and quadriradial figures as well as a variety of different rearrangements such as translocations, interstitial duplication, and marker chromosomes in fibroblasts. The rate of spontaneous chromosome breakage was increased compared to our control collective (0.45 vs 0.00-0.06 breaks/cell). Analysis of a fibroblast culture by flow cytometry detected no excessive rise in the G2 phase of the cell cycle after treatments with mitomycin C, which would be typical of FA. However, novel subtypes of FA have been reported recently and further studies will show if they apply in our case or if VACTERL association can be associated with chromosome instability in a context other than FA.

P015

Changes in phenotype of bloom´s syndrome with new manifestations in adult individuals

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Bloom´s syndrome results from autosomal recessive mutations in the BLM gene located on human chromosome 15 at 15q26.1, encoding a DNA helicase with homology to RecQ in E. coli (MIM 210900). Its phenotype includes (i) pre- and postnatal growth retardation, (ii) facial features with dolichocephaly and a narrow face, (iii) light-sensitive facial telangiectasia in most patients, (iv) manifestations of genomic instability as revealed by a 10-fold increase of spontaneous sister chromatid exchanges, breaks and homologous exchanges between chromosomes, an increased rate of somatic mutations, and (v) increased risk of cancer.
We report data of a longterm study of the natural history of 15 individuals with Bloom´s syndrome observed during the past 37 years in Germany. We found that the phenotype in adult individuals becomes less distinctive with age than it is in children. In spite of persistent feeding difficulties, such as lack of appetite or regurgitation, adult individuals tend to gain weight. A new finding is development of diabetes mellitus type 1 or type 2. This has been observed in 27 of 117 patients (23%) of individuals in the Bloom´s Syndrome Registry (J. German, M. Sanz, E. Passarge, unpublished data). The skin manifestations tend to improve with age. We diagnosed Bloom´s syndrome prenatally in a family known to be at risk. Retarded growth was evident during all stage of pregnancy and the affected infant weighed only 2000 g at birth at 40 weeks of gestation. However, he lacked the typical appearance of Bloom´s syndrome. We conclude that the phenotype of Bloom´s syndrome is wider than recorded previously.
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P016

Five novel mutations in the F13B gene resulting in mild FXIII deficiency

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Background: FXIII deficiency is a rare autosomal recessive disorder affecting approximatly 1 out of 1-3 million inhabitans. The disease is characterized by bleeding, impaired wound repair and spontanious abortions in females. Based on genotype there are two types of FXIII deficiency: A-subunit deficiency (F13A gene affected) and much rarely B-subunit deficiency (F13B gene affected). Both types result in absence of FXIII catalytical activity in plasma. To date, only 5 fami-

lies with isolated B-subunit deficiency have been described in the literature. Here we report 5 novel mutations, affecting F13B gene and resulting in FXIII deficiency.
Methods: F13A and F13B genes were analysed by direct sequencing.
Results and discussion: All patients have shown single heterozygous mutations in F13B gene.Patient E.M. (female, born 1947, FXIII activity 47-53%, postoperative bleeding) had a missense mutation in exon 2 (c.73 T>C, Cys5Arg) resulting in disruption of disulfide bond between amino acids Cys5 and Cys56. Patient H.D. (male, born 1983, FXIII activity 54%, epistaxis) showed a missense mutation in exon 3 (c.406C>T, Leu116Phe). It may cause instability of disulfide bond between neighbouring Cys117 and Cys71. Patient E.R. (female, born 1989, FXIII activity 53%, increased bleeding during menstruation) had an in-frame deletion (c.471-473delATT) resulting in deletion of Leu138. Patient O.S. (female, born 1983, FXIII activity 46-57%, bleeding after tonsillectomy) had a small insertion (c.1959insT) in the exon 12 changing the distal amino acid (634-641) sequence and predisposing a synthesis of additional 21 new amino acids at the Carboxy-termini of FXIIIB-Subunit. Patient P.G. (male, born 1974, FXIII activity 43%, bleeding after tooth extraction) had a splice site mutation in intron 3 (IVS3-1 G>C).
Conclusions: Our results suggest higher prevalence of FXIIIB deficiency as it was thought before. Patients having mild (heterozygous) FXIIIB deficiency may bleed upon provocations.

P017

Isolated Ectopia Lentis: A case report

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Ectopia Lentis is a displacement or malposition of the eye´s crystalline lens from its normal location and one of the major criteria in the clinical diagnosis of Marfan syndrome, defined by the Ghent nosology. Marfan syndrome is caused by mutations in the gene coding for fibrillin1 on chromosome 15 (FBN1). Dominant ectopia lentis has also been genetically linked to this locus.
A case report of a 25-year-old woman who was diagnosed with bilateral ectopia lentis (and myopia) at the age of two years, is presented. Clinical examinations of the patient did not reveal any further symptoms of Marfan syndrome. Her father also had been diagnosed with isolated ectopia lentis.
A molecular genetic analysis of the FBN1 gene was performed by direct sequencing of the 65 coding exons and flanking intronic regions of the gene. Mutational analysis revealed a novel Y63C missense mutation in exon 2 of the FBN1 gene. The same Y63C missense mutation was found in the father. The mutation is located in the highly conserved N-terminal region of the protein and directly adjacent to a previously reported R62C mutation in a case of isolated ectopia lentis.
In conclusion this familial case confirms ectopia lentis as a monosymptomatic manifestation of a fibrillinopathy, due to a mutation in the FBN1 gene.

P019

LIS1 mutations associated with different forms of neuronal migration disorders

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Lissencephalies are neuronal migration disorders leading absent or reduced gyration and a broadened, but poorly organized cortex. The most common form of lissencephaly is isolated, also referred to as classical or lissencephaly type I. The most frequent cause of lissencephaly type I is a heterozygous deletion of the entire LIS1 gene, while intragenic heterozygous LIS1 mutations or hemizygous DCX mutations are less common.
We assessed clinically and genetically 19 unrelated patients with neuroradiologically confirmed brain malformations, mostly with lissencephaly type I, but also patients with subcortical band heterotopia or cerebellar hypoplasia. The mutation analysis of the LIS1 gene revealed 13 novel and six previously described mutations. We observed 13 truncating (nonsense, n=2; frameshift, n=7; splice site, n=5), four missense and one in-frame deletion mutation. In particular, the in-frame deletion of six amino acids as somatic mosaic mutation resulted in a partial subcortical band heterotopia within the occipital-parietal lobes. Furthermore, we present two patients with novel LIS1 mutations in exon 10 (frameshift and missense mutation) associated with lissencephaly with cerebellar hypoplasia type a (LCHa).
Our results indicate that the assessment of LIS1 mutations should be generally taken into account for patients fulfilling criteria of the agyria-pachygyria-band-spectrum. In particular, patients presenting with mild cerebral malformations such as a variable degree of subcortical band heterotopia or cerebellar hypoplasia should also be considered for the genetic analysis of the LIS1 gene. In contrast to previous reports our data suggest, that neither type nor position of the mutation within the LIS1 gene allow an unambiguous prediction of the severity of the phenotype.

P020

Primary oxalosis type I with a novel mutation in the AGXT gene (S221P) in a patient with multiple congenital anomalies

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Mutations in the AGXT give rise to Oxalosis type 1, a non-syndromic disorder. Here we report a Turkish girl, the 2nd child of consanguinous parents, with a complex syndrome characterized by microcephaly, gothic palate, choanal atresia, lacrimal duct stenosis, vesicoureteral reflux and anal atresia with vestibular fistula. Later a severe inflammatory enteropathy developed. A sister with a similar composition of features had died in the age of 5 months. Elaborations of serum creatinine to 1 mg/dl and nephrocalcinosis at the age of four months together with increased oxalic acid levels (176 µmol/l in serum and 1044 mmol/mol creatinine in urine) were detected. AGXT activity in liver biopsy was low (2.0 µmol/h/mg protein). Direct sequencing revealed a homozygous novel mutation S221P. Modelling studies demonstrate that the S221P is located in the dimerisation domain. This might lead to inhibition of the dimerisation, protein instablity and rapid degradation as confirmed for the S205P mutation. The minor allele P11L which has disease aggravating effect on other mutations has not been found. Sequencing of the AGXT is a non-invasive method for the diagnosis of PH1; the results could be helpful for therapeutic decisions.
Because the associated malformations of the patient were not conclusively explained by the AGXT gene mutation, we searched for causes of the complex disease. Subtelomeric microdeletions, contiguous gene deletion syndrome and microdeletion 22q11.2 were excluded. The karyogram was normal. The origin of the malformation syndrome by a second autosomal recessive disorder unrelated to AGXT is plausible. While several features like choanal atresia, anal atresia and microcephaly were compatible with the CHARGE association, the presence of lacrimal duct stenosis, vesicoureteral reflux and chronic inflammatory enteropathy were not.

P021

Reduced penetrance in a family with X-linked dominant chondrodysplasia punctata

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X-linked dominant chondrodysplasia punctata (CDPX2) is characterized by short stature, stippled epiphyses, cataract, ichthyosiform erythroderma and patchy alopecia of the scalp. The disorder is caused by mutations within the emopamil binding protein (EBP) gene encoding a 3β-hydroxysteroid-Δ8,Δ7-isomerase. Intrafamilial variation of disease severity is a known feature of CDPX2 probably caused by skewed X-inactivation.
A 24-year-old gravida 1 was referred to our ultrasound unit at 22+4 weeks of gestation because of suspicion of a fetal skeletal dysplasia

on routine ultrasound screening. The detailed anomaly scan revealed platyspondyly, shortened limbs, slightly bowed femura, elbow contractures, ulnar deviation of the hands and a small, bell shaped thorax. After counselling of the parents, the pregnancy was terminated at 23+6 weeks of gestation. Pathological examination confirmed the ultrasound findings. In addition, postaxial polydactyly of both hands and ichtyotic skin lesions were noted. Radiography of the fetus showed punctate calcifications scattered throughout the spinal column, the costal cartilages, the clavicles, the pelvis and the epiphyseal centers of the extremities. The diagnosis of CDPX2 was confirmed by mutation analysis within the EBP gene (c.328C>T, p.R110X). This nonsense mutation was previously detected in a patient, who was diagnosed as CHILD syndrome. Surprisingly, the mother of our case carries the same mutation without having any signs of CDPX2. The analysis of the CAG repeat within the AR gene and of a second polymorphic dinucleotide repeat (ZNF261) revealed no evidence of skewed X-inactivation both in the mother and the fetus. Therefore, the intrafamilial variability in this case can not be explained, at least in blood cells, by skewed X-inactivation. In conclusion, we present a completely phenotypical healthy carrier female, who was pregnant with a severely affected fetus as a striking example of reduced penetrance in CDPX2.

P022

Fetal left isomerism (Polysplenia) and maternal uniparental isodisomy 14: evidence for a recessive gene for heterotaxia on chromosome 14?

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Genetics of heterotaxia is highly heterogenous and so far known heterotaxia genes account only for a minority of patients. Isomerism is characterised by abnormalities of the spleen and of visceral lateralisation with congenital heart defects. Occurrence is mostly sporadic and generally a recurrence risk of 5% is given. However, recessive inheritance is assumed in consanguineous families with several affected sibs.

Here, we report on the first pregnancy of a young, consanguineous Turkish couple. Ultrasound examination in the 17th week of gestation revealed an atrioventricular septal defect and tetralogy of Fallot. The inferior vena cava was interrupted with a continuity of the v. azygous suggesting the diagnosis of left isomerism (polysplenia). Cytogenetic examination of amniotic cells revealed a homologous chromosome 14 Robertsonian translocation [45,XX,rob(14;14)(q10;q10)]. Maternal uniparental isodisomy 14 was confirmed by molecular analysis. Karyotypes of the parents were normal. The pregnancy was terminated and autopsy confirmed polysplenia, left atrial isomerism, atrioventricular canal, bilobal lungs symmetrical liver and malrotation of the intestinum.

UPD 14 mat is one of the more frequently observed UPD´s characterised by a pre- and postnatal growth retardation, muscular hypotonia and early onset of puberty. To our knowledge cardiac defects in UPD 14 mat have only been observed in one patient with persistent ductus arteriosus (Miyoshi et. al. 1998). Thus, UPD 14 mat is unlikely to cause polysplenia in our patient. Polysplenia in this fetus could be due to unmasking of heterozygosity because of UPD 14 and point to a so far unknown gene for heterotaxia on chromosome 14.

P023

Ectodermal dysplasia: Mutations in the EDAR gene

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Ectodermal dysplasia (ED) is a genetic disorder characterized by malformation of teeth, sparse hair and the lack of sweet glands. Although most ED cases are due to mutations in the X-chromosomal EDA gene, autosomal forms are known. These ED phenotypes result from mutations in either EDAR gene or EDARADD gene. Remarkably, mutated EDAR can cause both, dominant and recessive forms of ED. Only a few publications reported on mutations in the EDAR gene. In order to evaluate the contribution of EDAR mutations here we analysed 8 families with clinical symptoms of ED. All patients where previously tested negative for ED1 mutations. In a large kindred with 9 affected family members the dominant mutation Arg420Gln was detected in EDAR.

The propositas is the youngest affected member of the family. She has only six molars. Missing teeth and reduced ability to sweat are known in this family. Only the affected grandmother shows very thin hair.

The mutation Arg420Gln was described in another american family before. Therefore it is likely that this mutation is a single founder mutation. In a sporadic case we detected a novel compound heterozygous point mutation: Glu379Lys and the splice site mutation IVS5+1ds G>A. Neither the mother nor the father show clinical features of ectodermal dysplasia suggesting a recessive trait.

However, the IVS5+1ds G>A affected the splice consensus site of intron 5. Therefore one would expect a dominant effect of this mutation. As a result of the variety of phenotyps it can not be excluded that IVS5+1ds G>A is a dominant mutation with a minor phenotype in the mother, which was not detected during clinical examination. Finally, in another family with 3 affected family members the novel dominant mutation 1165delGA was detected.

In conclusion we submit that mutations in the EDAR gene are much more common than it was thought. Therefore, testing for EDAR mutations should be considered in all cases tested negative for the X-linked ED1 form.

P024

Lowe syndrome versus dent disease 2 – does type and/or location of OCRL1 mutation predict phenotype?

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Background: Mutations in X-linked OCRL1, a gene that encodes a phosphatidylinositol 4,5-bisphosphate 5-phosphatase, are responsible for the two diseases Dent 2 and Lowe syndrome. These are characterized by impaired proximal tubular function associated with low molecular weight proteinuria, aminoaciduria, phosphaturia, glycosuria and often renal failure, as well as mildly elevated muscle serum enzymes. Whereas cognitive and behavioral impairment is more often present in Lowe patients, congenital cataracts are a sole and constant feature of Lowe syndrome.

Methods: 9 male patients from 7 families with a phenotype resembling Lowe/Dent 2 disease were analyzed for defects in the OCRL1 gene.

Results: The type/location of mutations identified in 6 of these families resembles earlier observations in that the resultant phenotype can be deduced from the respective mutation observed. On the other hand, the spectrum of OCRL1 mutations provides no clue for the phenotypic differences in both diseases and the formation of cataracts in the more severe Lowe syndrome.

Conclusion: Taken together with our previous results and a review of the literature, data imply that variations in (an)other gene(s), should be involved in the development of the two diseases.

P025

Autosomal dominant vocal cord and pharyngeal weakness with distal myopathy (VCPDM, MPD2, 5q31): refinement of the candidate interval and identification of a second VCPDM family

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Vocal cord and pharyngeal weakness with distal myopathy (VCPDM) is a late-onset, progressive, autosomal dominant neuromuscular condition. VCPDM had been mapped to an 11.8 Mb interval on chromosome 5q31 in a large family from the US. We were able to identify two newly affected patients within the original pedigree. Linkage analysis using densely spaced STR markers from the candidate interval disclosed a critical recombination event in both patients. This reduced the critical region to 5.3 Mb between newly established STR markers AC108764 and AC089765. This interval still contained 62 genes. The most promising candidate gene was myotilin (MYOT) as MYOT mutations cause a similarly progressive and adult-onset muscle disease. However, defects in myotilin were widely excluded by various experimental approaches. We studied additional families with a distal myopathy and with or without accompanying vocal cord paralysis by linkage analysis, identifying a large Bulgarian family that also showed linkage to the VCPDM region. The families do not share a common disease haplotype indicating that they probably carry different mutations in the so far unknown VCPDM gene. Mutation screening of candidate genes is currently underway.

P026

Pathology and phenotypic variability of fetuses with holoprosencephaly in the Meckel Anatomical Collections at the University of Halle, Germany

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In 1826, when Johann Friedrich Meckel the Younger, the founder of developmental pathology, described cyclopia ("Über die Verschmelzungsbildung"), he had studied this condition for over 15 years and had assembled a collection of 28 preparations - eleven piglets, seven lambs, five calves, a kitten, and four humans – which display this disorder in varying degrees of severity. Cyclopia is an extreme facial malformation and is almost always associated with the alobar form of holoprosencephaly (HPE), a major brain anomaly. There are variable types of HPE, ranging from severe alobar, semilobar and lobar type, to microforms with microcephaly, corpus callosum-, septum pelucidum-agenesis/dysgenesis and arhinencephaly. Facial dysmorphies associated with HPE include cyclopia, ethmocephaly, cebocephaly, premaxillary agenesis, ocular hypotelorism or a single maxillary central incisor. The etiology is extremely heterogeneous and involves environmental and genetic factors. Here, we present essential material upon which Meckel and his medical students based their descriptions. Moreover, our re-evaluation of the preparations led to the discovery of a wide range of HPE forms. The face dysmorphic fetuses in the Meckel Collections can be classified into synaphtolmia, ethmocephaly, cebocephaly, and premaxillary agenesis. Modern diagnostic techniques, such as MRI and CT scanning and comparative genetic hybridization (CGH) are currently being used to investigate these fetuses. In the present paper, we discuss the human fetuses with HPE under consideration of the embryopathology of the facial malformations.

P027

Dandy-Walker complex in a boy with a 5 Mb deletion of region 1q44 due to a paternal t(1;20)(q44;q13.33)

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A 10 year old boy with vermis hypoplasia, dilatation of the 4th ventricle, enlarged cisterna magna and aplasia of the corpus callosum, consistent with the Dandy Walker Complex (DWC), and slight facial dysmorphisms, severe motor and mental retardation (including strongly limited speech) is presented. Initial GTG banding at the 550 band level of our patient showed a normal male karyotype (46,XY). By combining data obtained with 3783 BAC-based Array-CGH, FISH, and Multiplex Ligation-mediated Probe Amplification (MLPA) we identified a 5 Mb deletion of the 1q44 -> qter region resulting from a paternal t(1;20)(q44;q13.33). The deletion breakpoint was located between the ZNF238 gene (nucleotide position: 240,540,626 through 240,546,817; retained) and the HNRPU gene (nucleotide position 241,343,004 through 241,353,868; deleted). This is the smallest 1q44 deletion in a DWC patient reported so far. This deletion enabled us to significantly narrow down the number of candidate genes for the DWC in this region. Since the ZNF124 transcription factor is strongly expressed in the fetal brain it may represent a candidate gene for the DWC at 1q44.

P028

Dural ectasia in patients with genetically proven Marfan syndrome

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Marfan syndrome is an autosomal dominant disorder involving different organ systems. The phenotype is described by the Ghent nosology which classifies the clinical manifestations in major and minor criteria. Marfan syndrome type 1 (MFS1) is caused by mutations in FBN1, and mutations in TGFBR1 and TGFBR2 lead to Marfan syndrome type 2 (MFS2) or a related disorder, Loeys-Dietz syndrome (LDS).
Dural ectasia is one of the major criteria for Marfan syndrome but it is rarely tested for. Dural ectasia is described as widening of the spinal canal, scalloping of the vertebral body posteriorly, increased thinning of the cortex of pedicles and laminae, widening of the neural foramina or the presence of a meningocele.
28 patients with identified mutations in FBN1 and two patients with mutations in TGFBR1 and TGFBR2 were examined for dural ectasia. Magnetic resonace imaging of the lumbar and sacral spine of these 30 patients were performed and the results were compared with 30 patients without Marfan syndrome.
Our results show that a high portion of the patients only then fulfill the clinical criteria of the Ghent nosology if a diagnosis of dural ectasia is made.
Established methods for measuring dural ectasia are of highly variable sensitivity and specificity. Our purpose is to compare these methods with a newly established method of assessing dural ectasia by measuring the transverse and sagittal width of the dural sac at different levels.

P029

A novel mental-retardation/multiple congenital anomalies syndrome associated with a de novo t(11;20)(p15;q13.3) translocation

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We report a 7 years old boy presenting with severe mental retardation and developmental delay, absences and focal seizures with generalisation, bilateral sensorineural hearing loss, submucous cleft palate, persistent ductus botalli, unilateral cystic-dysplastic kidney and frequent infections. He also showed dysmorphic facial features including a high forehead, high arched eyebrows, hypertelorism, a small mouth, a broad nasal bridge and a bulbous nasal tip. Prenatal cytogenetic analysis of chorionic villi specimens showed a normal karyotype. Subtelomeric screening by fluorescence in situ hybridisation (FISH) showed a apparently balanced translocation, which was in conjunction with high resolution GTG-banding described as 46,XY,t(11;20)(p15;q13.3). Parental karyotypes including FISH studies were normal. Molecular karyotyping using a 10 K SNP array revealed no evidence for a cryptic imbalance. Breakpoint

mapping and investigation of candidate genes in the breakpoint region is in progress.

P030

Detection of gene duplications and complex rearrangements in the 21-hydroxylase gene locus in patients with congenital adrenal hyperplasia

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Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder with a wide range of clinical manifestations. CAH is caused in 90-95% of cases by mutations in the CYP21A2 gene on chromosome 6. Molecular analysis of 21-hydroxylase deficiency is complicated due to the presence of an inactive pseudogene (CYP21PA1), by gene conversions between the two CYP21 genes and by deletions and duplications within the CYP21 gene locus. We studied three families with index patients showing only mild symptoms of 21-hydroxylase deficiency (hyperandrogenism, hirsutism, oligo-/amenorrhe). PCR and direct sequencing of the CYP21A2 gene from index patients, parents or other family members revealed compound heterozygosity for mutations, leading to complete loss of 21-hydroxylase enzyme activity. With the multiplex ligation-dependent probe amplification (MLPA)-method duplications of the CYP21A2 gene could be detected on one allele. These duplicated CYP21A2 genes can explain the relatively high residual enzyme activity which will be necessary for the prevention of classical CAH symptoms. In one patient, 17OH-progesterone serum level (basal and after ACTH stimulation) was in a range typical for heterozygous mutations of the CYP21A2 gene. The segregation of the duplicated CYP21A2 gene in-cis with one of the "classical" mutations could be followed in the patient's families. In conclusion, complex rearrangements and duplications in the CYP21 gene locus should always be considered in the analysis and genetic counselling of patients with CAH. Especially in cases with a disagreement between mild clinical symptoms of hyperandrogenism or CAH but detection of point mutations in the CYP21A2 gene leading to complete loss of enzyme activity in-vitro. MLPA is a sensitive and easily performed technique that allows the rapid identification of deletions and duplications of the CYP21 genes that remain undetected using direct sequencing and/or other screening methods.

P031

Using Multiplex Ligation-Dependent Probe Amplification (MLPA) assay for the detection of subtelomeric abnormalities

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Because it is known that unbalanced chromosomal submicroscopic abnormalities involving subtelomeric regions may cause mental retardation we decided to screen a group of patients showing abnormalities such as mental retardation, developmental retardation, facial dysmorphism, malformations or muscular problems. In all these cases G-banded chromosomes had been analyzed using light microscopy at the resolution of 500 bands. All the karyotypes appeared to be normal 46,XX or 46,XY. Using Multiplex Ligation-Dependent Probe Amplification (MLPA) assay we studied more than 50 patients, controls and controls with a known submicroscopically unbalanced structural anomaly at the telomere. For our screening we used the SALSA P036B probemix by MRC-Holland. This kit contains one MLPA probe for each subtelomeric region. So it is possible to detect deletions/duplications of these regions. Most probes are designed for a well-characterized gene near the telomere. MLPA PCR reactions have been analyzed by capillary electrophoresis on an ABI-Prism 3100 Genetic Analyser. Using GeneMapper software (Applied Biosystems) it was possible to export the data for statistical analysis. This has been done with the help of Sequence Pilot 2.3 software by JSI Medical Systems GmbH. Deletions of probe recognition sequences will be apparent by a 35-50% reduced relative peak area of the amplification product of that probe.
We were able to confirm subtelomeric anomalies in all control samples and detected several subtelomeric deletions and/or duplications in some of our patients.
We report on representative cases where MLPA assays have been the initial steps for further analyses (e.g. FISH) to characterize chromosomal subtelomeric abnormalities.
One of these patients is a girl, three and a half years old, dysmorphic, with general developmental retardation. We could detect a subtelomeric deletion on chromosome 1p using MLPA and we could confirm this by subtelomeric FISH.

P032

Marden-Walker-syndrome: case report, nosologic aspects, and genetic counseling

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The Marden Walker syndrome is characterized by an immobile face with blepharophimosis, cleft or highly-arched palate, congenital contractures, camptodactyly, arachnodactyly, muscle weakness, reduced muscle mass, and severe psychomotor retardation. Postnatal features consist of failure to thrive, growth retardation and recurrent respiratory infections. We here report on 6 new patients and review forty patients of the literature. The suggested criteria for diagnosis are critically discussed. More than half of the patients have microcephaly and cerebral and cerebellar malformations, suggesting that Marden Walker syndrome may be caused by a primary neurological disturbance, resulting in a more or less typical hypokinetic phenotype. Overlapping features to the fetal akinesia sequence are therefore not astonishing. The etiology is probably heterogeneous and most cases are sporadic. However, affected sibs and parental consanguinity point to autosomal recessive inheritance and should be considered in genetic counseling.

P033

Identification of a "cryptic mosaicism" involving at least 4 different small supernumerary marker chromosomes, derived from chromosome 9, in a potential infertile woman

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Here we report the case of a potential infertile woman without further clinical symptoms with a small supernumerary marker chromosomes (sSMC) present. In detail: a young, healthy, non-consanguineous couple with repeated miscarriages asked for genetic evaluation due to infertility. A mosaic sSMC was detected by GTG-banding and molecular cytogenetics revealed four distinguishable populations of cells with sSMC, all derived from chromosome 9. In each metaphase with sSMC only one or two markers were observed. Based on the fluorescence in situ hybridization (FISH) analyses the patient's karyotype was defined as 47,XX,+min(9)(:p12->q12:)/47,XX,+min(9)(:p12->q12::q12->p12:)/47,XX,+r(9)(::p12->q12::)/47,XX,+r(9)(::p12->q12::p12->q12::)x2/46,XX. It can be concluded that the presence of "cryptic mosaicism" for sSMC derived from the same chromosome could explain the high frequency of miscarriages in our patient. Additionally, the hypothesis that 9p12 chromosomal band is an euchromatic variant region without phenotypic impact other than a potential infertility is supported by this case report.

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P034

Impact of molecular diagnostic procedures on therapy decision making in Wiskott-Aldrich syndrome: a case report

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Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive disorder characterized by thrombocytopenia, small-sized platelets, eczema, recurrent bacterial, viral infections and an increased risk for autoimmunity and malignancy. X-linked thrombocytopenia (XLT) is characterized by chronic microthrombocytopenia. The gene responsible for WAS/XLT is the WASP gene. A correlation between genotype and phe-

notype was controversially discussed for a long time. Recently published two in-depth analyses describe a strong association between the clinical phenotype and presence or absence of WASP protein. The conclusion reached from these studies is that the effect of the mutation on protein expression may have implication on long-term prognosis and on the selection of therapies. A 2-year-old boy with WAS had thrombocytopenia and ecchymoses since birth. At the age of one year he developed eczema around the mouth. From the age of 18 months onwards he has regularly received platelet transfusion because of persistent symptomatic thrombocytopenia. Mutation analysis demonstrated a missense mutation in WASP gene exon 2 (WASP Exon E2 g.290 C>T, Arg86Cys). The protein expression was reduced. On review of the family history it was noticed that the grandfather also had a past history of reduced thrombocytopenia. The same WASP mutation and reduced protein expression was detected. The clinical variability in this family with WAS raises the question to what extent molecular diagnostic procedures can help the therapy decision making process? The therapy of WAS is based on clinical symptoms and includes platelet substitution and prophylactic treatment with immunoglobulins and/or antibiotics. Hematopoietic stem cell transplantation is the only curative therapy for WAS. Patients with protein expression usually have a milder course of disease, but is it safe, not to perform or delay a curative hematopoietic stem cell transplantation taking into consideration the significant morbidity and mortality?

P035

A contiguous gene syndrome with features of Saethre-Chotzen syndrome caused by interstitial del(7)(p21.3p15.3) with TWIST 1 gene deletion

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We report on a patient with craniofacial dysmorphisms including craniosynostosis, with mild hand and feet abnormalities, and mental and psychomotoric retardation due to a microscopically visible del(7)(p21.3p15.3).
We present results of FISH studies with a microdissection probe and with cosmids covering the TWIST 1 gene locus, as well as SNP analyses of the TWIST 1 gene coding region. These analyses confirm the cytogenetic result and demonstrate the heterozygous TWIST 1 gene deletion. The patient's Saethre-Chotzen features can be explained by TWIST 1 gene haploinsufficiency, overlapped with other symptoms within this contigous gene syndrome.

P036

Molecular studies on Pulmonary Arterial Hypertension (PAH) in children

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Pulmonary Arterial Hypertension (PAH) is a progressive cardiopulmonary disorder, which is usually diagnosed in the fourth decade of life. Nevertheless it sometimes affects children in the infancy (IPAH). Children with IPAH have been reported to develop severer symptoms than adults and before the introduction of long-term vasodilator therapy the mean survival time was only 10 months for children, whereas this was 2.8 years for adults. Although mutations of the Bone Morphogenetic Protein Receptor II (BMPR2) gene are commonly detected in familial and idiopathic adult cases, BMPR2 mutations have been reported in few affected children. We have performed screening of 42 children with manifest PAH for both point mutations and large rearrangements of the BMPR2 gene. These analyses revealed that only in three cases mutation of BMPR2 could have explained the infantile PAH. One child showed a de novo R491Q mutation, while two children had heterozygous deletions of BMPR2 exons 1 and 4/5, respectively. Interestingly the girl with the deletion of exons 4/5, who had the disease onset at the age of 7, has inherited the mutated allele from her apparently non-affected father. This situation supports observations of the reduced genetic penetrance of the disease and the female preponderance. Nevertheless in most children the genetic background of PAH remains unclear. Besides the BMPR2 gene, two additional components of the TGF-β pathway, the Activin A Receptor type II-like 1 (ALK1) and Endoglin (ENG) genes have been identified as causative factors in PAH. These genes are also involved in hereditary hemorrhagic telangiectasia. Therefore, in addition to the BMPR2 mutation screening in affected children we introduced analyses of the ALK1 and ENG genes with the use of direct sequencing and MLPA techniques. Here we will present the results of these analyses. Besides BMPR2, ALK1 and ENG, there will be other genetic and non-genetic factors involved in the pathogenesis of infantile PAH.

P037

Familial congenital non-immune hydrops, chylothorax, and pulmonary lymphangiectasia

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Congenital pulmonary lymphangiectasia (CPL) is is a rare lymphatic entity that is usually sporadic but can be associated with anomalous pulmonary venous connection and several malformation syndromes. Pulmonary lymphangiectasia is often associated with nonimmune hydrops and commonly fatal in the neonatal period. Familial occurrence of CPL and also non-immune hydrops fetalis are very uncommon. To date, only five sets of sibs with pulmonary lymphangiectasia or a related condition has been reported. Here we report two siblings with congenital non-immune hydrops, chylothorax, and pulmonary lymphangiectasia from healthy Turkish first cousins. This is the first report of consanguinity in this rare condition which gives even further support to an autosomal recessive inheritance pattern.

P038

Unbalanced subtelomeric translocation t(9;20) in a 6-year-old girl transmitted by father and grandfather– clinically unexpected results

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We report on a 6-year-old girl that was referred to the genetic department because of muscular hypotonia, developmental and speech delay and facial dysmorphism. She is the only child of healthy parents and was born by Caesarean section after 29 weeks of gestation (because of preeclampsia) with normal measurements (weight 1380g, length 40cm, and OFC 28cm). She could sit at 18 months, crawl at 20-22 months, walk at 27 months and spoke first words at 14 months. Now, at 6.5 years, she has normal body measurements (115cm and 49.7cm OFC), muscular hypotonia with articulation problems and facial dysmorphism (prominent forehead, high frontal hairline, hypertelorism, long philtrum). She attends a regular kindergarten, regular primary school is planned.
Chromosome analysis showed a normal female karyotype, but subtelomeric screening revealed an unbalanced subtelomeric aberration between the short arms of chromosomes 9 and 20 [46,XX,der(9)t(9;20)(p24;p13)(305J7-;D20S1157+)].
Mother's psychomotor development was also delayed, although she is of normal intelligence in adulthood. Childhood photographs of the mother showed strong facial similarities. Moreover the maternal grandmother had 4 pregnancy losses. Therefore, we suspected the mother to carry the translocation. On the other hand, the father had two mentally retarded relatives – a first cousin and an aunt.
Subtelomeric analyses of both parents showed –surprisingly to the family– that the father was carrier of the balanced translocation as well as the paternal grandfather. Investigation of the father's mentally retarded aunt showed the same unbalanced situation as in his daughter, results of the cousin are pending.
During genetic counselling of the parents together with the paternal grandparents it became clear that in more distant parts of the large paternal family tree there are 12 more relatives with up to now unclear retardations. If these are also related to unbalanced t(9;20)-aberrations has to be investigated.

P039

Holoprosencephaly, bilateral cleft lip/palate and ectrodactyly (Hartsfield syndrome): new cases and additional clinical findings

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The combination of holoprosencephaly, bilateral cleft lip/palate and ectrodactyly is rare and up to now only a few cases have been reported. We present two additional cases:
In case 1, holoprosencephaly and bilateral cleft lip/palate were seen by ultrasound in the 29th week of an otherwise uneventful pregnancy of healthy and unrelated parents. Prenatal cytogenetic diagnostics revealed a normal male karyotype (46,XY). In addition to the characteristic findings of lobar holoprosencephaly including bilateral cleft lip/palate, telecanthus, low-set ears and ectrodactyly of both hands and feet, cerebellar hypoplasia, hydrocephalus, an accessory umbilical vessel and amniotic bands were found in the pathological examination after termination of pregnancy. X-ray analysis revealed hypoplastic frontal bones and a wedged thoracic vertebral body which represents a previously undescribed feature in Hartsfield syndrome.
Case 2, a male newborn, presented with holoprosencephaly, bilateral cleft lip/palate, ectrodactyly of one foot, and unilateral syndactyly of the third and fourth fingers. By array-CGH we could not detect any causative submicroscopic changes in case 1 except for already known copy number variants. All reported cases were male and sporadic so far. Therefore, we speculate that a de novo mutation of an X-linked gene might be causative for this rare disorder or that sex is at least an important modifier in Hartsfield syndrome.

P040

Corticosterone methyloxidase deficiency type II due to a novel mutation in the aldosterone synthase gene (CYP11B2)

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The cytochrome P450 enzyme aldosterone synthase, encoded by the CYP11B2 gene, catalyzes the formation of aldosterone from deoxycorticosterone in a two-step reaction: 11-hydroxylation, leading to corticosterone, is followed by hydroxylation and finally oxidation at position 18. Corticosterone methyloxidase (CMO) deficiency type II is an autosomal recessive inherited disorder, caused by inhibition of the terminal oxidation step. Patients present in early infancy with severe electrolyte imbalance and dehydration, hyponatremia and hyperkalemia and poor weight gain. Steroid analysis shows low plasma levels of aldosterone, accompanied by elevated levels of corticosterone, 11-deoxycorticosterone and a high ratio of the precursor 18-hydroxycorticosterone to aldosterone. The index patient presented in early childhood with severe postnatal salt wasting. CMO deficiency type II was diagnosed clinically and biochemically by steroid analysis. Molecular genetic analysis confirmed the clinical diagnosis at the age of 15 years and was able to exclude the disorder in the newborn second child of the family.
Direct sequencing of PCR amplified DNA revealed a novel homozygous missense mutation of codon 451 (CTC⟶TTC) in exon 8 of the aldosterone synthase gene in the index patient, causing a substitution of leucine 451 by phenylalanine (L451F). In the highly homologous 11ß-hydroxylase (CYP11B1) the amino acids 443 to 463 are involved in the formation of the binding site of the heme group, so it is likely that the mutation at position 451 interferes with the binding of the heme group. Both parents are heterozygous carriers of this novel mutation, the second child of the family did not inherit the mutation. In conclusion, confirmation of the diagnosis of CMO deficiency type II by molecular genetic testing can have considerable implications for the early onset of therapy of the patient and affected family members.

P041

Ter Haar syndrome in two sons of a consanguineous Lebanese couple

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Ter Haar syndrome or Melnick-Needles-like syndrome is a rare condition with facial and skeletal anomalies. We saw the index patient at the age of 6 months and 3 ½ years. His parents are first cousins. No other disorders were observed in the highly consanguineous family of Lebanese descent. The boy was born with normal birth measurements and had normal body measurements at 3 ½ years of age. He showed a characteristic face with hypertelorism, strikingly large eyes with large corneae, full cheeks, wide mouth, microretrognathia and large ears. His fingers were short with short and broad terminal phalanges. He had a gibbus of the lumbar spine. His motor and mental development was normal. Cytogenetic investigations gave normal results (46,XY). X-rays of the spine confirmed the lumbar gibbus and showed abnormally shaped vertebral bodies, reminiscent of the so-called dysostosis multiplex. The radiograph of his pelvis showed broad ossa ilia and short femoral necks. The hand X-rays demonstrated a generalized shortness of all tubular bones and poor modelling of distal radius and ulna. In addition, there is reduced mineralization of these bones. The second son was born at term with normal birth measurements. He showed the same facial changes seen in his brother, but in addition he had a complex heart defect (VSD and transposition of the great vessels). Metabolic investigations, performed because of severe hypotonia, revealed L-2-OH-glutaric aciduria. The boy died at the age of 5 months of his metabolic and heart problems. The facial and radiological changes in the two boys were consistent with Ter Haar syndrome. The family was included in a large genome wide analysis of families with Ter Haar syndrome in order to identify the gene responsible for this autosomal recessive disease.

P042

Mutations in WNT4 are not a common cause for Rokitansky-Küster-Hauser syndrome

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The Rokitansky-Küster-Hauser (RKH) syndrome encompasses the absence of uterus and vagina in women with karyotype 46,XX. Heterozygous mutations in the WNT4 (wingless-type MMTV integration site family, member 4) gene encoding a secreted protein which represses male sexual development have been associated with RKH in humans. Furthermore, female mice deficient in WNT4 were masculinized showing absence of Mullerian ducts.
We describe here four women with suspected diagnosis of RKH. Patient 1 was presented with hypergonadotropic hypogonadism, uterus- and vaginal aplasia, while patient 2 and 3 has only an uterus aplasia, respectively. Patient 4 suffers from a vaginal agenesia in correlation with various other symptoms like short stature, dystrophia, anal atresia with vestibular fistula and esophago-tracheal fistula. Chromosomal analyses showed in all cases a normal female 46,XX karyotype.
Because of the findings in humans and mice we decided to perform sequence analyses of WNT4 gene in all four patients.
DNA of the patients were extracted from blood samples and the five exons of WNT4 gene were amplified by polymerase chain reaction from the DNA of the patients. The obtained PCR products were analysed by direct sequencing.
Mutational analyses revealed no mutation in the WNT4 gene of the four patients. Therefore, we conclude that mutations in the WNT4 gene are not a common cause for RKH. The two young women with mutations in the WNT4 gene described in the literature both showed signs of androgen excess like acne and hirsutism suggesting that WNT4 deficiency is a clinical entity that can be delineated from RKH.

P043

MLPA in diagnosis of 21-hydroxylase deficiency confirms the extreme heterogeneity of the CYP21 gene locus

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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. The pathogenic defects

in the corresponding gene (CYP21 on chromosome 6p) result from recombinations between the CYP21 gene and its highly homologous but inactive pseudogene (CYP21P). These recombinations lead to deletions/duplications of the CYP21 gene or to gene conversions with transfer of sections of the pseudogene (including its mutations) into the active gene. Large gene conversions together with gene deletions account for one third of CYP21 defect alleles and hence require detection. Since they escape the common PCR based mutation detection assays an alternative approach like Southern analysis has to be applied. We used real-time PCR for quantification of gene copy number. This method offers the possibility of direct quantification instead of comparative quantification regarding the pseudogene, thus, facilitating detection of heterozygous deletions of CYP21 plus CYP21P. Since MLPA has emerged as ultimate technique for the detection of large deletions and duplications we extended the application of MLPA to the analysis of the CYP21 gene. A retrospective re-analysis of the panel of patients was in complete concordance with the original results of the real-time PCR. Because of the widespread composition of the MLPA-kit (further gene loci in the CYP21 region are included into the analysis) the resulting patterns are complex and confirm the assumption that the overall gene locus structures are highly heterogenous.

P044

Alopecia-contractures-dwarfism mental retardation syndrome (ACD syndrome): – description of a new case and review

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We report on a patient with short stature and distinct dysmorphic features, e.g. microdolichocephaly, total alopecia, missing eyebrows and eyelashes, photophobia, hypertelorism, hypolacrimation, ichthyosis congenita, hyperkeratosis, ectrodactyly of the right hand, long fingers, contractures of knees and hips, consistent with Alopecia-Contractures-Dwarfism Mental Retardation Syndrome (ACD Syndrome). No abnormalities were found in abdominal sonography and echocardiography. At the age of three years, the patient developed seizures, which were adequately controlled by antiepileptic medication. We performed conventional chromosome analysis and subtelomere FISH analysis with normal results. No mutation was observed in the TP73L gene, which has been reported to be causative for several syndromes with ectodermal dysplasia. Therefore, we performed in addition high resolution array-CGH (Comparative Genomic Hybridization) analysis.

ACD syndrome is a very rare genetic disorder described first by Schinzel A. in 1980 and has a distinct phenotype including short stature, microcephaly, alopecia, ich-thyosis follicularis, photophobia, contractures, skeletal anomalies, and some dysmor-phic features. ACD syndrome shares a unique combination of features with the Ichthyosis follicularis-atrichia-photophobia syndrome (IFAP), the cardinal features of IFAP being congenital alopecia, ichthyosis follicularis, and photophobia, but patients with IFAP do not have contractures or skeletal anomalies. To our knowledge, there have been only 7 case reports with ACD syndrome so far. Laboratory diagnoses were inconspicuous, although in none of them, array-CGH analysis was performed.
We present the clinical findings of the patient at the age of 10 1/12 years as well as the laboratory findings, comparing our results with the available literature data, and discussing the available literature information of ACD and IFAP syndromes.

P045

Pseudoachondroplasia in a 3 year old boy with a mild phenotype

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Introduction: Pseudoachondroplasia is a rare autosomal dominant osteochondrodysplasia due to mutations in the cartilage oligomeric matrix protein (COMP)gene, with all cases being ascribed to mutations in exons 8-19.
We report on a three year old boy with disproportional short stature (short-limbed dwarfism, body lengh –7SD) and normal head circumference. In addition, the patient presented with contractures of the elbows and mild lumbar lordosis. His psychomotor development was normal.
Methods: X-rays showed shortening of the tubular long bones with irregular broad metaphyses, very small femoral epiphyses, short phalangeal bones, epiphyseal ossification of the hand is disturbed as well.
Results: Mutation analysis of the COMP gene was initiated and a heterozygous nucleotide exchange 1310A>G in exon 13 resulting in a substitution of amino acid 437 from aspartic acid to glycine of the COMP protein was verified.
Conclusion: According to the literature the heights of the majority of patients with mutations in exon 13 of the COMP gene were significantly short (mostly below –8 SD) than in patients with mutations in the other exons. The clinical phenotype of the presented child including the height and the radiological changes seems to be comparatively mild.

P046

Kohlschütter-Tönz syndrome, an autosomal recessive disorder with epilepsy and a defect of tooth enamel

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Kohlschütter-Tönz syndrome is a rare inherited disorder characterised by severe epilepsy followed by developmental stagnation or even regression and yellowish discoloration of teeth due to a developmental defect of tooth enamel. This amelogenesis imperfecta affects both primary and permanent dentition. About 20 cases have been reported, and inheritance is thought to be autosomal recessive. We present another family in which two affected children (a boy and a girl) were born to consanguineous parents from Morocco. The affected children had the typical features of Kohlschütter-Tönz syndrome: After uneventful pregnancy and birth they showed delayed psychomotor development and regression after the onset of epilepsy at the age of four and twelve months respectively. Subsequently both children showed severe mental retardation. Dentition was late and the teeth were yellow with an irregular surface. All investigations including cerebral MRI and a muscle biopsy performed in another hospital gave results within normal limits. The diagnosis of Kohlschütter-Tönz syndrome was made relatively late as the typical tooth abnormalities had been overlooked. The causative gene of this disorder has not yet been identified. As there are no biochemical abnormalities, the diagnosis has to be made on clinical grounds alone but is usually straightforward once the tooth abnormalities are recognised.

P047

Fine mapping and molecular characterization of a t(4;12) translocation breakpoint in a patient with hypertension and brachydactyly type E

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Arterial hypertension and brachydactyly are two independent conditions, usually not linked together by a joint cause. One notable exception is the Bilginturan Syndrome (OMIM 112410), which was first described in 1973 as a form of brachydactyly manifested by shortening of both phalanges and metacarpals and associated hypertension. Subsequent linkage analyses have localized the responsible gene(s) to chromosome 12p12.2-p11.2.
Here, we report a family with 5 affected members, who have proportionate short stature, brachydactyly type E and hypertension. In the 52-year-old female index patient as in other affected family members we detected a balanced translocation t(4;12), which was absent in unaffected family members. Array-based comparative genome hybridization (array-CGH) with ca.

0.5 MB resolution showed no significant loss or gain of genomic material, neither on the affected chromosomes nor anywhere else in the patient's genome.
Subsequent fine mapping with FISH localises the breakpoint to a "gene desert" on 12p11.22. Since no known gene appears to be hit directly, long range effects by removal or addition of cis-regulatory elements are likely to be involved in the pathogenesis of the disease. Currently, we are scanning the breakpoint region for putative enhancers and silencers of gene expression by co-localisation studies with interphase FISH. Such co-localisation studies have been successfully applied by Velagaleti et al. [Am. J. Hum. Genet. 76 (2005) 652-662], who identified two enhancers in a distance of approximately 1 MB from their target gene (SOX9).

P048

The long way to diagnosis: A family case of submicroscopic translocation t(7;22)(q36;q13)

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The index patient, second daughter of healthy, consanguineous parents, was first seen in 1997 at the age of 11 months because of short stature, microcephaly, agenesis of corpus callosum, uvea and opticus coloboma, single upper central incisor, agenesis of the right and hypoplasia of the left kidney and developmental delay. She had a mentally retarded aunt and two mute cousins, the later were not available for examination. Cytogenetic analyses of the index patient, her mother and her aunt results showed normal karyotypes. Also, Rubinstein-Taybi and DiGeorge syndrome were excluded for the index patient by FISH. She was seen again in 2005 and 2006. Because of her midline defects a mutation in the Sonic Hedgehog gene (SHH, 7q36) was suspected, but direct sequencing of the coding regions of the SHH did not reveal any mutation. To exclude chromosomal mutations in putative regulatory sequences of SHH, FISH with a subtelomere probe 7q was performed. Interestingly, this probe hybridized only to one of the girl's chromosome 7. Molecular analysis narrowed down the extension of the deletion and showed hemizygosity for the region 7q36.3 of the index patient and her affected aunt. Thus, hemizygosity 7q36.3 is likely to cause the holoprosencephaly spectrum present in this family. To study the reasons for this hemizygosity, FISH with wpc7 and the DiGeorge Region Probe (Abbott) was performed and revealed that the index patient's mother carries a balanced, submicroscopic translocation t(7;22)(q36;q13). The unbalanced state 46,XX,der(7)t(7;22)(q36;q13) was found in the affected family members. To our surprise, the marker ARSA from 22q13 was present on the terminal long arm one chromosome 7 of all three women. These results seemed to be not consistent with earlier FISH results obtained with Oncor's DiGeorge Region Probe in 1997, where the control probe from 22q13 hybridized only to the chromosomes 22. The explanations for this apparent contradiction will be discussed on our poster.

P049

An additional patient with craniodigital syndrome: a new case of Filippi syndrome?

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Filippi syndrome ia a rare autosomal recessive condition. Characteristic features are pre- and postnatal growth retardation, microcephaly, syndactyly of fingers and toes, psychomotor retardation and dysmorphic face. We report on a new patient resembling the clinical features of Filippi syndrome.
The boy was born in the 36th gestational week with a length of 42 cm (< 3rd centile), and a head circumference of 30 cm (3rd centile). Craniofacial anomalies included micro- and brachycephaly, round face, short nose, downturned mouth, thin lips, and micrognenia. There was soft tissue syndactyly of digits 3-5 and incomplete syndactyly of toes 2-3 and 4-5 bilaterally. The boy had an atrioventricular septal defect. At the age of 6 6/12 years height was 105 cm (< 3rd centile), and head circumference 43 cm (<< 3rd centile). He had a teeth anomaly - only three lower incisors. There was a severe physical and mental retardation.
He is the first child of nonconsanguineous parents. Two paternal half-brothers of the mother are mentally retarded.
Cytogenetic analysis was performed on peripheral blood lymphocytes. The analysis of G-banded chromosomes revealed a normal male karyotype 46,XY. Fluorescence in situ hybridization (FISH) analyses excluded microdeletions 22q11.2 and 17p13.3. Subtelomere FISH analysis excluded a cryptic subtelomeric rearrangement.
In 1985 Filippi [Am J Med Genet 22:821-824] gave a description of an apparently new syndrome in three of eight sibs comprising syndactyly of fingers and toes, microcephaly, and severe physical and mental retardation. In 2002 Franceschini et al. [Genet Counsel 13:343-352] compared the clinical findings in 17 cases of Filippi syndrome with other craniodigital syndromes. Sharif et al. 2004 [Clin Dysmorph 13:221-226] added two patients with syndactyly of fingers and toes, microcephaly, developmental delay, growth retardation, unusual teeth and hair. We want to contribute our patient as a further observation of this rare entity.

P050

A 400 kb deletion involving the aspartoacyclase gene as novel cause of Canavan disease in a child of consanguineous parents of Turkish descent

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Canavan disease is a progressive autosomal recessive disorder characterized by spongy degeneration of the brain. The biochemical marker for this disease which is rare in non-Jewish populations is an increased level of N-acetylaspartic acid (NAA) in cerebrospinal fluid and urine owing to a deficiency of aspartoacyclase (ASPA). This disorder is mostly caused by point mutations in the ASPA gene. Partial deletions of ASPA gene seem to be very rare. Here, we report the first occurrence of a homozygous microdeletion including the ASPA gene in a child presenting with Canavan disease.
The patient aged 7 years is the second child of consanguineous parents of Turkish descent asking for prenatal diagnosis for this disease. PCR failed to produce an amplification product for any of the exons of the ASPA gene on DNA of the patient. Sequence analysis of his parents' DNA revealed no mutation in the ASPA gene. SNP chip array analysis (Affymetrix 250k GeneChip) using DNA of the patient showed a gross homozygous deletion of about 400 kb in 17p13.2-3 involving not only the ASPA gene but also several neighboring genes. A FISH test is under development to offer prenatal diagnosis for this disorder in an ongoing pregnancy.
Molecular genetic testing for Canavan disease by targeted mutation analysis and / or sequence analysis in non-Jewish populations is expected to reveal less than 90% of all causative mutations in the ASPA gene. Our results suggest that SNP chip array analysis might be a valuable tool for testing those patients suffering from Canavan disease that cannot readily be characterized on a molecular level by standard assays.

P051

Autosomal dominant rigid spine without mutation in the LaminA/C gene

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We report on a three-generation family with autosomal dominant rigid spine of early onset and progression of symptoms up to young adulthood. The index patient was a 38 years old woman with fixed hyperlordosis of the thoracic and lumbar spine with minimal thoraco-lumbar scoliosis to the left, ventral tilting of the pelvis, and talipes varus. There was a significant loss of mobility and bending down was only possible by flexion in the hip joints. Apart from these findings and back pain in the morning the woman had no impaired sensibility, neurological symptoms or radiological signs of spondylarthosis or vertebral ankylosis. As in her son, first symptoms became evident at early childhood. Her son was born after uneventful pregnancy with bilateral club feet and showed first symptoms of progressive lumbar hyperlordosis at age 4 years. Two years later his spine was more or less stiff and neutralization of the physiological cervical lordosis and thoracic kyphosis was observed. No intraspinal lesions were seen at MRI examination. The 71 years old mother of the index patient and one sister with her two sons were similarly affected. A skeletal cause of the spine stiff-

ness was unlikely because of normal spine radiographs. None of the affected individuals had evidence of a known neuromuscular disorder; however, a muscle biopsy was not performed. Serum creatine kinase levels and cardiac examinations were normal. However, since a rigid spine can be the only symptom of a laminopathy we excluded a mutation of the LaminA/C gene. To our opinion, the symptoms, clinical course and mode of inheritance in our patients are different from autosomal recessive rigid spine syndrome as well as from any other muscular dystrophies described in the medical literature.

P052

Identification of a novel germline mutation (p.Ala208Thr) in MODY2 (GCK gene) in a family with diabetes mellitus

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Introduction: Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous autosomal dominant form of non-insulin dependent diabetes mellitus, characterized by a primary defect in beta-cell function and an early age of onset. Mutations in at least six genes have been shown to cause this type of diabets mellitus (DM). The most common type of MODY among Europeans is glucokinase-related MODY type 2. It is predominantly detected in children with mild hyperglycaemia.

Patient: The index case is a 3-year-old patient from Germany with pathologically glucose tolerance (elevated postprandial levels of glucose: 120-200mg/dl, HbA1c 7.1%). His mother has insulin-treated diabetes, and two uncles are also affected.

Methods: Genomic DNA was extracted from peripheral blood leukocytes. Coding regions (12 exons) and corresponding exon-intron boundaries of the GCK gene were amplified by PCR and subjected to direct sequencing.

Results: Sequence analysis of the GCK gene shows a novel heterozygous germline mutation p.Ala208Thr. The same mutation was proven in a cousin of the index patient with elevated level of blood glucose (142mg/dl) and HbA1c of 6.0 %, whereas in the sister of the index patient and in another cousin without pathologically glucose tolerance the proven mutation was not found. The novel missense mutation was not present in 100 unrelated healthy control subjects.

Conclusion: These data show that the mutation p.Ala208Thr cosegregates with the phenotype of DM within the family. Therefore this mutation is the genetic cause for MODY2 in the analyzed family. Careful genetic counseling and knowledge of the genetic cause of MODY has significant impact on clinical management and offers the possibility to identify family members at future risk.

P053

Report of one male infant with partial 1q trisomy mosaicism

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We report on a 14-months old male patient, first child of healthy unrelated parents. An amniocentesis in the 15th gestational week followed by chromosome analyses demonstrated mosaicism for a partial duplication on 1q in all cultures analyzed (karyotype: mos 46,XY,dup(1)(q21.1q32.1)[25]/46,XY[152]). Therefore, the amniocentesis and chromosome analysis was repeated in the 19th gestational week. In this analysis, the dup(1) was not found in 40 cells of the first culture, but was present in 56 of 57 cells of the second culture. The delivery was carried out at the 41th gestational week by caesarian section due to disturbances of the cardiotokogram. At birth, the weight of the patient was 2945 g, the length was 50 cm and the head circumference was 36 cm. The external morphologic examination revealed a general dystrophy, mild craniofacial dysmorphisms (brachycephaly, microphthalmia, retrognathia, high arched palate) and muscular hypotonia. A developmental evaluation at the age of 14 months showed a delay of 8-9 months. Thus, a severe developmental retardation was diagnosed. Postnatal cytogenetic analysis of blood lymphocytes revealed a normal karyotype (46,XY) in 50 cells analyzed. Therefore, a chromosome analysis was performed on skin fibroblasts, which were found to carry the dup(1) in all cells. Thus, mosaicism for a dup(1)(q21q32) leading to a partial trisomy 1q in certain cell types was confirmed postnatally. Partial trisomy of the long arm of chromosome 1 is a rare condition and to our knowledge, the described duplication 1q has not been published so far. In this work, we report the clinical features of a new mosaic partial trisomy 1q.

P054

Sotos syndrome due to a novel NSD1 mutation

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We report on a boy who is the second child of a non-consanguineous healthy couple. He was born after 37 weeks of pregnancy with normal birth weight (2985g) and length (52cm) with prolonged icterus neonatorum. Thyroid function was normal at that time. There was a triangular face and a pointed chin. At the age of 4 months failure to thrive and developmental delay prompted further investigations. Hypothyroidism due to exclusive ectopic thyroid tissue was diagnosed and thought to be the cause. However, despite a normalization of the hormonal situation psychomotor development remained delayed. At presentation he was 2 5/12 years old. Length and head circumference were above the 97th centile. He had a flat, long face with frontal bossing, hyperteleorism, down slanting palpebral fissures, strabismus, large hands and feet. MRI showed ventriculomegaly. Hearing tests (BERA) were normal. Radiographs of the hands showed advanced bone age. The clinical diagnosis of Sotos syndrome was proven by molecular analysis of the NSD1 gene. A novel heterozygous frameshift mutation (c.1216-1219delGAAA) was identified. Our case illustrates the change of the facial gestalt in early childhood in Sotos syndrome. Moreover, a developmental delay in presence of dysmorphic features should prompt further investigations and not be attributed to a postnatal hypothyroidism alone. Ectopic thyroid tissue and hypothyroidism have not been reported in Sotos syndrome so far. Although we cannot exclude that these findings are coincidental, we suggest that thyroid function should be evaluated in other patients with Sotos syndrome.

P055

Mosaicism of an isodicentric Y chromosome with a Turner karyotype in a boy with mild mental retardation and malformations

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Isodicentric chromosomes of the short arm of the Y chromosome combine a wide range of phenotypes including female patients with Turner syndrome, infertile phenotypically ormal males and males with mental retardation, malformations and dysmorphic features. Prediction of the resulting phenotype is compilcated by varying degrees of mosaicism of cells with 45,X karyotypes and karyotypes with an aberrant Y chromosome. The phenotype depends on the virilisation inducing ratio of testicular tissue.

We report on a 13 years old boy with short stature, mild mental retardation, hearing impairment, congenital heart defect, hypogenitalism and hypogonadism.

The cytogenetic and molecular cytogenetic analysis revealed the karyotype mos 46,X psu dicY(q11.22)[50]/45,X[8]. To analyse a further tissue mucosa cells from a buccal smear were investigated by FISH with a centromere probe for the Y chromosome. Mosaicism of 3% cells with a 45,X karyotype was ascertained. PCR analysis revealed a deletion of the AZF-B and AZF-C loci while SRY was present.

We will discuss the clinical, cytogenetic and molecular findings of the boy in the context of the literature.

P056

Cranioectodermal dysplasia (Sensenbrenner's syndrome): Report of two siblings

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Cranioectodermal dysplasia (CED, Sensenbrenner's syndrome, OMIM 218330) is a rare disorder characterized by dolichocephaly, rhizomelic dwarfism, dental and nail dysplasia, sparse hair and renal problems. Among about 20 cases reported to date, most are sporadic, but few familial cases suggest autosomal recessive inheritance. So far, the underlying genetic defect is unknown, and linkage analyses were not possible due to the limited number of patients and small family sizes. Here, we report on a 5-year-old girl and her 1-year-old brother with cranioectodermal dysplasia. Their healthy parents (16- and 25-year-old at the birth of the first child) are remotely consanguineous which supports the assumption of autosomal recessive inheritance. Clinical features included short stature with rhizomelic shortening of limbs, brachydactyly, narrow chest, craniosynostosis, dolichocephaly, full cheeks, telecanthus, broad nasal bridge, small and widely spaced teeth, dysplastic auricles and fine, sparse hair, bilateral inguinal hernia and hyperelastic skin. Psychomotor development is normal. Both children suffer from tubulointerstitial nephropathy with more severe features in the brother.
We present detailed clinical features of the patients in comparison to previously reported cases, as well as prenatal features of the syndrome detected on ultrasound examinations beginning from the 17th week of the 2nd pregnancy.

P057

An adult index patient with Currarino syndrome due to a novel HLXB9 mutation, c.336dupG (p.P113fsX224), presenting with Hirschsprung´s disease, cephalgia and lumbodynia

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Background: The symptom triad of autosomal dominant Currarino syndrom (CS; MIM #176450) consists of anorectal malformation, a sacral bone defect and presacral masses Mutations in the homeobox HLXB9 gene have already been described in a subset of sacrococcygeal anomalies characterised by partial sacral agenesis.
Case: We report a 28 year old male patient with Currarino syndrome due to a heterozygous novel frame-shift mutation c.336dupG (p.P113fsX224) in the homeobox HLXB9 gene.
Conclusions: Molecular diagnostics may be helpful in cases of Hirschsprung's disease accompanied by other symptoms suggestive for Currarino syndrome, since it can lead to major complications like perianal sepsis, meningitis, and malignant transformation.

P058

„Zimmermann-Laband syndrome": follow-up of new sporadic case

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"Zimmermann-Laband syndrome"(ZLS) is rare disorder, characterized by gingival fibromatosis, multiple abnormalities (MIM 13550). Most cases are sporadic, familial cases displayed autosomal dominant transmission.
We presented follow-up on 17-years old male patient - first child of young healthy nonconsanguineous couple. Pregnancy, labor were unremarkable. Male infant (BW=3100; BL=52cm) showed no visible abnormalities. Normal growth (W=10200g; L=77 cm), motor milestones delay, asymmetric cranium, mild gingival hypertrophy, small nails were observed at age of 1 years old. Psychomotor retardation was diagnosed since early childhood. Gingival fibromatosis was developed during first decade of life. Limitation of joints mobility, camptodactyly became more pronounced with age. At age of 17 years old patient showed severe mental retardation, absent speech, growth delay, neurological signs, long face, large ears, deep-set eyes, large nose, full lips, short philtrum, gingival fibromatosis, hypodontia, teeth abnormalities, scoliosis, spastic joints contractures, nails hypoplasia, muscular hypotrophy, hypertrichosis. No ocular, heart defects, hepatosplenomegaly were observed. Chromosomal, biochemical analyses were normal. Differential diagnosis was done with other similar genetic syndromes ("gingival fibromatoses"; "gingival fibromatosis-cherubism-seizures"; "gingival fibromatosis-corneal dystrophy"; "gingival fibromatosis-deafness"; "gingival fibromatosis-depigmentation-microphtalmia"; "gingival fibromatosis-hypertrichosis"). We have established ZLS based on association of peculiar face, gingival fibromatosis, nails hypoplasia. Clinical features of patient was compared with published data. Cases presented various expression and severity. Results indicated, that peculiar face, gingival fibromatoses, nails defects are the most striking features. Mental retardation ranging from mild to profound is not rare feature, which is important for genetic counseling affected families.

P059

Molecular genetic studies of a quadricuspid aortic valve of a Turner's syndrome patient

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Quadricuspid aortic valve is uncommon congenital anomaly that often associated with other cardiac disorders. The most reported cases of quadricuspid aortic valve are detected as incidentally during necropsy or aortic valve replacement, so that the potential clinical course still unclear remained. We present a case of a 47-year-old woman presented with aortic insufficiency grade III-IV, mild left ventricular dilatation with an end-diastolic diameter of 59mm. During surgery for aortic valve replacement according to Ross a quadricuspid aortic valve was identified. Two years after the successful Ross-procedure a molecular genetic studies of this rare anomaly was performed using karyotyping, fluorescence in situ hybridisation (FISH) and polymerase chain reaction. Cytogenetic analysis detected chromosomal aberration 45,X0/46,XX, indicating a low-level X chromosome mosaicism and repeat karyotypes was normal. This is the first case to be reported on quadricuspid aortic valve in a woman with Turner's syndrome. For further studies in the future we hypothesize and support the conventional assumption that an involvement of X-linked factors and cardiac defects in Turner´s syndrome may exist.

Cytogenetics

P060

DNA degradation during maturation of erythrocytes—molecular cytogenetic characterization of Howell–Jolly bodies

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Howell–Jolly bodies (HJBs) are small DNA-containing inclusions of erythrocytes and are often present after splenectomy. The genetic composition of HJBs is unknown at present. We measured the sizes of HJBs in erythrocytes from a splenectomized patient using an inverted microscope. Two-dimensional positions of HJBs were projected onto a virtual erythrocyte. We also isolated individual erythrocytes that had inclusion bodies from five splenoctomized patients and performed DNA amplification using DOP-PCR with subsequent reverse painting on normal male metaphase spreads. The average size of HJBs was 0.73 ± 0.17 µm (range, 0.4–1.1 µm). Most HJBs were located in the center of the erythrocyte. Small HJBs contained DNA from one or two centromeres and larger HJBs contained DNA from up to eight different centromeres. Centromeric DNA from chromosomes 1/5, 7, 8, and 18 were most frequently observed. Signals from the centromeric regions of chromosomes 3, 4, 9, and 10 were not observed. Signals from euchromatic regions were detected in a few cases. We hypothesize that, in addition to enucleation and nucleus fragmentation, DNA degradation during maturation of erythrocytes preferentially eliminates euchromatic DNA. Similarities between these processes and those described for embryogenic stem cells suggest that most stem cells are able to degrade DNA in a genetically controlled manner.

P061

A critical region for severe mental retardation and corpus callosum dysgenesis of about 1 MB maps to chromosome region 1q44

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Patients who have terminal deletions of the long arm of chromosome 1 with the breakpoint in 1q43 are known to be affected with severe mental retardation, corpus callosum agenesis or dysgenesis, congenital malformations like cardiac malformations, epilepsy, as well as various dysmorphic features. We report on a patient who was evaluated for severe mental retardation, corpus callosum dysgenesis, epilepsy and vertebral malformation. Chromosome analysis revealed a paracentric inversion of 4q with a karyotype described as 46,XX,inv(4)(q21q35). The inversion was a de novo event. Cytogenetically, the inversion was balanced and various FISH studies could not detect any copy number change on chromosome 4. In order to obtain a high-resolution profile of genomic imbalances, arrayCGH was performed using a 44K-chip platform (Agilent). Chromosome 4 displayed a balanced genomic status but, interestingly, a cytogenetically cryptic deletion of about 1,1 MB was detected in chromosomal band 1q44. FISH probes were generated using various BAC clones spanning and flanking the deleted region. The cells from the patient showed a deletion in 1q44, confirming the arrayCGH findings. In contrast, both parents regularly showed a normal signal constellation indicating that the deletion had occurred de novo. In two other patients with a cytogenetically proven del(1)(q43), FISH for the newly identified candidate region in 1q44 confirmed involvement of the same region. Those patients exhibited a similar phenotype like the index patient with severe mental retardation, epilepsy, corpus callosum agenesis, and dysmorphic features. We postulate that the commonly deleted region in 1q44 contains a gene/genes which is/are associated with severe mental retardation and corpus callosum dysgenesis.

P062

Three cryptic deletions detected by 317 K SNP array within a de novo pericentric inversion 6 (p11.5q15)

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We reinvestigated a patient with an institial deletion 6q14 contained within a pericentric inversion with breakpoints in 6p11.2 and 6q15 determined by high resolution cytogenetic analysis (E. Passarge, Cytogenet. Cell Genet. 91: 192-198, 2000). Fluorescence in situ hybridization with region-specific BACs to the patient's chromosomes revealed a deletion from 84.6 Mb (RP4-676J13) to 96.5 Mb (RP11-702G19) in the inverted part of her chromosome 6. The inversion breakpoint in 6q15 could be narrowed to a small interval, whereas mapping of the breakpoint in 6p11 produced conflicting results. A segmental aneuploidy profile of the patient and her parents obtained with an Infinium 317 K SNP Array (Illumina, San Diego, CA, USA) revealed three separate deletions within 6q14 in 6p12, 6p12.3, and 6q14;q16. The deletion 6p12 comprised 1.15 Mb of DNA containing the PKHD1, IL17, MCM3, EFHC1, and TRAM2 genes. The deletion in band 6p12.3 involved a 360 kb stretch of DNA containing the genes RHAG, CRISP1, 2, and 3, and PGK2. The third deletion of 11.9 Mb in band 6q14;q16, affects 27 known genes, including genes involved in growth regulation and tissue remodeling during development, and pain sensation. The latter is remarkable, because this patient has reduced pain sensitivity associated with her distinctive phenotype. This includes characteristic dysmorphic facial features, a striking, non-progressive deficit of motor control, lack of speech development and abstract mental abilities, while well adapted to family life and to a school for mentally retarded persons. We assume that haploinsufficiency of some of the genes involved may account for this patient´s phenotype. We hypothesize that the de novo pericentric inversion 6p12;q16 caused two flanking deletions: 46,XX,inv(del(6))(6pter->6p12.3::6p12.3->6p12.2::6p12.2-> 6p12.1or 6p11.2::6q14.2->6p12.1or6p11.2::6q16.1->qter). This is the first complete mapping of adjacent, but non-contiguous, deletions with a 317 K SNP array system.

P063

Advances in parental origin determination (pod) FISH – evaluation and applications

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The discrimination of homologues (chromosomal) regions could up to now be done exclusively by molecular genetic methods using microsatellite or SNP-analysis. Only in exceptional cases a distinction on a single cell level by chromosomes was possible. Therefore, we developed a method called parental origin determination fluorescence in situ hybridization (pod-FISH) that is suited for a single cell analysis and can distinguish every homologues chromosome pair on the basis of copy number polymorphisms (CNP). Presently we selected 225 of over 2191 reported polymorphic regions in the human genome and use them as pod-FISH-probes. As it is useful to work with more than one polymorphic BAC probe at the same time chromosome specific pod-FISH sets in 5 colors were developed for every chromosome. For larger chromosomes like chromosomes 1, 2, 3, 4, 6, 9 and X it was more convenient to create chromosome arm specific pod-FISH sets for an easier analysis and to prevent double labeling. The evaluation of pod-FISH probes can be done in several ways: (1) by eyes using a fluorescence microscope, (2) by analyzing fluorescence profiles with an appropriate software and (3) by measuring signal intensity with a software that has been shown to be suited for measuring FISH signal intensities before. Furthermore, the usefulness and feasibility of this new approach is compared to conventional microsatellite analysis as well as its application in UPD analysis by chromosomes, origin determination in three cases with trisomy 8 associated AML and an outlook is given on future applications and new questions to be answerable now.

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P064

Evolutionary history of human Y-chromosomal segmental duplications in primates

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Segmental duplications (SD) are highly homologous euchromatic sequences (>90% nucleotide sequence identity over >1kb) predominantly located in euchromatin/hetero-chromatin transition regions. Altogether a minimum of 5 % of the human genome is composed of such duplicated sequences. Representing an constitutive architectural feature of many genomes, they are discussed as playing an important role in genome evolution. This assumption is based on the coincident location of genomic alterations and SDs. Among all human chromosomes the Y chromosome has the highest content of SDs. All four euchromatin/heterochromatin transition regions on the human Y chromosome are characterised by the presence of SDs.
To explore the evolutionary history of these transition regions we comparatively analysed human and non-human primate species by combined cytogenetic (FISH) and whole genome analysis comparison (WGAC). We detected an increase in the complexitiy of the genomic SD distribution pattern over the primate phylogenetic timescale of the last 35-40 Myr. Concordant results were obtained by FISH as well as WGAC analyses. However significant differences in the genomic SD distribution pattern between Yp11- and Yq11-derived SD were disclosed by their direct comparison. Whereas FISH and WGAC of Yq11-derived SDs present almost identical results, both approaches provided vastly inconsistant results for Yp11-derived SDs. This apparent contradiction originates from the different sensitivity levels of both methods. The methodological complement therefore might be the "silver bullet" to give a comprehensive overview of the evolutionary history of SDs in primates.

P066

A molecular basis for the cytogenetic co-localization of fragile sites, evolutionary conserved breakpoints and tumor breakpoints

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One of the molecular pathways for chromosomal rearrangements (rearr.) starts from so called fragile sites (FS) (Schmidt et al., 2005). These are specific break prone regions in the genome that can be induced by a number of different culture conditions. A possible association of FS evolutionary conserved breakpoints (bp) and cancer associated bp is discussed since Dutrillaux (1979) suggested a common mechanism for their formation. Similar to low copy repeats/segmental duplications they can play a role in pathogenesis especially in tumorigenesis. Nevertheless, only a few such sites are already investigated on a molecular level. In contrast, evolutionary conserved bp are well defined for great apes, especially for the chimpanzee, since the release of its complete genomic sequence. This fact made it possible now to choose BAC clones for these evolutionary conserved bp regions and apply them for direct comparison on aphidicolin induced FS. Until now there are 33 molecular characterized evolutionary bp reported in great apes that are caused by macro rearr.. Only 14 of them are localized in the same cytogenetic region as FS. After analyzing 8 of these 14 regions on a molecular cytogenetic level 4 of them were mapped within a common FS. Besides the well known macro rearr. a number of micro rearr. were published for chimpanzees. Focusing on these submicroscopic bp we could show a molecular colocalization for 11 common FS until now. These results suggest a remarkable role of FS regions to act as a basic event/feature for chromosomal rearr. Nevertheless, not all rearr. loci seem to have this feature and may be driven by other sequence properties like segmental duplications. Further investigations addressing a colocalization also with tumor breakpoints are hence needed before final conclusions can be drawn on the molecular mechanisms of chromosome rearr.

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P067

Spectrum of sex chromosome abnormalities and previously not described structural X-chromosome changes in pre- and postnatal diagnostics: a laboratory survey of cases with suspected Ulrich-Turner syndrome from 1997-2006

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Turner Syndome is one of the most common chromosome abnormalities with an incidence of approximately 1:3000 in female newborns. A variety of chromosomal constitutions are known to cause Turner syndrome. The literature describes a 45,X karyotype in half of patients analysed cytogenetically. About 30-40% of the cases are in mosaic state (45,X/46,XX, 45X/47,XXX and 45,X/46,XX/47,XXX).
Approximately 12-20% cases show a structurally rearranged X-chromosome. Less than 10% of probands are affected of mosaicism with a cell population wherein an aberrant Y-chromosome is detected. Here we present the results of a nearly ten year comprising case survey of pre- and postnatal cytogenetic diagnostics. More than 350 cases were identified to present structural or numerical abnormalities of X-chromosomes respectively mosaicism that causes a Turner phenotype. Of these, 204 cases revealed a 45,X karyotype without mosaicism. For 55 patients mosaicism with numerical aberrations of the sex chromosomes was found. Structural abnormal X-chromosomes such as isochromosomes, deletions of p or q-arm and ringchromosomes were detected in 70 cases. For 24 cases X0/XY mosaicism or structural abnormal Y-chromosomes were found to cause a phenotype. We present our data in detail with special respect to the spectrum of chromosome abnormalities described in the literature and 5 previously not described structural alterations of the X-chromosome.

P068

Different expression of FRA16B in metaphase and interphase between mother and aon: Increasing size of repeat-expansion through transmission?

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The rare non-folate-sensitive human chromosomal fragile site FRA16B contains AT rich minisatellite repeats and is located at 16q22.1. We report on a 2 10/12 old boy who presented with behavioral problems, psychomotor retardation, mild dysmorphisms (round facies, prominent forehead, hypertelorism, small nose with anteverted nares).
Subtelomer screening with the MLPA (Multiplex Ligation Dependent Probe Ampflication) method showed no pathological results. Standard Chromosome analyses of the patient's blood showed a spontaneous heterozygous expression of FRA16B in 10% of the metaphases whereas both parents did not show the fragile site. By DAPI treatment of the lymphocyte cultures we could show that the mother of the patient is the carrier for an inducible FRA16B expression. The cells from the patient did not grow with addition of DAPI or thymidine suggesting a failure of cell division by DNA-replication-stress. We have developed a FISH BAC-probe set, which flanks the FRA16B site. Using this BAC probe set we have studied G0 phase lymphocytes from the patient and his parents. The FISH results demonstrated for the first time that the manifestation of FRA16B can be identified in interphase and confirmed the cytogenetic results. Interesting was the heterozygous expression of FRA16B in 17% of the interphase cells from the patient and 7% of the cells from his mother. The use of a specific PCR to amplify the repetitive minisatellite showed a repeat expansion in mother and son. To test whether uniparental disomy (UPD) is responsible for the high expression of FRA16B in the patient we used polymorphic microsatellite markers from chromosome 16 (D16S514, D16S408, D16S308, D16S320) and could exclude UPD 16. This case exhibits an interesting opportunity to study the correlation between repeat expansion and spontaneous manifestation of FRA16B in metaphase and Interphase. It remains to be determined whether the symptoms from the patient are caused by the high expression of FRA16B.

P069

Generation of high-quality chromosome painting probes from single chromosomes after FISH

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The versatility and resolution of FISH depends critically on the used probe set. Here, we describe a novel approach for the generation of specific DNA probes from single chromosomes. In a first step single chromosomes or single chromosomal regions were microdissected by laser pressure catapulting and amplified using linker-adaptor PCR. The probes were labeled and tested in various scenarios including multicolor-FISH experiments employing up to seven different fluorochromes. The quality of the obtained painting probes is sufficient to elucidate even small structural chromosomal aberrations as demonstrated by microdissecting chromosomes from a carrier of a t(2;3)(q37;p25) translocation [1]. To further extend this technology, we tested whether painting probes can be generated from a single chromosome to which previously a DNA probe had been hybridized. Single chromosomes with hybridization signals were processed by microdissection and amplification as described above. The previous hybridization did not hamper the result and we again obtained specific probes, which evenly stained the respective chromosomal regions. In a further step we applied two new commercially available genomic DNA amplification kits, GenomiPhi (GE Healthcare) and GenomePlex (Sigma-Aldrich). These kits are optimized to produce microgram quantities from minute amounts of starting material within a few hours. To test the quality and feasibility for downstream applications, amplified DNA from these microdissected single chromosomes was hybridized on the 1 Mb array [2]. Our results suggest that the sequential application of FISH and subsequent isolation and unbiased amplification of single chromosomes for the generation of FISH probes is feasible. If applied to an array platform to achieve a high resolution, this approach should be especially helpful for the detailed characterization of complexly rearranged chromosomes, which are frequently encountered in tumor cytogenetics.

P070

Complex interstitial deletions detected by array-CGH and SNP-chip analysis in a patient with an apparently balanced translocation t(5q;9q)

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We report the further characterization of a translocation t(5;9) – apparently balanced as judged by light microscopy - by array-CGH and SNP-chip analysis. The now 14 year-old female patient is the only child of healthy and non-consanguineous parents. She was born spontaneously in the 36th week of gestation after an uneventful pregnancy. She exhibited some dysmorphic features, but no organ malformations. Later she developed a mild psychomotor retardation and recurrent seizures at the age of 13 years. At the age of 6 years, cytogenetic analysis revealed a translocation t(5;9)(q14-q15?;q22.3), at that time considered to be balanced; the parental karyotypes were normal. Subsequent FISH-analyses could not detect any deletions, duplications, or a complex rearrangement.
In order to further analyse the translocation we now performed a microarray based comparative genomic hybridization (array-CGH) using a microarray with 1 Mb resolution. Using this method we could find a deletion of three non-consecutive clones in band 5q15; three flanking and two intercalating clones showed ratios just below the threshold.
For fine-scale examination we genotyped the DNA on an Illumina HumanHap300 Genotyping Bead Chip. Two regions on chromosome 5 appeared homozygous and with attenuation of the fluorescence signal of the SNPs contained in these regions. The distal region (5q15) was about 5,25 Mb in size, the proximal (5q14.3) about 1,9 Mb, in between there were about 4 Mb containing heterozygous SNPs and normal intensities. We currently interpret this finding as either two independent deletions or as a contiguous deletion resulting from an inversion. It seems plausible that haploinsufficiency of genes in the deleted regions are causing the observed phenotype.

P071

Molecular and molecular cytogenetic characterization of four fragile sites of chromosome 1

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Fragile sites (FS) are specific chromosomal (chr) regions that tend to result in breaks and gaps on metaphase spreads. These interrupting regions of the chr structure were first described in folate decreased cultures. Later on, it was found that FS can be induced by a variety of different culture conditions. Here we treated human lymphocytes with aphidicolin to induce common FS. We mapped four of these break prone regions on chr 1 by fluorescence in situ hybridization (FISH) applying locus-specific BAC-probes of the corresponding regions. We found FRA1K (1q31) and FRA1F (1q21) in 8 of 3420 mitosis, each. For FRA1K (1q31) the FS region was narrowed down by BAC-mapping to a gene poor region proximal to RP11-166A4 (192.5 Mb). FRA1F (1q21) could be located distal to RP11-300L20 and thus is located within a neuroblastoma breakpoint gene family cluster, which is known to be involved in chromosomal rearrangements. Furthermore, we mapped FRA1A (1p36, detected in 18 of 3420 mitosis) to a 2 Mb spanning gene rich region between BAC RP11-285P3 and RP4-636F13. The most frequently observed FS on chr 1 FRA1E (1p21.2, seen in 201 of 3420 mitosis) was recently mapped within the DPYD gene at 97.7 Mb (Hormozian et al., 2006), a finding which could be confirmed by our data. However, we were unable to detect a FS FRA1D in 1q22. Whenever a FRA1D was suggested cytogenetically and studied by BAC-FISH we characterized it to be indeed a FRA1E located at 97.7 Mb. Thus, we suggest that FRA1D does not exist but these reports were misclassified FRA1E. In summary, we mapped FRA1A, FRA1E, FRA1K and FRA1F by BAC-FISH. According to this data no variations within each of the four breakpoints were observed. However, as FS are reported to vary in size between 400 kb to up to several Mb, more FS of chr 1 have to be studied to come to final conclusions.

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P072

Balanced multiple chromosomal rearrangements: Report on three new cases

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Complex chromosomal structural rearrangements are characterized by three or more breakpoints located in two or more chromosomes. There are three major categories of CCRs: 1.: Three way exange of three chromosomal segments. 2.: More complicated CCRs. 3.: Douple two way exchange, in which there are two seperate reciprocal translocations. This category is not regarded as true CCR and is preferentially described as a double or multiple chromosomal rearrangement (MCR).
We report three new cases of balanced multiple chromosome aberrations of the 3. category. Parental karyotypes were normal in two cases with de novo MCR. Familial MCR transmitted throught the mother is described in case three. In a two-year-old girl with short stature a karyotype showing two reciprocal translocations one between the chromosomes 7 and 11 and the other between chromosomes 9 and 12 was observed [karyotype: 46,XX,t(7;11)8q11.2;q22),t(9;12)(q21.2;q22) de novo]. A complex rearrangement involving all four chromosomes was excluded using whole chromosome paints.
In the second case amniocentesis in 34th g.w. revealed a karyotype of 46,XX,inv(1)(q31.1q43.12),t(12;13)(q24.1;q34) de novo. Prenatal ultrasound revealed abnormal fetal movements, retrognathia and cerebellar hypoplasia. In the third case, amniocentesis was performed in 15th g. w. because of recurrent abortions. A double translocation involving chromosomes (1;16) and (3;13) [karyotype 46,XX,t(1;16)(q32;q13),t(3;13)(p21;q14) mat] was ascertained. Sonografic examination did not find any fetal abnormality. Reproductive risks for families with MCR seem to be very specific for each family. The risk for miscarriages appears to be higher than that of a simple balanced reciprocal translocation carrier. Reviewing the literature showed that de novo apparently balanced MCR seems to have a high risk for abnormal phenotypes increasing with the number of breakpoints. 60% of cases with prenatally diagnosed CCRs or MCRs had phenotypic abnormalities.

P073

A familial case of subtelomeric deletion 1q indicating the transition between pathogenetic deletion and benign variant

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We present the clinical, cytogenetic and molecular data of a familial case with an end-to-end translocation in tandem resulting in a terminal deletion 1q associated with normal phenotype [45,XX,der(1)t(1;13)(1pter⟶q44::13q11⟶qter),-13.ish del(1)(q44)(D1S3739-)]. Our both propositas increase the number of described polymorphisms up to 24 of all 41 relevant subtelomeric regions and therefore give an impulse to a critical view of apparently pathological results after subtelomere screening. The reduced expression of the involved predominately putative genes and/or pseudogenes resembling olfactory receptors seems to have no detrimental effect of development and function. Such polymorphisms may be benign. However, full knowledge of the complete character of these polymorphisms is essential prior to clinical application. As a consequence of the frequent reports of subtelomeric polymorphisms and the putative interpretative dilemmas, a method previously illustrated for chromosome 16p and 17p has been elaborated, enabling to detect the exact boundary between pathogenic terminal imbalances and benign variants. A first step towards the establishment of a validated “molecular ruler” for the ending of chromosome 1 is made by the present cases and a formerly reported patient. Our observations in combination with these results determine the exact transition point between polymorphism and pathogenetic copy-number alteration for 1q. Our cases thus demonstrate the necessity of the establishment of a “molecular ruler” for each analysed subtelomeric region. Dosage polymorphisms seem to be common, therefore studies of control subjects as well as the use of DNA probes, not detecting polymor-

phic or variant loci are required. Subtelomere screening is an effective diagnostic tool, but our cases further demonstrate that some precautions must be taken to avoid data difficult to interpret by the confounding effects of polymorhisms to ensure the most benefit.

P074

Ring chromosome 5 without apparent loss of euchromatin in an 17 year old male with microcephaly, epilepsy and mild dysmorphic features

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Ring chromosome 5 is a rare cytogenetic abnormality. The majority of ring chromosomes result from distal breakage of the short and long arm of a chromosome and rejoining of the ends. As a consequence, most previously reported ring chromosome 5 cases resembled the clinical picture of cri-du-chat-syndrome due to loss of portions of the 5p-arm. In contrast, ring chromosomes without apparent deletion of the telomeric ends are rare. To our knowledge, only three patients with a complete ring chromosome 5 have been described. However, the methodologies available at the time of this report (McDermot et al. 1990), i.e. chromosome banding, scanning electron microscopy, and flow karyotyping, have a poor resolution probably not allowing the identification of smaller deletions. Here, we present a 17 year old male patient with prenatal onset microcephaly (HC: 32.5 cm), mild facial dysmorphism, clinodactyly and mild psychomotor delay. Since age of 9 years he has epilepsy requiring anticonvulsant treatment. G-banding analysis revealed a mosaic karyotype: mos 45,XY,-5[2]/46,XY,r(5)[25]/47,XY,r(5),+r(5)[2]/46,XY[2]. Surprisingly, array-CGH failed to detect any deletion or duplication in this patient, especially not at the distal regions of chromosome 5. FISH with clones for the subtelomeric region of 5p (BAC-clone 114-J18) and 5q (PAC-clone GS-240-G13) indeed confirmed the presence of these regions within the ring chromosome. Hybridisation with a probe for the telomere repeats (TTAGGG)n revealed the loss of these repetitive sequences in the ring chromosome. So the phenotype of this patient is most likely attributable to ring formation and the resulting mosaicism. We will also present a detailed comparision of the clinical picture of our patient with those previously reported.

P075

Collection of chromosome abnormalities in females and males prior to IVF/ICSI - a new database

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In 2005 a review of somatic chromosomal abnormalities in infertile men and women (Cytogenet Genome Res 111:317-336) made it nescessary to collect the different cytogenetic abnormalities of the largest and most important surveys. In a new database these different abnormalities are listed in detail (separate tables for anomalies in males and females, sorted by type of structural abnormality, breakpoints, hitherto existing experiences with IVF/ICSI, et cetera). It is planned to add data of more surveys. Individual cases could be added as well. The database might become a useful resource for genetic counselors and gynecologists involved in IVF/ICSI.

P076

Detection of a 4.7 Mb deletion in 13q33.3-q34 in a family with translocation t(12;13) and mental retardation by array-CGH

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This report concerns a 4-year-old boy and his mother with a translocation t(12;13)(q13.3;q32.3) and mental retardation. The patient was born at 36 weeks gestational age by caesarean section. At birth his weight was 2159 g (3rd centile) at a length of 48 cm (25th centile). Head circumference was 31 cm (3rd centile) and thorax circumference 28 cm (3rd centile). An APGAR-score of 7-7 was observed.
Clinical manifestations in the patient at the of age two years included facial dysmorphic features, bilateral clinodactyly of V fingers and toes, marbled skin, hypotonia, cryptorchidism, and single transverse crease of the right hand. His psychomotor development was delayed. On examination, the mother showed mild mental retardation, microcephaly and a heart defect. Further investigation by submegabase resolution array-based comparative genomic hybridisation (array-CGH) identified a 4.7 Mb deletion of chromosome 13q33.3-q34 in the patient and his mother. Constitutional deletions of the long arm of chromosome 13 are rare. Until now only about 20 cases with partial deletions of the long arm of chromosome 13 have been described in the literature. Typical findings in patients with 13q deletions include mental and growth retardation, craniofacial dysmorphism including eye and ear abnormalities, hypoplasia or aplasia of the thumbs, heart defects as well as genital abnormalities. Twenty known genes are located within the region 13q33.3-34 which is deleted in this family, including the LIG4 gene. Defects in LIG4 are the cause of LIG4 syndrome [OMIM:606593]. This disease is characterized by immunodeficiency as well as developmental and growth delay. In addition to that, the so far reported patients display unusual facial features, microcephaly, pancytopenia, and various skin abnormalities. Therefore we presume that LIG4 haploinsufficiency is responsible for the phenotype we observed in the patient described here.

P077

Familial small supernumerary marker chromosomes are predominantly inherited via the maternal line

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In a recent paper (Dalprà et al., Genet Med 2005;7:620-625) authors suggested familial small supernumerary marker chromosomes (sSMC) to be inherited in a 2:1 ratio concerning maternal/paternal inheritance. As such a correlation was never described before, we analyzed the recently summarized data on all in detailed reported sSMC cases for this question (sSMC-homepage: http://mti-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC.htm or http://markerchromosomes.ag.vu). Of over 2100 cases with sSMC 1182 were de novo, 102 inherited and for the remainder no information on the parental origin available. Here we find 72 sSMC inherited from the mother versus 30 sSMC inherited from the father, which is a similar 2.4:1 ratio as suggested already in the paper of Dalprà et al. for the parental origin of sSMC. Thus, the observation that familial small supernumerary marker chromosomes (sSMC) are predominantly inherited via the maternal line is substantiated by this analysis. However, an explanation still lacks. One has to suggest that a yet unknown effect is driving selection via fertilization-success of sperm without an sSMC. Problems in connection with sSMC-replication arising predominantly in the more rapidly progressing sperm meiosis or a 'weight-effect' making sperm without more rapid than those with an sSMC, similar to the effect known from Y- versus X-chromosome carrying sperm could be envisaged as possible mechanisms. In summary, there is a yet unexplained doubled transmittance-rate of sSMC via the maternal compared to the paternal line. Nonetheless, there is a 1:1 sex-ratio in the offspring of familial and de novo sSMC-cases.

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P078

Clinically abnormal case with paternally derived partial trisomy 8p23.3 to 8p12 including maternal isodisomy of 8p23.3

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We report on a patient with various clinical malformations and developmental delay in connection with an inverted duplication event, involving chromosome 8p. The karyotype was characterized in detail by multicolor banding (MCB), subtelomeric and centromere-near probes as der(8)

ins inv dup (pter->p23.3::p12->p23.3::p23.3->qter). Additionally, microsatellite analysis revealed the paternal origin of the duplication and gave hints on a mitotic recombination involving about 6MB in 8p23.3. There are several cytogenetically similar cases reported in the literature, however, in none of those cases the chromosomal rearrangement was studied and described that comprehensively. The present case is compared to previously reported ones and the mode of formation of this chromosomal aberration is discussed. In summary, inverted duplications on chromosome 8p are observed rather frequently due to technical improvement in routine cytogenetics. More complex karyotypes are being delineated by widely available use of newly developed tools. In conclusion, the present patient suggests that there might be a certain predisposition to chromosome 8p for more complex aberrations other than inverted duplications, which should be considered during the cytogenetic evaluation.

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P079

Parental origin and formation of a 47,XY,i(X)(q10) karyotype in an infertile man

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Klinefelter syndrome is found in about 1 : 1000 newborn males and up to 3.1 % of infertile men, respectively. Most patients have a 47,XXY karyotype, some have higher-grade chromosome aneuploidies, and only few cases displayed other karyotypes.
Our patient presented with hypergonadotropic hypogonadism and azoospermia. Endocrinological investigations revealed high FSH and LH and normal or decreased testosterone values. Testes were small (2 ml and 3 ml, respectively) and soft. Testis biopsy histology resulted in only Sertoli cells and hyperplasia of Leydig cells. No sperms or spermatozoa were found in both testes and epididymidis. Clinically the patient was of normal height and had no thrombosis or any other Klinefelter syndrome associated anomalies.
Cytogenetic investigation from peripheral lymphocytes showed a 47,XY,i(X)(q10) karyotype in 20 metaphases. Both parents have normal karyotypes. Microsatellite marker haplotype analysis demonstrated a maternal origin of both the normal X chromosome and the isochromosome. Formation might be either completely in maternal meiosis I or by non-disjunction in maternal meiosis I followed by formation of the isochromosome in meiosis II or in an early mitosis. Investigations to determine whether the isochromosome is a true isochromosome or a Xq/Xq translocation are on progress.

P080

Prenatal diagnosis of a direct intrachromosomal duplication 11p12⟶11q11.1~12.1, a one year follow up

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Amniocentesis was performed after a positive test result of the first trimester screening: risk for Down syndrome 1:27 with increased nuchal translucency (NT) of 3.4 mm and missing nasal bone. Prenatal cytogenetic analysis revealed the karyotype 46,XX,dup(11)(p12p11)dn. FISH analysis was performed to confirm the breakpoints. A dicentric chromosome 11 with a direct duplication of 11p12⟶11q11.1~12.1 was identified. The following fetale karyotype was observed:
46,XX,dup(11)(p12p11)dn.ish dup(11)(p12q11.1~12.1)(wcp11+,AN+,D11S324+,WT1+,dJ1053P10+, bA12C11+, D11Z1+,dJ1053F10+,bA12C11+,D11Z1+,bA77M7+)
Because major ultrasound abnormalities could be excluded parents decided to continue the pregnancy. A healthy girl was born at term. At the age of one year she was referred to our genetic counselling centre for a follow up examination.
The girl had no developmental delay and no major abnormalities. There was only mild craniofacial dysmorphism: round facies, flat occiput, hypertelorism, wide nasal bridge, broad nasal tip and a deep philtrum. The clinical examination showed no other physical or neurological abnormalities except of a strabismus divergens alternans and mild hyperopia with astigmatism. To our knowledge this is the first report of a dup(11)(p12q11).
E. Goossens [1] described a patient with a similar duplication dup(11)(p12). His patient was a 53- year- old male with mild to moderate mental retardation without gross dysmorphic stigmata. The stocky built man (height 1.61cm, weight 69.6 kg) with a round facies, flat occiput and short neck could manage simple jobs.
Both patients with dup11p12 show similar clinical signs: round facies, flat occiput and a strabismus without severe dysmorphic features and no internal malformations were detectable. While the man shows a mild to moderate mental retardation the psychomotoric development of the girl at the age of one year was normal.
[1] Goosens et al. 1999, Genetic counselling, vol 10, No 2: 137-140

P081

Partial trisomy 5qter and monosomy 10qter: unbalanced subtelomeric aberrations as a cause for a Rubinstein-Taybi like phenotype

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Dysmorphic features are variants of the body, that can indicate a specific malformation syndrome, however they can be observed rarely also for healthy individuals. Although occurrence of dysmorphic features is thus not directly demonstrating a specific genetic cause, it should be taken into account for strategies to identify the underlying cause of familial occurring entities. Here we describe signs and symptoms of a 3 months old baby with unspecific dysmorphic features whose 8 year old mentally retarded cousin was clinically diagnosed as a Rubinstein-Taybi syndrome (RSTS). The healthy parents sought for genetic counselling since it was unclear whether their child may be affected by any impairments. Conventional chromosome analysis revealed a normal female karyotype. Since RSTS is an autosomal dominant entity and parents of both children were healthy, for formal genetical considerations a correlation between the familial cases could not be determined initially. However, clinical course and familial history gave reason to decide for performing subtelomer screening by fluorescence in-situ hybridization (FISH). Taken together, the results of the subtelomeric and modificated FISH analyses ("wcp5" combined with "10pter") indicated a partial trisomy 5qter and a partial monosomy 10qter for the index patient (46,XX.ish der(10)t(5;10)(pter+,D10S2490-,wcp5+,D5S2907+)). FISH on paternal chromosomes revealed a balanced reciprocal translocation t(5;10)(q35.1;q26.3). For the cousin affected with clinical signs of RSTS the same unbalanced karyotype as for the index patient was observed with specific subtelomeric probes. A balanced translocation corresponding to that of the index patients father was detected for the healthy mother of this child. In conclusion, for familial cases with dysmorphic features, in combination with confirmed or not confirmed mental retardation, it should be considered that subtelomeric screening can be the only method to identify the appropriate diagnosis.

P082

Characterization of a 16 Mb interstitial chromosome 7q21 deletion by tiling path array CGH

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We report on a 42-year-old female patient with an interstitial 16 Mb deletion in 7q21.1-21.3 and a balanced reciprocal translocation between chromosomes 6 and 7 [karyotype 46,XX,t (6;7)(q23.3;q32.3)del(7)(q21.1q21.3)de novo]. We characterized the size and position of the deletion by tiling path array Comparative Genomic Hybridization (CGH), and we mapped the translocation breakpoints on chromosomes 6 and 7 by FISH. The clinical features of this patient – severe mental retardation, short stature, microcephaly and deafness – are in accordance with previously reported patients with 7q21 deletions. Chromosome band 7q21.3 harbors a locus for split hand/split foot malformation (SHFM1), and part of this locus, including the SHFM1 candidate genes SHFM1, DLX5 and DLX6, is deleted. The absence of limb abnormalities in this patient suggests either a location of the SHFM1 causing factor distal to this deletion, or reduced penetrance of haploinsufficiency of a SHFM1 factor within the deleted interval.

P083

Oligo-based-Array-CGH as a diagnostic tool: first experiences

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Array-CGH is a novel diagnostic tool for the detection of submicroscopic chromosomal imbalances. In June 2006 Oligo-based-Array-CGH analysis was diagnostically implemented in our genetic service in order to reach a resolution that extends far beyond routine cytogenetic analysis. Array-CGH was performed using a 44k or 105k Oligo-array (Agilent).
We report on a one year old female patient with severe psychomotor delay, intractable epilepsy and hypertelorism. CT and MRT analysis revealed pachygyria and polymicrogyria mainly on the right hemisphere. Cytogenetic analysis showed an insertion on Xp22. Using Oligo-Array-CGH a 23.7 Mb duplication (Xp11.22-p21.1) was detected. Interestingly, in 2000 Portnoi et al. described a female patient with a Xp11.2-p21.2 duplication having a very similar phenotype.
Additionally, we report on a male patient showing mental retardation. Standard cytogenetic analysis revealed that he carried a de novo apparently balanced translocation, 46,XY,t(7;12)(q32;q14). Searching for cryptic chromosome abnormalities, Oligo-Array-CGH was performed. The patient showed a deletion on chromosome 12q12 with a loss of about 1.5 Mb. At least 5 genes are located within the deleted region, which are involved in neuronal function.
Finally, various practical aspects of using Oligo-Array-CGH in genetic diagnostics will be discussed. These include the detection of CNVs ("copy number variations") and validation of significant array CGH aberrations by MLPA. In conclusion, our current strategy enables us to routinely use this powerful tool to detect submicroscopic imbalances and thereby to unravel the causative defects of unclassified syndromes or mental retardation. Additionally, Oligo-Array-CGH can be used for translocation breakpoint analysis.

P084

Congenital bilateral ptosis, hip dysplasia and pes calcaneus in a newborn girl with mos 46,X,psu idic(Y)/46,X,r(Y)/45,X

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We report on a newborn girl with multiple dysmorphic features. Most obvious was the bilateral ptosis, but also mandibular retrognathia, bilateral congenital hip dysplasia, pes valgus and calcaneus and a simian crease on the right hand were found. She had a round face and a short neck with redundant skin. Instead of a second X, Chromosome analysis of 20 lymphocyte mitosis showed a Y-like chromosome that lacked the Y-typical heterochromatin. Instead of that, Distamycine/DAPI-staining showed slightly positive band on the long arm, indicating an isochromosome with an extra inactive centromere. FISH demonstrated the presence of the male determining gene SRY on both terminal ends and, therefore, the Y-origin of this isochromosome. By extensive workup, 3 metaphases were found that lacked the derivative Y chromosome (45,X). Hence, the girl's complete lymphocyte karyotype was mos 46,X,psu idic(Y)(q11.2)[133]/45,X[3]. The 45,X cell line and the mosaic state explained the female sex in contrast to the vast majority of the SRY-containing blood cells and the features of Turner syndrome present in this patient. The increased risk of gonadoblastoma due to SRY containing cells in the gonads led to laparoscopic gonadectomy at 8 months of age. A skin and an ovarian biopsy were cultured and cytogenetically analysed. A third cell line was identified that lacked both the second X and the psu idic(Y), but contained a very small marker, presumably a small ring chromosome. FISH with a Y centromere probe confirmed the Y-origin of the small ring. The distribution of the three cell lines in the ectodermal tissue skin and the ovary was quite similar, with approximately 50% of 45,X-cells, 25% 46,X,psu idic(Y) und 25% 46,X,r(Y), clearly contrasting the pattern of distribution in the mesodermal blood cells. The cytogenetic analysis of three different tissues allowed a close view on the meiotic and mitotic events that had led to the rare sex chromosome mosaicism found in this girl.

P085

Cyto-Quiz Part 2: Test and train your abilities to recognise chromosome aberrations

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As in the preceding congress several karyotypes with various chromosome aberrations are displayed. Different levels of difficulty might be interesting for beginners as well as for experienced cytogeneticists.
The viewer is invited to test and train herself/himself if she/he is able to recognise the represented aberrations. Additionally, some clinical aspects of the different cases are presented.

P086

Scheme for straightforward characterization for small supernumerary marker chromosomes (sSMC) by simple molecular and molecular cytogenetics approaches

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Small supernumerary marker chromosomes (sSMC) are still a major problem especially in prenatal cytogenetic diagnostics and counselling. sSMC are structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are generally equal in size or smaller than a chromosome 20 of the same metaphase spread. They are reported to be present in 0.043% of newborn infants and 0,075% of prenatal cases. For their characterization molecular cytogenetics is necessary and many highly sophisticated approaches are available throughout the literature for their comprehensive description. However, as in many diagnostic laboratories such techniques are not available, we suggest here a straight forward scheme to characterize at least the sSMC's chromosomal origin as quick as possible. Based on this, it is possible to compare the own case with similar cases from the literature, which are summarized on the sSMC homepage (http://mti-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC.htm or http://markerchromosomes.ag.vu). For a more wide-ranging sSMC characterization a specialized laboratory should be contacted, like e.g. that of the last author.

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P087

Cyto-Quiz: Test and train your abilities to recognise variant chromosomes

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Several karyotypes with frequent and rare/unusual variant chromosomes are displayed. Different levels of difficulty might be interesting for beginners as well as for experienced cytogeneticists.
The viewer is invited to test and train herself/himself if she/he is able to recognise the represented variants.
Besides, images of additional stainings and more details of the different cases are presented.

P088

Adjacent I malsegregation of a balanced submicroscopic translocation 8/11 results in unbalanced karyotypes in four offspring
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Subtelomeric rearrangements are a common cause of both syndromic and non-syndromic mental retardation. Here we describe a family that includes two siblings, a boy and a girl with mental retardation, speech delay, behavioural abnormalities and facial dysmorphism. Karyotyping and FISH analysis in the boy was performed elsewhere and had revealed a submicroscopic deletion of 8p. Another sibling, a girl appeared normal at birth. We studied the karyotypes of the unaffected parents and detected a cryptic balanced translocation t(8;11)(p23.1;q25) in the mother using subtelomeric FISH probes of chromosome 8p and 11q. Reinvestigation of both of her mentally retarded children and of her seemingly unaffected daughter detected an unbalanced karyotype (partial unbalanced duplication of 8p and a partial deletion of 11q) in all three children. The now 28 year-old mother is pregnant again and karyotyping of amniotic fluid cells demonstrated a 45,X karyotype plus the reversed type of adjacent 1 malsegregation with a deletion of 8p and a duplication of 11q. We demonstrate the diagnostic procedure in the family and compare the clinical picture of the children with respect to their chromosomal imbalances.

P089

Characterization of a prenatally diagnosed de novo small supernumerary marker harbouring material of chromosome 16
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Small supernumerary marker chromosomes (sSMC) are detected in about 0,05% of newborn children. The majority of these sSMC is proven to be de novo. The outcome of a sSMC is difficult to predict as they can have different phenotypic consequences due to differences in the euchromatic content of DNA, differences in the degree of mosaicism and / or existence of a uniparental disomy (UPD) of the chromosomes homologous to the sSMC.
We report here on the prenatal detection of a small supernumerary marker chromosome derived from chromosome 16. Prenatal cytogenetic analysis of cultured amniocytes was performed at week 17 of gestation. In all cells analyzed a male chromosome complement with a paracentric inversion of the short arm of the X chromosome was found. Furthermore, in 18 out of 25 cells investigated a small supernumerary marker chromosome was detected. Multi color banding revealed that the marker chromosome consists of heterochromatic and euchromatic regions of chromosome 16, leading to a partial trisomy of 16 p11.21 -> 16q11.1. With microsatellite analysis a maternal uniparental disomy for chromosome 16 was proven. After 39 weeks of gestation the delivery occurred spontaneously. The male child had a birth weight of 2960g. Apgar score was ?/10/10. Apart from a posterior plagiocephaly, the clinical examination has so far revealed no indication of malformations or abnormalities of internal organs. Clinical consequences of the chromosomal findings can not be predicted with certainty, because only few cases of prenatal diagnosed de novo supernumerary marker chromosomes 16 have been reported. Thus, further careful follow-up examinations such as a chromosome analysis of fibroblasts are indicated. Among the cases that were published, only one case seems to have comparable breakpoints to the case we present here. For this casuistic, a 3 month old girl with dysmorphic features, mild gross motor delay, asymmetric lower extremities and macroglossia was described.

P090

Interstitial deletion (2)(q11.2-q21) in a newborn with multiple malformations
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Here we describe the clinical symptoms of a newborn with the largest proximal deletion of the long arm of chromosome 2, which has ever been described in a child.
The pregnancy was complicated by an IUGR, further ultrasound tests were reported to be normal. The girl was born by cesarian section in 33th week of gestation in context with a silent CTG. She exhibited multiple abnormalities such as microcephaly, complex heart malformation, cleft palate, hypoplastic corpus callosum, partial bilateral syndactyly of toes II and III as well as syndromic signs. The child revealed a thrombocytopenia and an unexplained cholestasis. The girl died at the age of 3 months.
Karyotyping revealed an interstitial deletion of chromosome (2)(q11.2-q21). Until time of writing the abstract the parents denied karyotyping. Only a few long arm deletions of chromosome 2 partially overlapping with the deletion of child shown here have been described scientifically up to now. The symptoms of the patient will be discussed in respect to literature.

P091

First inherited small supernumerary chromosome marker generating complete trisomy 18p
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Small supernumerary marker chromosome (sSMC) have been describes from all human chromosomes with different sizes and shapes. However, it is always hard to know the clinical manifestations associated with them, because it depends on the size, presence of euchromatic material, degree of mosaicism and/or uniparental disomy (UPD). Trisomy of the whole arm of chromosome 18 (18p), has been described in just a few cases and the general consensus seems to be that it is related with just a slight phenotype effect. Here we present a newborn male who presented an atrial septal defect (ASD) and a foot anomaly. The high resolution G-band karyotype (550-850 bands) and the molecular cytogenetic techniques revealed in all cells the presence of an sSMC, which was a complex derivative from the short arm of a chromosome 18 (18p) and a centromere of a chromosome 13/21. His healthy mother had the same sSMC in all analysed cells. With the present case we support the previous concepts that this unusual chromosome trisomy 18p, has little clinical repercussions.

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P092

De novo subtelomeric unbalanced rearrangement (der(9)t(9;22)) resulting in partial monosomy 9pter and duplication 22qter
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We describe a 6 year old male with developmental retardation (especially severe speech delay), abnormal behaviour with autistic traits, stereotypy, flappy ears, flat occiput and small upper lip. Pregnancy and birth were uneventful. As a newborn the patient presented poor feeding. He is the first of two children of healthy parents. Cytogenetic analysis revealed a normal karyotype. Subtelomeric screening detected a monosomy 9pter-p24.2 and a duplication 22q13.3-qter as consequence of an unbalanced de novo translocation. Subsequent FISH analysis with the locus specific probe ARSA (22q13.3) demonstrated the inclusion of this locus in the duplicated region. Cytogenetic analysis of both parents resulted in normal karyotypes.
In literature numerous deletions of the short arm of chromosome 9 have been described. However, the majority of these deletions span larger regions compared to our patient. Five children having a 9pter-p24.3 deletion with developmental retardation, speech delay and small upper lip have been reported. Additionally they also showed cataract, cleft lip and palate as well as congenital heart defects and pyloric stenosis in some of the patients.
To our knowledge, duplications of 22q13 to 22qter have been described in a few patients, but no comparable case with corresponding deletion and duplication has as yet been published. The symptoms of the patient will be discussed in respect to literature.

P093

Partial monosomy 7q34-qter and 21pter-q22.13 due to a de novo cryptic unbalanced translocation in a patient with multiple congenital malformations
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We report on a case of de novo unbalanced translocation in connection with a der(7)t(7;21) associated with congenital malformation including mental retardation, motor development delay, craniofacial dysmorphism and skeletal abnormalities. The case could be misclassified as a "full monosomy of chromosome 21" because the translocated part of chromosome 21 has formed GTG-banding patterns similar to original ones of chromosome 7. Molecular cytogenetic analyses with a set of chromosome 7 and 21 specific DNA probes allowed to precise chromosome abnormality in the reported case as 45,XX,der(7)t(7;21)(q34;q22.13),-21. The clinical phenotype was found to be similar to previously described cases of ring chromosome 21, and a number of cases with del(7)(q34). The present report points out the possibility that at least a certain percentage of "full monosomy of chromosome 21" in liveborn is cases of unbalanced translocation involving chromosome 21 and similar cases like that are to be expected in future.

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Cancer Genetics

P094

Binary state pattern clustering: A digital paradigm for class and biomarker discovery in cancer expression profiles
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Comparison of expression profiles of clinical specimens from different kinds of tumor can be useful to classify both genes and phenotypes. Often, these classification problems are linked, in that one desires to find marker genes that are differentially expressed in certain types of tumor. A number of so called biclustering methods have been developed for this purpose. However, most of these algorithms do not necessarily take all clinical specimens into account when defining the biclusters, which has clear negative implications for cancer biomarker discovery. We have developed a new data mining technique, which we call Binary State Pattern Clustering (BSPC) that is specifically adapted for class and biomarker discovery in cancer and other categorical datasets. BSPC is capable of uncovering statistically significant sample subclasses and associated marker genes in a completely unsupervised manner by means of a digital paradigm, where the expression level of each potential marker gene is treated as being representative of its discrete functional state. Multiple genes that divide samples into states along the same boundaries form a kind of gene-cluster that has an associated sample-cluster. BSPC is an extremely fast deterministic algorithm that scales well to large datasets. Here we describe results of its application to three publicly available oligonucleotide microarray datasets. Statistically significant clusters reproducing many of the known sample classifications were identified along with associated biomarkers. Using simulated datasets, we have shown that BSPC has substantially greater noise tolerance than several other comparable methods and confirmed the accuracy of our calculations of statistical significance. We additionally present a user-friendly, open-source application with which BSPC analysis can be performed on microarray datasets.

P095

Variable expression of trancriptionally active HERV-K(HML-2) proviruses in tissues and cell lines
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About 8% of the human genome mass consists of human endogenous retroviruses (HERVs); relics of ancient germ line infections of exogenous retroviruses millions of years ago. Most HERVs are no longer functional due to accumulated mutations. The human endogenous retrovirus family HERV-K(HML-2) is exceptional. Several HML-2 proviruses encode intact retroviral genes and are expressed at significant levels in germ cell tumors (GCT). HML-2 proteins trigger specific immune responses in GCT patients and HML-2 encoded proteins may be involved in tumorigenesis. While expression of HML-2 in GCT is well established, little is known about transcriptional activity of specific HML-2 proviruses in GCT and other disease and normal conditions. For that reason, we identified HML-2 proviruses that are transcriptionally active in GCT and other conditions. In a straightforward approach we cloned and sequenced HML-2-specific RT-PCR products from various biological specimens: GCT, lung and mammary tumors, corresponding normal control tissues, and from brain samples from patients with schizophrenic and bipolar disorders. We assigned cDNA sequences to HML-2 proviruses by means of nucleotide differences between HML-2 proviruses. We identified, in total, 22 transcriptionally active HML-2 proviruses. Some proviruses were expressed only in tumor or respective control samples. More proviruses were found active in GCT and brain. Three proviruses were constitutively active in all tissues, with two of them being active at higher levels, as indicated by cloning frequencies. Our results demonstrate (i) the transcriptionally active HERV-K(HML-2) loci in the human genome and (ii) variable expression of specific HERV-K(HML-2) proviruses in different tissues. Our analysis furthermore indicates that HERV sequences in EST databases are probably insufficient to comprehensively identify transcribed HERV loci.

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P096

Identification of genomic aberrations associated with shorter overall survival in patients with oligodendroglial tumors
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Deletions on chromosomes 1p and 19q are associated with favorable prognosis in patients with oligodendroglial tumors. The aim of our study was to identify additional genomic aberrations linked to patient survival. We performed a genome-wide screen for genomic imbalances by comparative genomic hybridization on tumors from 70 patients, including 40 oligodendrogliomas, 30 oligoastrocytomas (21 WHO grade II tumors, 49 WHO grade III tumors). Data were correlated with overall patient survival (OS, median follow-up: 5.8 years). The most frequent aberrations were losses on chromosome 19q (64%), 1p (59%), 9p (26%), 4q (21%), 10q (19%), 18q (17%); gains on 7q (24%), 19p (19%), 7p (17%). In univariate analyses, combined 1p/19q and 19q loss were significantly associated with longer OS, and gains on 7, 8q, 19q, 20, losses on 9p, 10, 18q, Xp with shorter OS. Multivariate analyses showed the best prognostic factors for OS of patients with any oligodendroglial tumor to be WHO grade (odds ratio (OR) 8), 7p gain (OR 6), 9p loss (OR 3); for OS of patients with anaplastic tumors to be 7p gain (OR 10), 8q gain (OR 5), 18q loss (OR 3). Patients with anaplastic oligodendroglial tumors containing one or more prognostically unfavorable genomic aberration had a poor outcome independent of the 1p/19q status. In summary, we identified several independent genomic markers of shorter survival in patients with oligodendroglial tumors. Thus, molecular diagnostic testing should not be restricted to 1p/19q deletion analysis but also assess prognostically unfavorable genomic aberrations.

P097

A homozygous MSH6 splicing defect causes signs of NF1 and three different malignancies in two siblings of a consanguineous marriage

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We report here of two siblings of unaffected consanguineous parents, who both developed early-onset malignancies and multiple café-au-lait spots a hallmark of neurofibromatosis type 1 (NF1) in young children. The girl was diagnosed with medulloblastoma at the age of 6 years and with myelodysplatic syndrome (MDS) two years later. She died from acute myeloid leukaemia (AML) into which the MDS has evolved. Her younger brother died of a high grade glioblastoma at the age of 10 years. Comprehensive NF1 mutation analysis performed in the boy revealed no evidence for a pathogenic NF1 gene alteration. Recently approximately 20 children of 10 families with signs of NF1 and early-onset CNS tumours and/or hematological malignancies have been reported to carry biparentally inherited mutations in one of the mismatch repair (MMR) genes. Therefore, we suspected homozygous MMR deficiency to be the genetic alteration causing cancer predisposition and NF1 symptoms also in the here presented siblings. Autozygosity mapping of loci associated with the mismatch repair genes, MLH1, MSH2, MSH6, PMS1 and PMS2 showed homozygosity in both children only for the MSH2/MSH6 locus. Subsequent RNA-based mutation analysis as developed in our laboratory for these genes uncovered a novel complex alteration affecting the 3'splice site of the last intron of the MSH6 gene. The mutation leads to two different aberrantly spliced transcripts that both allow for the translation of polypeptides with potential residual function. This can possibly explain the lack of a clear cancer predisposition in family members carrying the mutation in a heterozygous state. This report is significant in bringing attention to the accumulating evidence that autosomal recessive inheritance of mutations in genes important for DNA repair cause a cancer predisposition syndrome that is characterized by signs of NF1 and early-onset CNS tumours, hematologic malignancies and occasionally early onset gastrointestinal neoplasia.

P098

SGNE1 is epigenetically altered and transcriptionally down-regulated in human medulloblastomas

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In a genome-wide screen using DMH (differential methylation hybridization) we have identified a CpG island within the 5' region and untranslated first exon of the secretory granule neuroendocrine protein 1 gene (SGNE1) that showed hypermethylation in medulloblastomas compared to fetal cerebellum. Bisulfite sequencing and COBRA was performed to confirm the methylation status of this CpG island in primary medulloblastomas and medulloblastoma cell lines. Hypermethylation was detected in 16/23 (70%) biopsies and 7/8 (87%) medulloblastoma cell lines but not in non-neoplastic fetal (n=8) cerebellum. Expression of SGNE1 was investigated by semi-quantitative cRT-PCR and found to be significantly down-regulated or absent in all but one primary medulloblastomas and all cell lines compared to fetal cerebellum. After treatment of medulloblastoma cell lines with 5-aza-2'deoxycytidine, transcription of SGNE1 was restored. No mutation was found in the coding region of SGNE1 by SSCP-analysis. Reintroduction of SGNE1 into the medulloblastoma cell line D283Med led to a significant growth suppression and reduced colony formation. In summary we have identified SGNE1 as a novel epigenetically silenced gene in medulloblastomas. Its frequent inactivation as well as its inhibitory effect on tumor cell proliferation and focus formation strongly argues for a significant role in medulloblastoma development.

P099

Combined bioinformatic analysis of array-CGH and gene expression data from hepatocellular carcinoma and adenoma

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Chromosomal aberrations play a major role in etiology and development of cancer. We performed array comparative genomic hybridisation (aCGH) to study chromosomal gains and losses on 24 hepatocellular carcinomas (HCC) and 10 hepatocellular adenomas (HCA) with the goal to find molecular differences between the adenomas and carcinomas and to understand molecular mechanisms leading to dedifferentiation of hepatocellular carcinomas. Data analysis was done with R software using bioconductor packages (marray, aCGH, GLAD for breakpoint detection) supplemented with new tables and graphs. Clones printed on the array at two different locations were used to assess different normalisation methods. A graph summarises the gains and losses of all experiments per chromosome, indicating minimally altered regions. Results are automatically summarised in tables.
As a complementary approach, expression arrays of HCC and HCA were performed and analysed with GSEA (gene set enrichment analysis) for differentially expressed groups of genes, with genes of one group belonging to the same cytogenetic band. The results were largely concurrent with the array-CGH data. In particular, gains in 1q were found to be the most frequent aberration in HCC with array-CGH, and four cytogenetic bands from 1q were found to be sig-

nificantly enriched in HCC compared to HCA with GSEA.

P100

AF10 interacts with the lymphoid regulator Ikaros and reduces the transcriptional repression activity of Ikaros

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The t(10;11)(p13;q14) translocation leads to the fusion of the CALM and AF10 genes. This translocation can be found as the sole cytogenetic abnormality in acute lymphoblastic leukemia, acute myeloid leukemia and also in malignant lymphomas. Previous studies have shown that the expression of CALM/AF10 in primary murine bone marrow cells triggers the development of an aggressive leukemia in a murine bone marrow transplantation model (Deshpande et al., Cancer Cell, Nov 15, 2006). However, the mechanism of CALM/AF10 dependent leukemogenesis, remains unkown. Recently, we could show that AF10 interacts with the transcription factor Ikaros (IKZF1) in yeast-two-hybrid assays. Interestingly, Ikaros is a key regulator of hematopoesis, required for normal differentiation and proliferation of B- and T-lymphocytes. In various forms of leukaemia an aberrant expression pattern of Ikaros has been found. In a murine model, the expression of a dominant negative isoform of Ikaros causes leukemias and lymphomas. Using various AF10 deletion mutants, the Ikaros interaction domain of AF10 was mapped to the Leucine Zipper domain of AF10, which has also been shown to be required for malignant transformation both by the CALM/AF10 and the MLL/AF10 fusion protein. The interaction between AF10 and Ikaros was confirmed by GST-pulldown and co-immunoprecipitation. Immunofluorescent staining of FLAG-AF10 after co-expression with YFP-Ikaros reveals an overlapping distribution pattern in the nucleus. In reporter gene assays, the transcriptional repressor activity of Ikaros is influenced by the presence of AF10. These results suggest that CALM/AF10 might have a dominant negative effect on Ikaros, and thereby block differentiation of the leukaemia propagating cell in CALM/AF10 positive leukemias. Our studies provide new insights into the mechanism of CALM/AF10 induced leukemia and might thereby facilitate the development of new therapies.

P101

Differential in vivo and in vitro expression of valosin-containing protein (VCP, p97) in acute lymphoblastic leukemia cells with and without glucocorticoid treatment

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The response to initial glucocorticoid therapy in childhood acute lymphoblastic leukaemia (ALL) reliably predicts the response to multiagent chemotherapy. Patients resistant to glucocorticoids (prednisone poor responders (PPR)) have a poorer event-free survival compared to glucocorticoid-sensitive patients (prednisone good responders (PGR)). In a case control study to investigate differential protein expression in leukaemic blasts from PGR and PPR childhood ALL patients, the valosin-containing protein (VCP) was identified as one of the proteins overexpressed in PPR patients. The aim of this study was to validate the overexpression of VCP both on the transcriptome and the proteome level. The human B cell precursor leukaemic cell lines MHH cALL 2 (PPR) and MHH cALL 3 (PGR) were studied after induction with glucocorticoids (3µg/mL Solu-DecortinH®). Western blot analyses using an anti-p97 antibody were performed on whole cell lysates, nuclear, membrane and cytosolic fractions. In addition, the results were evaluated in bone marrow samples from 10 patients with childhood ALL. Real-time PCR was performed on the PPR and PGR cell lines and on patient samples to quantify the amount of VCP expression.
However, VCP RNA expression in PPR cells was twofold that in PGR cell lines. In agreement with these findings, VCP protein was also overexpressed in the PPR cell line. After glucocorticoid induction, VCP RNA expression increased twofold in both cell lines. Most importantly, in three of five matched pairs, VCP RNA and protein expression was increased in the PPR patients. The results of this study confirm that valosin-containing protein expression is associated with glucocorticoid resistance in B-cell precursor childhood ALL and might indicate that the proteasome-ubiquitin degradation pathway, in which VCP is integrated, determines multi-agent chemotherapy resistance and treatment outcome in childhood ALL patients.

P102

Cytogenetic data on MDS in childhood from the EWOG-MDS 98 study

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To determine the clinical significance of cytogenetic findings in childhood myelodysplastic syndromes (MDS), chromosome analyses were performed in 395 consecutive patients with MDS enrolled in the prospective observational study EWOG-MDS 98. This is the first study analysing cytogenetic data of children with MDS in the context of a prospective multi-center study. A karyotype at diagnosis could be analyzed in 258 of the 395 patients (65%). Most unsuccessful analyses were noted among patients with refractory cytopenia (RC) (29/215, 14%) compared to those with RAEB (9/142, 6%) and RAEB-T (0/38, 0%) ($p<0.01$). For 143 patients, cytogenetic data follow-up was analyzed. Overall, 220 cytogenetic findings during follow-up were included. In primary MDS, monosomy 7 alone (n=32) or with one additional abnormality (n=13) accounted for 55% of all abnormal karyotypes at diagnosis, and was more frequently seen in RAEB and RAEB-T than in RC ($p< 0.01$). At the same time, a normal karyotype was significantly overrepresented in RC compared to RAEB and RAEB-T ($p<0.01$). Trisomy 8 as sole abnormality or with one additional aberration was the second most common abnormality noted in 6 and 2 children with primary MDS, respectively. Morphologically, these 8 patients (11% of primary MDS) were classified as RA in 3 cases, RAEB in 4 and RAEB-T in 1. Trisomy 21 with or without one additional abnormality was seen in 5 patients with primary MDS (7%). Deletions in 5q and 20q as sole aberration were not observed. Among 20 patients with congenital bone marrow failure syndromes, 8 patients (47%) carried a complex karyotype. Monosomy 7 was seen in 8 out of 14 patients (57%) with familial hematological neoplasias. Complex aberrant clones were found significantly more frequently in patients with secondary MDS (17/57; 30%) compared to patients with primary MDS (6/205; 3%). In summary, childhood and adult MDS seem to differ with respect to the frequency of recurrent aberrations.

P103

Gene expression profiling in hepatocellular carcinoma and adenoma: Upregulation of genes in amplified chromosome region 1q22

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Molecular cytogenetic analyses of hepatocellular carcinoma (HCC) and adenoma (HCA) have revealed gains of chromosome 1q as the most significant differentiating factor besides morphological criteria. However, no studies are available comparing these amplification events with gene expression of candidate oncogenes. Therefore, based on array comparative genomic hybridisation (aCGH), we performed gene expression profiling on the same set of tumours to investigate which of the genes in the amplified region 1q22 are overexpressed.
Analysis was carried out on 24 HCC and 8 HCA cytogenetically characterised by aCGH. Expression profiles of mRNA were determined using a genome-wide microarray containing 43000 spots. Hierarchical clustering analysis branched all HCA from HCC. Significance analysis of microarray (SAM) demonstrated 171 upregulated and 551 downregulated genes in HCC compared with HCA. Additionally performed gene set enrichment analysis (GSEA) detected groups of genes located in chromosome bands 1q22, 1q31, 1q41 and 1q42 significantly upregulated in HCC. These bands were also seen as the most frequently gained regions by aCGH in HCC. Comparison of SAM and GSEA narrowed down the number of dysregulated genes to 18 with 7 genes localised on 1q22 (SCAMP3, IQGAP3, PYGO2, GPATC4, ASH1L, APOA1BP, and

CCT3). In HCA, 26 genes in bands 11p15, 11q12, and 12p13 were upregulated. However, the respective chromosome bands were not gained in HCA.
In conclusion, gene expression analysis and aCGH identified a coordinated upregulation of genes in amplified regions, in particular for chromosome arm 1q. These results may serve as a basis to further narrow down the number of candidate driver genes in hepatocarcinogenesis.

P104

Expression analysis of genes lying in the NF1 microdeletion interval points to four candidate modifiers for neurofibroma number and onset

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominantly inherited tumor diseases. One of the typical hallmark symptoms are multiple dermal neurofibromas. To explain the interfamilial variability of the number of neurofibromas, the existence of modifying genes has long been proposed. NF1 patients with microdeletions spanning the NF1 and several contiguous genes with yet unknown function have an earlier onset and higher number of dermal neurofibromas as classical NF1 patients. In the region deleted in these patients, 21 genes and pseudogenes have been described. Therefore, it seems likely that one or more of these genes contributes to the increased tumor load. We examined the expression of 20 of the 21 candidate genes in neurofibromas, nerve tissue, cultured cells and the homologous genes in various murine tissues. We found only four genes, CENTA2, RAB11FIP4, C17orf79 and UTP6, to be expressed in all of the dermal neurofibromas examined. One gene, OMG, was not expressed in any neurofibroma. In contrast, primary human fibroblasts express 11 of the 20 genes investigated, the neurofibrosarcoma cell line NFS-1 and the mast cell line LAD2 even more (18/20). Additional investigation of murine tissues revealed a similiarly high number of expressed genes. The four genes expressed in all dermal neurofibromas examined are strong candidates for the modification of neurofibroma number and onset and will be further investigated.

P105

Translocation t(1;19) is associated with early treatment sensitivity in childhood acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. In vivo response to initial therapy, as assessed by determination of minimal residual disease at 5 and 12 weeks of treatment, has evolved as the strongest prognostic factor in pediatric ALL patients treated according to the BFM regime. In this study, 25 patients with a poor treatment response (MRD load $\geq$ 10-3 at week 12, MRD high risk, HR) and 25 treatment-sensitive patients (no measurable MRD at weeks 5 and 12, MRD standard risk, SR) were investigated by means of high-resolution bacterial artificial chromosome (BAC) array-based comparative genomic hybridization (array-CGH). To ensure homogeneity with regard to prognostic factors, the following inclusion criteria were used: B cell precursor or common ALL, DNA index of 1.0, no BCR/ABL, no MLL/AF4, no TEL/AML1 rearrangements. By means of array-CGH, a gain of chromosome 1q23-qter was found in 10/25 SR patients, whereas none of the HR patients showed a 1q gain of 1q23-qter. This gain may be due to an unbalanced translocation der(19)t(1;19)(q23;p13). Cytogenetically, two forms, a balanced t(1;19)(q23;p13) and an unbalanced der(19)t(1;19)(q23;p13) exist, both giving rise to the E2A/PBX1 fusion gene. To prove whether additional cases may carry an unrecognized balanced t(1;19), we performed RT-PCR to detect the E2A/PBX1 fusion transcript. Consistent with array-CGH, all cases with a gain of 1q23-qter showed the fusion transcript. The fusion transcript was detected in one additional SR patient. Interestingly, this patient showed a different gain from 1q25 to qter, which may be explained by an accompanying deletion of 1q23-1q25. No recurrent genomic imbalances (>1-2 Mb) were found in the HR group.

P106

Microarray analysis of the DNA repair transcriptome in Fanconi anemia cells

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Fanconi anemia (FA) patients are characterised by congenital abnormalities, bone marrow failure, and a high risk of developing certain types of cancer. Somatic cell fusion and biochemical analyses have delineated twelve different complementation groups (FANC-A, B, C, D1, D2, E, F, G, I, J, L, and M), and the disease genes underlying these groups have already been cloned. Cells of all complementation groups are highly susceptible to the induction of chromosome breaks by DNA cross linking agents, indicating that mutations in the different FANC genes cause a similar DNA repair defect(s). Several lines of evidence suggest a role of the FANC gene network in homologous recombination-mediated repair and the cellular response to oxidative stress. We have used a customized cDNA microarray containing several hundred genes involved in DNA repair and cell cycle control to compare the expression profiles of FA fibroblast cells of the different complementation groups (FA-G, A, D1, F, D2, B and E) with a pool of control fibroblast lines. Genes that were found to be differentially regulated by microarray analysis were validated by quantitative real-time PCR. These genes were found to be up regulated and down regulated in the different complementation groups. This allowed us to delineate specific expression profiles for each complementation group. Interestingly, we also identified two genes, CTSB and PLK2 that were similarly deregulated in all complementation groups. Collectively, our data may provide novel biomarkers for the diagnosis of FA and help to develop novel therapeutic strategies.

P107

Distinct genomic aberrations characterize late relapse of childhood acute lymphoblastic leukemia

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In childhood acute lymphoblastic leukemia (ALL), approximately 25% of patients suffer from relapse. In recurrent disease, despite intensified therapy, overall cure rates of 40% remain unsatisfactory and survival rates are particularly poor in certain subgroups. The probability of long-term survival after relapse is predicted from well-established clinical factors, e.g. time of relapse with respect to frontline treatment. However, the underlying biological determinants of this prognostic factor are poorly understood.
Aiming to identify genomic aberrations associated with time of relapse we performed microarray-based comparative genomic hybridization (array CGH) with a resolution of 1Mb, of 41 DNA samples of first relapse patients enrolled on the relapse trial ALL-REZ BFM 2002 of the Berlin-Frankfurt-Münster study group.
Aberrations most frequently detected by array CGH were losses of/on chromosome 9p [11/41, 27%] and gains of/on chromosome 21 [13/41, 32%]. Additional recurrent genomic aberrations included losses of/on 3p [4/41, 10%] 6q [6/41, 15%], and 12p [7/41, 17%] and gains of/on chromosome 1q [7/41, 17%], 10 [8/41, 19.5%], 14 [7/41, 17%] and X [10/41, 24%]. Patients with an early, on treatment relapse, that typically face a dismal prognosis, had significantly lesser aberrations in their leukemic blasts than late, off treatment relapse patients. Deletion of 9p was frequently detected in both groups and no significant prognostic value was found, consistent with published data. In contrast, gains and partial gains of chromosome 21 and X as well as deletions on 3p were exclusively observed in late relapse patients.

In summary, we could demonstrate that patients with a late, off treatment relapse show a characteristic pattern of genomic aberrations which affect chromosomes 3p, 21 and X. To further characterize the impact of these observations we intend to correlate the genomic patterns with gene expression data obtained from the same samples.

P108

Polymorphisms of DNA repair and detoxification genes in B-cell chronic lymphocytic leukemia

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B-cell chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in the Western World. As its clinical course is highly variable, prognostic factors for disease progression such as particular cytogenetic abnormalities, the mutational status of the immunoglobulin heavy chain (IgH) genes, LPL, or CD38 expression, have been identified. However, a convincing prediction of disease progression can not be made in early stages of the disease. Further prognostic factors would be helpful for meaningful prediction of disease progression. There is evidence that multiple low-penetrance genetic factors, including genetic polymorphisms, predispose to CLL or modify its clinical course. The aim of this study was to identify such allelic variants of genes, primarily concentrating on DNA repair and detoxification genes. We studied the distribution of 50 polymorphisms in 500 CLL-patients and 500 control persons using PCR amplification followed by restriction enzyme digestion. Whereas for most polymorphisms an association with CLL could be excluded, our analysis of a polymorphism in the DNA repair gene XRCC1 revealed different frequencies of the allelic variants between DNA samples of CLL-patients and DNA of control persons. Further investigations are now under way to proof the relevance of this polymorphism in the clinical course of CLL and its putative significance for CLL in combination with other gene polymorphisms. The results of this study will lead to new insights into the etiology of CLL and may help to predict disease progression.

P109

Comprehensive characterization of genomic aberrations in gangliogliomas

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Gangliogliomas (GG) are neuroepithelial tumors frequently associated with epilepsy. They are composed of dysplastic neuronal as well as neoplastic glial elements and are generally benign (WHO grade I or II). As little is known about their molecular pathogenesis, we aimed to identify genomic aberrations involved in GG tumorigenesis. Fifty-two GGs (47 WHO grade I, 5 WHO grade II) were screened for chromosomal imbalances by comparative genomic hybridization (CGH). Genomic aberrations were detected in 60% of GGs with an average of 1.85±0.34 (mean±SEM) alterations per tumor (range: 0 to 13). Recurrent gains were identified on chromosome 7 (17% of tumors), 5 (13%), 8 (12%), Y (8% of tumors from male patients), 20 and X (8% each), 12 and 19 (6% each), 4, 9q and 17 (4% each). Recurrent losses were found on chromosomes 22q (17%), 9 (10%), Y (8% of tumors from male patients), 16 (8%), 17, 18, 20 and 21q (6% each), 10q, 13q and 19 (4% each). Combined gains of chromosomes 7 and 8 were detected in six cases. Analysis of 21 GGs with available high molecular weight DNA by high-resolution array-based CGH confirmed the aberration pattern identified by chromosomal CGH and additionally detected four cases with smaller imbalances (losses of 10q21.1-q26, 10q22.3-q25.3, 15q11.2-q22.2; gain of 12q13.3-q14.1). By interphase fluorescence in situ hybridization to tumor tissue sections, some of the array-CGH results were verified and the involved cells were identified to be a subpopulation of glial cells. In the dysplastic neuronal cells, no chromosomal imbalances were found. Two GGs had recurred as malignant glioblastomas (GBM) of WHO grade IV. These GBMs contained a loss of CDKN2A/CDKN2B in one case and an amplification of CDK4 in the other case. Interestingly, the loss of CDKN2A/CDKN2B and a gain of CDK4 were already detectable in the primary GGs of WHO grade I and II. In conclusion, our study provides the first comprehensive overview of genomic alterations in gangliogliomas.

P110

An integrative approach to analyse the interplay of genetic and epigenetic changes in lung carcinomas

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Tumorigenesis is characterized by the accumulation of genetic and epigenetic alterations, which act in concert to generate an individual tumor phenotype. However, our knowledge of the complex interplay of DNA copy number changes, global methylation patterns and gene expression is poorly understood. In this integrative approach we present the analysis of 24 primary lung carcinomas and four tumor cell lines by means of array CGH, MeDIP (Methylated DNA Immunoprecipitation) and oligobased gene expression analysis. The overall patterns of chromosomal aberrations were in line with results from chromosomal CGH, however, the about hundred-fold higher resolution of submegabase resolution array CGH revealed the presence of small high copy amplicons not reported before (up to twelve in one case), and which may assist in the identification of oncogenes and potential therapy targets. Differential diagnosis employing supervised clustering of array CGH data was superior to clustering based on gene expression data. Changes of global methylation did not affect all samples to the same extent. Proceeding on the meta-analysis of changes in DNA copy number, methylation and gene expression, we suggest that the consequences of a specific chromosomal change is varying depending on the overall genetic and epigenetic environment in the individual tumor.

P111

Frequent hypermethylation of the OXTR gene in uveal melanoma

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Uveal melanoma (UM) is the most common primary intraocular tumour with an incidence of six per one million per year. About 50% of the patients die of metastases. Metastatic disease is significantly correlated with loss of one copy of chromosome 3 (monosomy 3) in the tumour. The aim of this study was to identify epigenetic alterations of chromosome 3 genes that might represent the second hit in UM tumour suppressor gene (TSG) inactivation. We also wanted to know whether epigenetic alterations might serve as a marker for tumour DNA in the serum of patients. We analysed the methylation of the CpG island associated with the OXTR-gene, which is located within a smallest region of deletion overlap (SRO) on chromosome 3p25-26. Methylation analysis was performed by bisulfite modification of the genomic DNA, followed by PCR amplification and direct sequencing of the PCR products. We analysed 39 uveal melanomas, 24 tumours with monosomy 3 and 15 tumours with disomy 3. Fifty percent of the tumours with monosomy 3 and eighty percent of the tumours with disomy 3 showed hypermethylation of the CpG island. The degree of CpG methylation varied between the tumour samples. Overall, the degree of methylation increased from 5` to 3`. We could not detect any OXTR methylation in 17 blood DNA samples from normal controls. Thus, OXTR methylation appears to be a UM specific epigenetic alteration.
The location of OXTR within the SRO and its recurrent hypermethylation in UM suggested that OXTR might be a TSG. We analysed OXTR expression by quantitative RT-PCR-analysis of RNA from UMs with and without OXTR methylation, but could not detect a correlation between methylation and mRNA levels. This does not support a role of OXTR as a TSG in UM. The

suitability of the OXTR methylation as marker for tumour DNA in serum is currently under investigation.

P112

Frequent loss of chromosome region 13q21.1 in primary multiple myelomas and identification of novel chromosome translocation breakpoints in multiple myeloma cell lines

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Multiple myeloma (MM) is a B-cell neoplasm characterized by the accumulation of clonal malignant plasma cells in the bone marrow. The pathogenesis of the disease remains still unclear. The deletion of chromosome 13, in particular of region 13q14, is of important prognostic value for therapeutic decisions because of its association with shorter survival times and lower response to treatment. We used Array-CGH-analysis for the detection of DNA gains and losses in the genome of 32 primary multiple myelomas. In addition to the deletion of chromosome region 13q14 we detected a frequent loss of chromosomal region 13q21.1. The importance of this finding should be corroborated in larger studies and possible candidate genes should be analyzed.

Furthermore, the detailed analysis of numerous chromosome translocations has remained limited, because cell cultures and metaphase preparations of primary malignant plasma cells are difficult to accomplish. Therefore we analyzed three MM cell lines (INA-6, ANBL-6 and MM.1S) by SKY-, Array-CGH- and expression analysis. We detected eight novel chromosome translocations that showed a striking correlation between DNA copy number changes and the transcription level. For instance, the translocation t(4;9)(q22.3;q13) was accompanied by the partial deletion of the bone morphogenetic protein receptor (BMPR1) gene on 4q22.3, which is involved in the TGF-beta/BMP pathways. The relevance and importance of this and other genes for the pathogenesis of MM may now be studied in larger numbers of primary tumors.

P113

Association of prostate cancer in Germany to a confirmed susceptibility region on 8q24.12

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Prostate cancer (PCa) susceptibility has recently been mapped to 8q24.12 by genome wide linkage and subsequent association analyses in the population of Iceland. The suggested risk locus has gained broad attention, first, because its reliability was strengthened in the initial work by the utilization of additional study populations, and second, it contains only two candidate EST genes. We used the mircosatellite DG8S737 and the SNP rs1447295 (C>A), which tagged the maximum risk in the Icelandic sample, in order to assess the impact of 8q24.12 on sporadic and familial PCa in Germany. Consistent with the previous report the risk allele at DG8S737 (corresponding to 22 CA repeats) was observed twice as frequent (0.091 vs. 0.049) in a total of 515 affected compared to 207 healthy men (allelic OR = 2.1, p-value = 0.0044). The A nucleotide of the genotyped SNP, which in general is more common than DG8S737 allele 22, presented a less prominent maldistribution among cases and controls (0.153 and 0.092, respectively), but was likewise significantly associated (OR = 1.8, p = 0.0022). In a family based association test both variants showed tendencies of overtransmission towards affected members within pedigrees of PCa clustering. However, probands grouped by positive or negative family history revealed similar frequencies of risk alleles in sporadic and familial PCa, indicating the moderate penetrance of the causal variant(s). Nevertheless, the population attributable risk that depends on frequency and relative risk of carriers is 0.09 to 0.13 for allele 20 and A, respectively, in the present study sample. Our study independently replicated association of PCa to 8q24.12, which may account for 10% of PCa incidence in Germany, regardless of familial disease history. The causal variant and pathogenic mechanism remains unknown. Current investigation aim for correlations of risk genotypes with somatic changes of two closely mapped EST genes and of the neighbouring c-myc oncogene.

P114

Genome-wide linkage analysis in prostate cancer families with accumulation of somatic TMPRSS2-ERG fusions

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Hereditary prostate cancer seems to exhibit extreme heterogeneity which renders identification of risk genes difficult. One possible approach to this is the selection of homogeneous subgroups of families defined through specific epidemiological criteria. Nevertheless, to date criteria of this kind, e.g., early average onset of cancer, do not provide consistent assignment of susceptibility genes. In this context, the recent identification of somatic fusions of the TMPRSS2 gene and members of the ETS oncogene family in prostate carcinomas may result in a novel criterion based on a common underlying pathological mechanism. In order to investigate this, we screened patients from 27 families for fusion status. The families were taken from a sample previously analyzed in a genome-wide linkage scan using 500 microsatellite markers and had been investigated concerning the prevalence of TMPRSS2-ERG rearrangements. Of these, 12 pedigrees contained at least two tumors each with a fusion event. The latter were reanalyzed using both parametric (LOD) and model-free (NPL) linkage approaches. In this analysis, affection status was still defined as presence or absence of prostate carcinoma. As a result, amongst others, suggestive linkage was observed in the chromosomal regions 6q24-27 (LOD = 2.26, p = 0.001), 9q13-21 (LOD = 2.56, p < 0.001) and 18q (LOD = 2.40, p < 0.001). However, none of these loci attained significant genome-wide linkage. It is noteworthy that the genomic loci of TMPRSS2 and ERG remained inconspicuous concerning linkage, indicating that the fusion region itself does probably not contribute to the rearrangements. However, several loci possibly involved in the underlying mechanism of translocation and/or cancerogenesis could be suggested. Candidate genes for chromosomal rearrangements might preferably be found in the functional context of DNA repair or of genome integrity.

P115

The number of genomic imbalances in the tumor correlates with the overall survival in children with adrenocortical carcinoma

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Adrenocortical tumors in children are rare and often associated with poor prognosis. We analyzed 14 adrenocortical carcinomas (ACC) and 1 adenoma of children and detected genomic imbalances in all of them by comparative genomic hybridization (CGH). The most common imbalances were +1q (57%), +12p (50%), +12q (50%), +1p (43%), +7q (42%), +9q (42%), +15q (42%), and -4q (57%), -11q (57%), -4p (42%), and -16q (42%). The total number of genomic imbalances ranged from 1-17 in individual tumor samples. This number did not correlate with sex, hormone level or tumor size. The median number of genomic changes was 5,5 (n=8) in pT1-pT2 and 15,5 (n=6) in pT3-pT4 tumors. Meanwhile, it was 4 in the 8 cancer patients who remain in remission more than 51 months, while it was 15,5 in 6 patients, who have died from the disease within

44 months. Moreover, all 7 patients with less than 10 individual imbalances were in remission (median follow-up 72 months), while all but one patients with 10 and more individual imbalances (n=7) have died from the disease (median survival time 30 months). The number of CGH imbalances separate patients with good and unfavourable prognosis and therefore has a predictive value for overall survival in paediatric ACC. Currently, we perform a chip based copy number analysis with affymetrix 50K-100K arrays. This is to augment data and to confirm our classification by an alternative approach. Furthermore, SNP based copy number analysis should be capable to detect smaller aberrations as well as copy-neutral LOHs that may refine categorisation.

P116

Identification of GLI1 target genes in human rhabdomyosarcoma cell lines

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Inappropriate hedgehog signaling plays a fundamental role in an increasing number of malignancies, including those of the skin and brain. Even though the core components of the cascade through which signaling occurs are known (Hedgehog, Patched, Smoothened, Gli, all of which were found to be altered in human cancers), the precise allocation of the hedgehog signal in specific tissues and/or tumors remains largely elusive. Interestingly, new data point to an important role of hedgehog signaling in the genesis of human rhabdomyosarcoma, the most common soft-tissue sarcoma in childhood. In order to uncover the precise downstream targets of hedgehog signaling in rhabdomyosarcoma we have set up an in-house real-time PCR assay to simultaneously screen for the expression of 88 genes in the GLI1-amplified RMS-13 cell line. The expression levels were then compared to three non-GLI1-amplified RMS cell lines. With this approach we have identified several known direct transcriptional targets of GLI1, including FOXF1, FOXA2, BMP4, GADD45A, CCND2, TGFB2, NMYC, and SNAIL. Additionally, we have detected putative GLI1 target genes, such as TLR4, WISP1, LEF1, IFNG, NOS2A, and IFNA1. GLI1 consensus DNA-binding sequences were subsequently identified in the 5'-regions of these genes, suggesting that they represent immediate down-stream targets. Moreover, we were able to group the RMS cell lines with and without GLI1-amplification on the basis of similarities in their gene expression patterns by using a hierarchical clustering algorithm. We believe that "smart" PCR-based expression assays will increasingly become an important tool to study the molecular make-up of human tumors particularly with regard to refining tumor diagnosis and therapy.

P117

The four and a half LIM domain protein 2 (FHL2) interacts with CALM and is highly expressed in AML with complex aberrant karyotypes

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The translocation t(10;11)(p13;q14), which leads to the CALM/AF10 fusion gene, is found in malignant lymphoma, acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukaemia (T-ALL). The CALM/AF10 fusion gene has been shown to cause an aggressive biphenotypic leukemia in a murine bone marrow transplant model (Deshpande et al., Cancer Cell, Nov 15, 2006). AF10 is a putative transcription factor likely involved in processes related to chromatin organization. The CALM protein plays a role in clathrin mediated endocytosis and trans Golgi network trafficking. To learn more about the function of the CALM/AF10 fusion protein, we searched for protein interaction partners of CALM using a yeast-two-hybrid assay and identified FHL2 as putative CALM interacting partner. FHL2 is a TP53 responsive gene known to interact with proteins in both nucleus and cytoplasm and it can function as a transcriptional cofactor. High expression of FHL2 in breast cancer has recently been shown to be associated with an adverse prognosis.
The CALM-FHL2 interaction was confirmed by GST-pulldown and co-immunoprecipitation experiments. In co-localization studies, a shift of CALM from the cytoplasm to the nucleus is seen in FHL2 expressing cells. Expression analysis (Affymetrix-based) in different AML subtypes showed a significantly higher expression of FHL2 in AML samples with complex aberrant karyotypes compared to AML with normal karyotypes or balanced chromosomal translocations like the t(8;21), inv(16) or t(15;17). Reporter gene assays using a GAL4-DNA binding domain CALM fusion protein and a GAL4 responsive luciferase reporter were reported to transactivate CALM. Currently, the effect of FHL2 co-expression on this aspect of CALM function is investigated. It is thus conceivable that FHL2 plays an important role in CALM/AF10-mediated leukemogenesis by tethering the CALM/AF10 fusion protein to various nuclear transcription factor complexes.

P118

Amplification and translocation of 3q26 with overexpression of EVI1 in Fanconi anemia derived childhood AML with biallelic FANCD1/BRCA2 disruption

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Fanconi anemia (FA) is a recessive inherited disease with congenital abnormalities and an extreme risk of acute myeloid leukemia (AML). Genetic alterations occurring during malignant transformation in FA and the biology of FA-associated AML are poorly understood, but are often preceded by the development of chromosomal imbalances involving 3q26q29 in bone marrow cells of FA-patients (Tönnies et al. 2003). We report here the molecular cytogenetic characterization of FA-derived AML cell lines SB1685CB and SB1690CB by conventional and array CGH, FISH and SKY. We identified gains of a 3.7MB chromosomal region on 3q26.2-q26.31, which preceded transformation to overt leukemia. This region harbors the oncogenic transcription factor EVI1. A third FA-derived cell line, FA-AML1, carried a translocation with ectopic localization of 3q26 including EVI1. Rearrangements of 3q, which are rare in childhood AML, commonly result in overexpression of EVI1, which determines specific gene expression patterns and confers poor prognosis. We detected overexpression of EVI1 in all three FA-derived AML compared to other AML cell lines included in our study (OCI-AML5, MUTZ-3, K562, and MUTZ-2).
Our results suggest a link between the FA-defect, chromosomal aberrations involving 3q and overexpression of EVI1. We hypothesize that constitutional or acquired FA-defects might be a common factor for the development of 3q abnormalities in AML. In addition, cryptic imbalances as detected here might account for overexpression of EVI1 in AML without overt 3q26 rearrangements.

P119

Phenotype-genotype correlation in women with simultaneous germ line mutations in both BRCA genes, BRCA1 and BRCA2

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Germ line mutations in BRCA1 and BRCA2 predispose to early breast and ovarian cancer. Only a few families have been described in which more than one pathogenic BRCA mutation segregated. Due to the extreme rarity of women with double heterozygosity (DH) for BRCA1 and BRCA2 mutations phenotypic consequences and clinical implications of DH remain still unclear. We describe here 3 women with DH for

pathogenic mutations in BRCA1 and BRCA2. To delineate a specific phenotype we compared the clinical data with those of affected female carriers of the same cohort harbouring either a BRCA1 (n=40) or a BRCA2 (n=20) mutation and with those of affected female relatives heterozygous for either the familial BRCA1 (n=3) or the familial BRCA2 (n=3) alteration. The interfamiliar comparison showed that the women with DH developed breast cancer in average 6.7 years earlier than BRCA1 carriers and 11.7 years earlier than BRCA2 carriers. The intrafamiliar comparison showed that the DH women developed breast cancer 19 years earlier than their affected relatives who carried only a mutation in one of the genes. Women with DH had more primary cancers (average 2.3) than non-related carriers of a BRCA1 (1.3) or BRCA2 (1.1) mutation and than their single heterozygous relatives (BRCA1:0.3; BRCA2:1.0). Despite all 3 women with DH developed several metastasis they are still alive 10, 11 and 26 years after initial diagnosis, respectively. Two women received alkylating treatment (cisplatin, methotrexat, 5-fluorouracil) and one woman possibly benefited from bilateral mastectomy and ovarectomy. Despite valid statistical analysis is hampered by the small number of women with DH, there is a clear trend of a more severe phenotype with a younger age at onset and more synchrone/ metachrone manifestations in women with DH compared with heterozygosity for only BRCA1 or BRCA2. Therefore, a second mutation should always be excluded in families with a positive history in the maternal and paternal family.

P120

The additive effect of p53 Arg72Pro and RNASEL Arg462Gln genotypes on age of disease onset in Lynch syndrome patients with pathogenic germline mutations in MSH2 or MLH1

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Background: p53 and the prostate-cancer-susceptibility gene RNASEL are tumour suppressor genes involved in apoptosis. We have previously reported that the common, functionally different variants Arg72Pro in p53 and Arg462Gln in RNASEL are associated with the age of disease onset of colorectal cancer (AO) in Lynch syndrome patients (Krüger et al. J Med Genet 2005;42:769-773; Krüger et al. Lancet Oncol 2005;6:566-572). Here we assess the combined effect of both variants.
Methods: 246 unrelated Lynch syndrome patients with a pathogenic germline mutation either in MSH2 (n=138) or in MLH1 (n=108) and colorectal cancer as first tumour, and 245 healthy controls were analysed.
Results: The global log rank test revealed significant differences in the AO for the genotypes of each variant (p=0.0176 for p53 and p=0.0358 for RNASEL) and for the combined genotypes of both variants (p=0.0174). The highest difference in median AO was seen between homozygotes for the wild-types in both genes (42 years [range 22 – 75]) and homozygotes for the variant alleles in both genes (30 years [range 26 – 47]). A multivariate Cox regression model indicated that only the p53 and RNASEL genotypes had a significant influence on age of disease onset (p=0.016 for p53 and p=0.014 for RNASEL) in an additive mode of inheritance, and that the effects of both variants are purely additive.
Conclusions: Here we demonstrate that the combined effect of p53 codon 72 and RNASEL codon 462 genotypes on AO in Lynch syndrome is additive, supporting the notion that the p53 and RNase L pathways do not interact. These findings may be relevant for preventive strategies in Lynch syndrome.

P121

Constitutional and somatic mutations in the SMARCB1/SNF5/INI1gene in patients with rhabdoid tumours (RTs) or atypical teratoid/rhabdoid tumours (ATRTs)

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Rhabdoid tumors (RTs) are a rare but highly malignant group of neoplasms that usually occur in children less than 2 years of age. Extrarenal RTs [which were often described as atypical teratoid/rhabdoid tumours (ATRTs)] are found in various locations, but most frequently in the central nervous system. Unifying features include morphology, immunohistochemical characteris-tics and inactivation of the putative tumour suppressor gene SMARCB1 (hSNF5/INI1) located in 22q11.2. Constitutional mutation in one SMARCB1 allele gives rise to the term "rhabdoid tumour predisposition syndrome".
We report here 16 patients with RTs or ATRTs analysed by FISH and/or DNA-sequencing for aberrations of the SMARCB1 gene locus. Somatic homozygous deletions of the SMARCB1 gene locus were detected by FISH in four cases, three of them showed no mutation by sequencing, whereas the fourth was not investigated by sequencing. Six cases displayed a hemizygous deletion of SMARCB1 gene locus by FISH. In three of them, a mutation of the second allele was detected by sequencing. Two additional cases were normal by FISH, but showed a homozygous mutation in the SMARCB1 gene. Taken together, biallelic inactivation of the SMARCB1 gene was detectable in 9 of 16 cases by FISH and sequencing. Four cases were normal by FISH and sequencing. Constitutional heterozygous mutations/deletions in the SMARCB1 gene were detected in 2 of 16 patients by sequencing and in 1 of 16 patients by FISH. All these constitutional mutations/deletions in the SMARCB1 gene appeared de novo.

P122

New chromosome aberrations in B-cell chronic lymphocytic leukemia

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B-cell chronic lymphocytic leukemia (CLL) is a common leukemia that more frequently affects elderly people. The clinical course of CLL is variable and in early stages a prediction of disease progression is difficult. Specific karyotype deviations are important prognostic factors, but within the group of patients with a normal karyotyp that is in general associated with a good prognosis a number of patients show an aggressive clinical course. The aim of our study is the detection and characterization of new chromosome aberrations by classical and molecular cytogenetics. In comparison to fluorescence in situ hybridization (FISH) conventional cytogenetic analysis by classical chromosome banding plays a minor prognostic role in CLL due to the low proliferation rate of malignant B-cells in vitro. As traditionally used sets of mitogens result in poor proliferation response of leukemic B-cells, new cultivation techniques using optimal mitogen combinations (OMC: TNFα + IL-2, SAC + IL-2, TNFα + TPA10) and immunostimulatory CpG-Oligodinucleotide DSP 30 (COD) plus IL-2 were established in order to enhance the yield of detectable chromosome aberrations in CLL cells. Successful metaphase stimulation by culturing the cells with OMC and/or COD plus IL-2 was observed in 45 CLL cases. Most of these karyotypes showed the same aberrations as obtained by parallel performed interphase FISH. In addition six novel chromosomal aberrations were identified and characterized. Detailed depiction of novel aberrations was possible by FISH using whole chromosome painting probes, centromere-specific, single copy probes and/or multicolor FISH. The relevance of these novel chromosome anomalies in CLL detected by using refined culture techniques and the combination of classical chromosome banding and molecular cytogenetic analyses with respect to other prognostic parameters and to the clinical course will be examined.

P123

New methodical approach for the quantitative and qualitative analysis of the chromosomal instability in Fanconi anemia patients

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Fanconi anemia (FA) is a rare inherited chromosome instability disorder with congenital abnormalities, a high risk of acute myeloid leukemia (AML), and solid tumor predisposition. FA can be caused by pathogenic mutations in at least 12 different genes interacting together with others (ATM, RAD51, NBS1 etc.) in the cellular DNA damage recognition and repair network. FA-cells are characterized by spontaneous chromosomal breakage and increased sensitivity to DNA cross-linking agents as mitomycin C (MMC).
Genetic diagnosis of FA is routinely based on a conventional chromosome breakage test which is performed on Giemsa stained chromosomes after MMC treatment. Mainly chromatid breaks and radial figures are counted and compared to these of normal control cells without any further information about the single chromosome(s) involved. Translocations are mostly not detectable by this test.
To get a better insight in specific breakage events and to circumvent time consuming breakage counting, we currently established a semi-automated, molecular-cytogenetic assay ("wcp-assay") for the detection of chromosomal breakage in FA cells. This assay is based on the use of whole chromosome painting probes for single chromosomes together with an automated scanning microscope and appropriate software.
Here we report first results of our "wcp-assay" in comparison to the normal conventional breakage test. Our data indicate that a successful FA diagnostic can be performed by using this assay. Additionally, we get more detailed insights in the involvement of specific chromosomes in the single breakage events, which are known as unbalanced translocation partners in FA-AML cells. Furthermore we expect a higher gain of information concerning the individual instability or genetic reversion of each FA patient over time. One goal of our study is to correlate these detailed instability data with the risk of emerging hematological complications (e.g. AML) for each FA complementation group.

P124

Monoallelic expression of the candidate tumor suppressor gene fls485 in uveal melanoma

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Uveal melanoma (UM) is the most common primary tumour of the eye. Approximately 50% of the patients die of metastases. Loss of an entire chromosome 3 (monosomy 3; M3) is strongly associated with metastatic disease. Comparing the extent of partial chromosome 3 deletions in UM, we have defined a candidate tumour suppressor gene region (SRO) in 3p25-26. In various tumours, recurrent loss of genetic material is part of a two step inactivation mechanism of tumour suppressor genes. Inactivation of the second allele is usually caused by point mutations, deletions or epigenetic silencing. Expression analyses had revealed genes on chromosome 3 that are only expressed in UM with D3 but silent in tumours with M3. One of these genes, fls485, is located within the SRO in 3p25-26, but no genetic alteration that might explain the loss of expression has been detected so far.
We have evaluated the possibility that fls485 is expressed only from one allele in UM precursor cells. Loss of the active copy in the course of tumourigenesis might explain the complete loss of fls485 expression in UM with M3. To test this hypothesis, we determined the expression ratio of both alleles in tumours with D3. We screened five chromosome 3 genes (fls485, LMCD1, CHL1, SYNPR, CNTN3) showing strongly reduced or loss of expression in tumours with M3 for transcribed SNPs. The expression ratio of both alleles was determined by primer extension analysis (SNaPshot) of cDNA from tumours with D3. Monoallelic expression of CHL1 was observed in one out of five (20%) informative samples. LMCD1, SYNPR and CNTN3 did not show unbalanced expression in any of the samples (1<ratio<2.5). In 4 out of 12 (33%) informative tumours, fls485 is expressed almost exclusively from one allele (ratio>10). These results suggest that monoallelic expression of fls485 in UM precursor cells contributes to its inactivation in UM.

P125

Identification of target genes of the leukemogenic CALM/AF10 fusion protein

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The t(10;11)(p13;q14), which is found in patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and malignant lymphoma, results in the CALM/AF10 fusion gene. Expression of the CALM/AF10 fusion gene in primary murine bone marrow cells leads to the rapid development of an aggressive leukemia in a mouse bone marrow transplant model. We analyzed samples from 20 patients with different types of acute leukemia and a t(10;11). The molecular analysis did not show any correlation between disease phenotype and the location of the breakpoints in CALM or AF10. The expression profile (Affymetrix) of ten of these samples was compared to expression data from normal bone marrow cells and 12 other defined leukemia subtypes. This analysis revealed high expression levels of BMI1, MEIS1 and several HOXA cluster genes. Interestingly, four of the most prominently upregulated genes in the CALM/AF10 samples are located immediately adjacent and centromeric to the translocation breakpoint on chromosome 10 band p12. These genes are DNAJC1, COMMD3, BMI1, and SPAG6. To determine whether this phenomenon was a position effect caused by the translocation itself, we analysed the expression levels of these genes in a mouse leukemia cell line in which the CALM/AF10 fusion gene is expressed from a retroviral construct. Bmi1, Commd3 and Dnajc1 but not Spag6 were found to be highly expressed in these cells suggesting that Bmi1, Commd3 and Dnajc1 are direct or indirect target genes of CALM/AF10. To study the direct target genes of the CALM/AF10 fusion, we established an inducible cell line model. Preliminary results show that 48 hrs after CALM/AF10 induction only a small number of genes is up or down regulated more than 2 fold. Surprisingly, after this short period of induction CALM/AF10 there is no correlation between differentially regulated genes seen in the patient samples and in the inducible cell line. We are in the process of looking at longer induction periods.

P126

Identification of epigenetically inactivated tumor suppressor genes in oligodendroglial tumors

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In contrast to the majority of astrocytic tumors, oligodendroglial tumors more frequently respond to radio- and chemotherapy and show a significantly better prognosis. Even though these characteristics are associated with loss of heterozygosity (LOH) on the chromosome arms 1p and 19q, the relevant genes and the underling molecular mechanisms are still unknown. There is now firm evidence, that epigenetic DNA-modifications, especially promotor hypermethylation constitute an abundant and effective mechanism for gene silencing. The purpose of this study was to investigate the role of differential methylation in oligodendroglial tumors. Therefore, we compared the methylation status of 7680 CpG-rich fragments of the human genome in 10 anaplastic oligodendrogliomas (WHO-grade III) with and without combined LOH on 1p and 19q by differential methylation hybridization (DMH) analysis. Using this approach, we identified 182 genes, a number of ESTs and mRNAs showing evidence of differential methylation between tumors with retention or loss of 1p and 19q alleles. So far, differential methylation of 42 candidate genes has been validated by sodium-bisulfite sequencing of anaplastic oligodendrogliomas. Expression analyses of 5 selected candidates by real-time reverse transcription-polymerase chain reaction and methylation analysis in an expanded series of 40 oligodendroglial tumors of different histological subtype and WHO-grade is presently ongoing.

P127

The EZH2 promoter and its affection by methylation assayed on reporter gene constructs

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The EZH2 gene is overexpressed in the majority of prostate cancers. We hypothesized hypomethylation as underlying mechanism for EZH2 upregulation, prior genomic amplification that is commonly observed in metastases. We analysed four pairs of tumor and adjacent normal tissues by bisulfite-sequencing of 204 CpG sites located in the GC rich EZH2 promoter (F0) and three flanking fragments (F1-F3). F1 and F2 were hypermethylated in the tumors whereas F3 was hypomethylated and F0 did not reveal a difference. In order to characterize the relevance of the diverging methylation patterns we examined various combinations of the EZH2 promoter with F1, F2 and F3 using a dual luciferase assay system. The unmethylated F1 and F2 both increased activity by 2.3 fold, while F3 repressed the promoter 3.4 fold. Methylated constructs generally decreased promoter activity compared to the unmethylated constructs to a tenth, an effect which reduced the promoter activity close to the baseline levels. However methylation of F1 and F2 did not significantly contribute to the observed repression as compared to promoter methylation itself. We conclude that methylation of F1 and F2 did not have much effect on the expression of EZH2 in the setting of our experiment. In a second step we investigated EZH2 regulation by members of the E2F family and further, if a given transactivation could be influenced by methylation. E2F2, E2F4 and E2F6 were cotransfected along with the promoter constructs. While E2F6 had no effect on the transcription, E2F2 and E2F4 were both capable to induce the EZH2 promoter (27 fold respectively 19 fold). Though several E2F consensus sites were predicted by in-silico analysis within F1, F2 and F3, E2F binding occurred only in F0, as revealed by combination with the SV40 promoter. Therefore, the observed hypermethylation in EZH2, which presumably is associated with a growth advantage in tumor cells and E2F transactivation are independent regulatory mechanism.

P128

RNAi-based cell arrays for high-throughput cancer research

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Application of the recently developed transfected-cell array technology in RNA interference-based functional analysis of pro- and anti-apoptotic genes will be presented. The study comprises issues of siRNA specificity, development of apoptotic signal detection assays, and siRNA multiplexing. Finally, application of cell arrays for apoptosis studies in different cell types will be discussed.

P129

Detection of chromosome aberrations in bone marrow and blood cells of Fanconi anemia patients by interphase-FISH and automatic scanning: Progress and case report

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Fanconi anemia patients have a high risk for bone marrow failure, aplastic anemia (AA), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and, later in life, for epithelial malignancies. The ongoing debate about the relevance of clonal aberrations in patients with Fanconi anemia prompted us to initiate a systematic, prospective cytogenetic follow-up in bone marrow and peripheral blood cells of FA patients. Our data reveal that gains of the chromosomal segment 3q26-29 and monosomy 7 are strongly associated with a poor prognosis and represent an adverse risk factor in FA. The overall survival of patients with 3q gains was extremely poor compared to FA patients without aberrations. None of 23 FA patients with 3q gains survived without HSCT. In this respect, it is noteworthy that the 3q region is also involved in multiple solid tumors indicating that its tumor promoting potential may be not tissue specific but generally involved in tumor initiation and/or progression. Out of 18 patients with 3q gains eight had an additional monosomy 7. In at least six of these eight patients the monosomy 7 has developed in the 3q aberrant clone as a secondary event. Our data imply that gains of 3q might increase the risk for developing a monosomy 7 subsequently. To detect clonal aberrations, we analyze interphase nuclei of patients bone marrow and peripheral blood with fluorescence in situ hybridization (I-FISH) using specific probes for the most common aberrations. Evaluation by an automated fluorescence microscope allows a high throughput and, thus, the analyses of many FA patients at regular intervals (6 months up to 1 year) ensures the early detection of adverse aberrations. Their occurrence suggest a very strong clinical care precaution: the finding of any chromosomal abnormalities, especially the abnormalities of chromosomes 3 and 7, warrant very close clinical follow-up, as they may signal the development of MDS or AML.

P130

Structural aberrations of chromosome 22q in T-PLL with inv(14)

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Approximately 80% of T-cell prolymphocytic leukemia (T-PLL) display an inv(14)(q11q32) or its variant, the translocation t(14;14)(q11;q32). These lesions are regarded as primary chromosomal aberrations in T-PLL and lead to an upregulated expression of oncogenes at the TCL1 locus. Additionally, T-PLLs show a highly characteristic pattern of secondary aberrations with frequent gains and losses, proximal and distal of chromosome 22, respectively. Neither any of the genes that are affected by chromosome 22 alterations, nor the underlying mechanisms promoting tumor progression are known. Therefore this study aimed at further characterizing structural aberrations on chromosome 22 in T-PLL. Affymetrix Human Mapping 50K analyses and Interphase-FISH in a panel of 12 cytogenetically characterized T-PLL with TCL1 break revealed deletions and gains on 22q in four cases and one case, respectively. The commonly deleted region extended from the centromere to 22q11.23. The distal borders of the deletion were not conserved, but scattered over a region of 11Mb within 22q11.23-22q12.3. One of the candidate genes in that region is the tumor suppressor gene CHEK2. However, a mutation screening of CHEK2 in the four T-PLLs by direct sequencing revealed no mutations. The gained region extended from 22q12.1 up to the telomere. In line with the pattern of centromeric losses and telomeric gain detected in T-PLL, gene expression profiling previously performed on the Affymetrix U133A platform (Dürig et al, submitted) showed an enrichment of genes with reduced expression in the centromeric and of genes with increased expression levels in the telomeric region. These findings suggest that the changes of expression might in part be caused by a gene dosis effect. The molecular mechanisms associated with deletion events on chromosome 22 underlying the pathogenesis of T-PLL need to be further explored.

P131

Leupaxin regulates cell adhesion and migration of prostate cancer cells via the ERK pathway and p120CTN expression

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Leupaxin is a cytoskeletal adapter protein belonging to the group III of LIM domain containing paxillin proteins. In a recent study we could show that leupaxin is differentially expressed in prostate carcinomas and that it interacts and transactivates the androgen receptor in a ligand-dependent manner. Its specific downregulation using RNAi leads to apoptosis in androgen-dependent LNCaP cells and to a reduction in invasiveness and migratory ability in androgen-independent PC-3 and DU 145 cells.
In the present report we could show that leupaxin interacts and transactivates the glucocorticoid and the progesterone receptor. But in contrast to the androgen receptor, these interactions are hormone-independent. Furthermore, leupaxin is involved in the ERK pathway. The downregulation of leupaxin expression in LNCaP cells re-

sults in a significant reduction of phosphorylation of MEK1/2 and ERK2, whereas PC-3 and DU 145 cells do not show any difference in the phosphorylation status of MEK1/2 and ERK1/2, respectively. In addition, no difference could be observed in the phosphorylation status of AKT neither in LNCaP nor in PC-3 and DU 145 cells. These results support the hypothesis that leupaxin triggers apoptosis in LNCaP cells specifically via the ERK signaling pathway. Moreover, after downregulation of leupaxin expression in LNCaP, PC-3 and DU 145 prostate carcinoma cells an upregulation of the cadherin associated protein p120CTN was detected. The overexpression of leupaxin in LNCaP cells using the Tet-On-System results in the downregulation of p120CTN expression. The protein p120CTN is known to be involved in cell adhesion and migration and is downregulated in 45% of prostate carcinomas.
In conclusion these results indicate that leupaxin functions in cell adhesion and migration of prostate cancer cells by influencing the ERK pathway and the expression of cell adhesion molecules.

P132

Optimization of the epigenetic marker MGMT as a tool for the molecular diagnostic of brain tumors

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Background: Resistance to chemotherapy is a major complication during treatment of cancer patients. Hypermethylation of the MGMT-gene alters DNA repair and is associated with longer survival of glioblastoma patients treated with alkylating agents. Methylation of MGMT is also found in several other cancer entities. Therefore MGMT plays an important role as a predictive epigenetic marker for chemotherapy resistance. To adopt this established correlation into a molecular diagnostic procedure, we compared different technologies with regard to their sensitive and reproducible detection of methylation in frozen and paraffin embedded tissues.
Method: DNA was extracted either from frozen or formalin-fixed, paraffin-embedded (FFPE) GBM samples. Following bisulfite treatment and PCR, sequencing of individual clones, COBRA, SIRPH, and Pyrosequencing was used to investigate altered methylation of MGMT.
Results: We identified most informative CpG positions in the promoter region discriminating glioblastoma DNA from normal brain tissues. The results of this comprehensive investigation were used for development of different assays to assess the methylation state at these positions. Finally, we compared these approaches with regard to their robustness, significance and reproducibility on frozen as well as FFPE clinical specimen.
Summary: Based on the results presented here we have established a robust, cost-efficient, and easy-to-use clinical diagnosis platform for the sensitive and quantitative assessment of MGMT methylation as a reliable clinical tool.

P133

FISH-, expression- and methylation analyses of p16 correlated with response to TMZ chemotherapy in patients with glioblastoma

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The clinical course of glioblastomas is mainly determined by the biological behavior of the tumor cells and their response to radiation and chemotherapy because a surgical cure is virtually impossible. Temozolomid (TMZ), a second-generation imidazotetrazine, increase the median survival time of patients with glioblastomas, especially those with deletions of the short arm of chromosome 9. The aim of our study was to investigate whether the tumor suppressor gene CDKN2A/p16 on 9p is responsible for the positive effect of TMZ of the survival time of patients with glioblastomas.
We used two-color FISH with a gene specific probe for CDKN2A in combination with a centromere probe for chromosome 9 on paraffin embedded tissues. Further on, we correlated the deletions of CDKN2A with p16 on protein level by immunohistochemical analyses. A possible inactivation of CDKN2A by hypermethylation of the promotor-region was analysed by MS-PCR. FISH analysis revealed in 8/19 cases homozygous deletions and in 10/19 cases heterozygous deletions of p16. Patients with homozygous deletions had a decreased survival time (9.2 months) in contrast to patients with heterozygous deletions (20.4 months). The immunohistochemistry was performed on 20 paraffin embedded tissues of glioblastomas. Patients treated with TMZ chemotherapy showed an increased survival time in univariate analysis when they express p16 (p=0,06). In the same way patients with cytoplasmic expression of p16 had an increased survival time in contrast to patients who express p16 in the cell nucleus. The MS-PCR revealed an inactivation of CDKN2A by promotor hypermethylation in 5/45 cases.
This study demonstrates that the loss of CDKN2A/p16 might be correlated with a worse prognosis. However, these patients seem to respond to TMZ chemotherapy in an increased survival time. Further on, patients who still express p16 showed evidence for an increased survival time, especially when they express p16 mainly in the cytoplasm.

P134

Common genetic pathways of tumor progression in retinoblastomas with and without biallelic RB1 inactivation

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Objectives: Inactivation of both RB1 alleles is a commonly accepted prerequisite for the initiation of retinoblastoma (Rb). Molecular genetic investigations allow for RB1 mutation detection in up to 96% of Rbs. In the few Rbs without detected RB1 mutations it is an open question if molecular changes were missed or are not present. It is conceivable that development of some Rbs is initiated by mutations in other genes than the RB1 and that progression of these tumors involves alterations of pathways distinct from those altered in Rbs initiated by RB1 inactivation. We therefore investigated the copy number changes (CNC) in unilateral isolated Rbs without biallelic RB1 inactivation.
Methods: Rbs of 31 patients with mutations detected in one or no RB1 allele were investigated using chromosomal comparative genomic hybridization (cCGH). The results were compared with previous cCGH findings in unilateral isolated Rbs with biallelic RB1 mutations.
Results: The median age at diagnosis was 24.7 months (range 1.1-112 months). CNC were observed in 26 cases (91%). The median number of CNC/abnormal tumor was 2 (range 1-11). Gains were most frequent at 6p (12 cases; 46%), 1q (8; 31%), 2p (5; 19%) and 13q (4; 15%), whereas losses were most frequent at 16q (6; 23%). In three of five cases with 2p gains high level amplifications at 2p22p25 were observed. In unilateral Rb with biallelic RB1 mutations with a median age at diagnosis of 20 months (2-91,7 months) the tumors showed CNC less frequently (73%), more CNC per abnormal tumor (median 3.5 CNC/tumor), and higher rates of gains of 6p (72.4%), of 1q (52.6%) and of 2p (27.6%), as well as of losses at 16q (47.4%).
Conclusion: cCGH analysis revealed no difference in the chromosomes involved in additional aberrations in Rb with and without biallelic RB1 inactivation. Our data do not support the hypothesis that there may be different pathways of tumor progression in Rb.

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P135

NUP214- ABL1 amplifications in adult T-cell acute lymphoblastic leukemia

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Objectives: NUP214-ABL1 amplifications can be found due to a cryptic episomal fusion of a 500 kb region of chromosomeband 9q34 in acute lymphoblastic T-cell acute leukemia (T-ALL)

blasts. Episomes are not detectable by conventional cytogenetics but by fluorescence in situ hybridization (FISH). The episomes occur in variable copy numbers due to unequal mitotic division. The NUP214-ABL1 fusion protein acts similar to the BCR-ABL1 fusion, which can be found in CML and is sensitive to tyrosine kinase inhibitors (TKI). Resistance against TKI has been described in a single case with NUP214-ABL1 amplification.
Methods: We screened a consecutive series of 73 T-ALL patients for NUP214-ABL1 amplifications by FISH using probes for the genes ABL1 and NUP214. The pattern of the FISH signals in 7 T-ALL patients with NUP214-ABL1 amplifications was investigated. The exons 4 and 6 of the ABL1 gene, which encode the p-loop, catalytic domain and activation loop of the ABL1 tyrosine kinase, were screened for mutations by denaturing high performance liquid chromatography (DHPLC) in 5 cases with NUP214-ABL1 amplification.
Results: A NUP214-ABL1 amplification was identified in 3 of 73 T-ALL patients (4%). A wide range of 5-50 signal pairs per cell were observed. The average number of amplified signals per case was 5-10. The proportion of cells with aberrant signals ranged from 1.7-86.7%. In none of the five cases with a NUP214-ABL1 amplification DHPLC analysis revealed a mutation in the ABL1 tyrosine kinase catalytic domain.
Conclusions: NUP214-ABL1 amplifications may be detected in less than 2% of T-ALL blasts by using FISH. The frequency of NUP214-ABL1 amplifications of 4% in T-ALL patients affirms data of previous studies which showed this abnormality in 2.5-6% of T-ALL patients. DHPLC screening revealed no evidence for ABL1 mutations. Thus, primary resistance against TKI may be due to the NUP214-ABL1 amplification itself.

P136

Screening of 180 epithelial ovarian carcinomas for mutations in the gene SPOC1

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy among women in developed countries, yet little is known about the molecular events that drive the initiation and progression of this disease. Especially for early diagnosis of EOC, prognostic factors, which may be used to identify groups of patients in whom more specific biological treatments or more aggressive therapy is indicated, are of great medical importance.
We have recently identified a novel human gene (SPOC1) which encodes a protein with a putative chromatin interacting PHD domain (plant homeo domain). We could show that expression of SPOC1 was clearly associated with worse prognosis in tumour tissue of patients with epithelial ovarian cancer. The median survival time was 1596 days for patients with low SPOC1 expression versus only 347 days for patients with high expression.
Since the present data suggested that SPOC1 functions as an oncogene in epithelial ovarian carcinogenesis, we therefore started to search for activating (gain-of-function) mutations in tumour tissues. Here, we report the screening of the complete coding sequence of SPOC1 in DNAs from 180 epithelial ovarian carcinomas. In total, we detected four heterozygous mutations resulting in an amino acid exchange. While three mutations were located in the N-terminal half of the SPOC1 protein, one mutation affected an evolutionary highly conserved amino acid in the C-terminal PHD domain. We are currently investigating the functional consequences of the mutations.
In any case, the results demonstrate that mutations in the coding sequence of SPOC1 are rare in epithelial ovarian carcinomas and that rather a disregulation of SPOC1 expression by changes in regulatory regions, by gene amplification or by mutations in upstream genes of SPOC1 occurs.

P137

Genetic heterogeneity in gliomas

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Gliomas display a wide range of histopathological features and biological behavior, and an inherent tendency to progress to a highly malignant phenotype. Molecular- and cytogenetic studies have delineated that different grades of gliomas correlate with specific genetic alterations. Glioblastomas, the most malignant form of gliomas, may develop de novo (primary glioblastomas) or through progression from low-grade or anaplastic astrocytomas (secondary glioblastomas).
Cell culture analysis may be biased by clonal selection artifacts. Homogenized tissue lacks control over the tissue composition and permits contamination of the tumor specimen with pre-existing and reactive nonneoplastic tissue. Moreover, gliomas exhibit a diffuse infiltrating growth pattern into normal brain so that no tumor area contains a uniform cellular composition. Genetic heterogeneity can hardly be detected by conventional methods. In order to evaluate an intratumoral genetic heterogeneity we performed FISH investigations and microdissection analysis in paraffin-embedded glioma tissue. Using this method we examined 124 tumor areas from 41 gliomas. Furthermore we correlate the cytogenetic data with the histomorphology of the given tumor areas.
Low-grade astrocytomas most often showed normal karyotypes, by conventional cytogenetic methods. However, we were able to identify numerous alterations in low-grade astrocytomas, especially in areas with a gemistocytic appearance. Primary glioblastomas and secondary glioblastomas showed consistant as well as different genetic findings, which correlate partial with the histomorphological features of the investigated areas. Our results provide clear evidence of inter- and intratumoral genetic heterogeneity in gliomas. In order to develop genetic prognostic criteria, the distinct genetic heterogeneity of these tumors should be considered.

P138

CFTR and SPINK1 mutations are not associated with pancreatic cancer in German patients

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, as well as mutations in the serine protease inhibitor Kazal type 1 (SPINK1) gene are associated with an elevated risk for idiopathic chronic pancreatitis. Chronic pancreatitis is a known risk factor for pancreatic cancer. Therefore, we hypothesised that CFTR and/or SPINK1 mutations may be associated with pancreatic cancer (PC). DNA was extracted from paraffin embedded tissue samples from 124 PC patients, 54 of which were classified as having had chronic pancreatitis. 33 of the most frequent CFTR mutations were analysed by an oligonucleotide ligation assay (OLA), polycrylamide gel electrophoresis (PAGE) and direct sequencing, covering 90% of cystic fibrosis (CF) alleles in the German population. Additionally, we sequenced SPINK1 gene exon 3 and flanking intronic regions for the detection of the most common mutations, e.g. N34S, R65Q and IVS3+2T>C.
Of 124 PC samples tested, one was compound heterozygous for F508del and I148T, one patient was heterozygous for the 5T (12TG) allele in CFTR intron 8, and seven patients were heterozygous for 5T (11TG). The distribution of CFTR mutations was not significantly different between PC patients with or without pancreatitis. The CFTR mutation/variation frequency in PC patients was even lower, albeit statistically not significant, in comparison to 136 healthy controls, mostly partners of CF patients or carriers. In the SPINK1 gene, heterozygosity for IVS3+2T>C was observed in one PC patient with pancreatitis, only. Compared to the expected SPINK1 mutation prevalence in the German population, no elevated frequency was observed.
We conclude that CFTR or SPINK1 mutations do not confer an elevated risk of pancreatic cancer.

P139

Data analysis of German Familial Pancreatic Cancer (FPC)

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Background: Familial Pancreatic Cancer (FPC) is an established hereditary tumour entity which accounts for approximately 2%-3% of all pancreatic cancer (PC) cases. First degree relatives of patients of FPC families have a high relative risk for PC. Germline mutations in CDKN2A and BRCA2 are recognized inherited risk factors. The practicability and efficacy of a clinical genetic risk assessment for FPC family members including genetic testing and risk adapted surveillance are currently being investigated in the German National Familial Pancreatic Cancer Case Collection (FAPACA).
Methods: Families presenting with a PC patient and at least one first-degree relative with pancreatic cancer or malignant melanoma (MM) are enrolled in FAPACA. Clinical data including ages at diagnosis of PC or other cancers were recorded in a three generations pedigree. First degree relatives of a PC or MM patient or family members carrying a CDKN2A or BRCA2 mutation were regarded as individuals at high risk for PC. All high risk family members over 40 years or 5 years younger than the youngest affected family member were offered a surveillance program for early pancreatic neoplasias which included endoscopic ultrasonography (EUS) and magnetic resonance tomography (MRT).
Results: Up to date 80 families have been enrolled in FAPACA. A total of 389 family members had a clinical genetic risk assessment of which 166 turned out to be at high risk for developing PC (40%). 53 high-risk family members underwent screening for PC. A total of 90 surveillances were performed. 32 individuals showed pancreatic lesions. Of four patients who underwent surgical exploration of the pancreas due to a suspected malignant process, two showed benign lesions, two had potential preneoplastic lesions.
Conclusion: Clinical-genetic analysis of FPC families provides a useful tool in the identification of patients at high risk for PC. The benefit of the risk assessment for the respective individuals has to be evaluated with more families being enrolled in the FAPACA and a longer period of the observation of family members being at risk for developing PC.

P140

Functional studies of unclassified variants of the human MMR protein hMLH1 in the yeast Saccharomyces cerevisiae

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The HNPCC-syndrome (Hereditary non polyposis colon carcinoma) is associated with germ line mutations in the „mismatch“ repair genes hMLH1, hMSH2, hMLH3, hMSH6, hPMS2 and hPMS1. Beside clearly pathogenic truncation or splice site mu-tations many missense mutations are found, whose functions usually remain unclear.
In the database of the German HNPCC consortium, all mutations found in hMSH2, hMLH1, and hMSH6 are registered. Many of the mutations are missense mutations of undefined pathogenicity (unclassified variants, UV).
In this study assays in the yeast S. cerevisiae were performed to determine the functional significance of specific amino acid replacements observed in HNPCC patients.
We examined missense mutations of the ATPase and the protein interaction domain of the hMLH1 protein. The functional consequences of such missense mutations have to be clarified experimentally in a yeast two hybrid assay to study the interaction between the proteins hMLH1 and hPMS2.
In the German database there are 25 UVs mapping to these domains of which four were already studied in a yeast two hybrid system. All of these mutations were not functional (Kondo et al. 2003).
Six of these UVs map to the ATPase domain and 15 map to the protein interaction domain of the hMLH1-protein.
In the yeast two hybrid assay between DNA binding domain fused hMLH1 constructs containing missense mutations and transcriptional activation domain fused hPMS2 either β- gal activity is shown, if mutations were not functional, or there is no β- gal activity.
In these experiments we could show that three mutations in the ATPase domain have a normal protein interaction. The results with the remaining three mutations are currently performed. Seven mutations in the interaction domain at the C-terminus show interaction, two have a reduced interaction and one shows no interaction. These results provide a first insight into the functionality of missense mutations in specific protein domains.

P141

Investigation of single nucleotide polymorphisms in putative NF1 modifying genes

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominantly inherited tumor diseases. Several symptoms of NF1 as the dermal neurofibromas show a high interfamilial variability. To explain this variability the existence of NF1 modifying genes has long been proposed. In a very rare variant of NF1, the familial spinal neurofibromatosis, patients have spinal neurofibromas but few or no dermal neurofibromas. The NF1 mutations found in some of these patients were also detected in patients with classical NF1. On the other hand, patients with microdeletions spanning the NF1 and several contiguous genes have an earlier onset and a higher number of dermal neurofibromas as classical NF1 patients. We suggest that one of the genes additionally deleted in these patients contributes to the increased number of neurofibromas and may be one of the NF1 modifying genes. Our expression studies in dermal neurofibromas revealed four putative NF1 modifying genes: CENTA2, UTP6, C17orf79 and RAB11FIP4. In these four genes, we will now investigate single nucleotide polymorphisms (SNPs) of the coding region (non-synonymous only) and the 3'- and 5'-UTR with a low allele frequency in the european population. Patients with familial spinal neurofibromatosis will be compared to classical NF1 patients with many dermal neurofibromas. First results will be presented.

P142

Analysis of deletions in 11p13, including the PAX-6 and WT1 gene using MLPA

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A deletion of the AN-gene, Pax6, which is located 700kb distal of the WT1-gene leads to Aniridia, a congenital malformation of the eye with a complete or partial absence of the iris. Newborn children with aniridia have a 30% risk for developing a Wilms Tumor (WT). Only those patients with a deletion encompassing both, the Pax6- and WT1-genes, have a higher risk to develop a Wilms tumor (WT).
In order to establish a faster and easier way to screen Anirida (AN)-patients for WT1 deletions, we performed a WT1 specific MLPA-analysis. In addition we have included several cases with different clinical features, with a high likelihood for the presence of a WT1 mutation. These cases were previously analyzed for WT1 gene mutations using SSCP and none were found. With this MLPA kit deletions of single or several exons of WT1 can be detected, which would escape the SSCP mutation analysis. We analysed a total of 28 cases and found that deletions in the WT1 gene are rare in sporadic Wilms tumor patients. However MLPA-analysis successfully detected deletions in patients with the WAGR-syndrome or patients with WT and GU without Aniridia. In this study, we could establish that MLPA is an easier way to screen AN-patients for WT1 deletions, in order to improve and simplify the diagnostical screening. In addition we were able to exactly determine the position of four WT1 deletions.

P143

Genetic characterization of anaplastic gliomas by FISH investigations on cryostate tissue sections

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Gliomas are the most common human brain tumors. The WHO divides them according their malignancy grade, but in the majority of studies no differentiation between high grade gliomas (grade III+IV) occurs. Our group has access to a large number of gliomas grade III to investigate in particular this tumor grade.
In total, we examined 37 patients with grade III gliomas, and 9 precursor lesions and recurrences, respectively. We performed two-color FISH on cryostate tumor tissue slides of all tumors with centromere-specific probes for chomosome 7 and 10 and locus specific probes for 1p36 and the telomere-region of 19q on par-

allel slides. The results were compared with the histopathological diagnosis and the patients progression-free and overall survival time.
Near all astrocytomas grade III (10/11) showed gains of #7 and losses of #10. Deletions of 1p36 were observed in half of the cases (6/11). All tumors showed deletions of 19q (11/11). Although oligoastrocytomas showed in nearly all cases gains of #7 (12/14) and losses of #10 (13/14). Furthermore, in 6/14 cases deletions of 1p36, and in all cases (14/14) deletions of 19q were detected.
Surprisingly, pure oligodendrogliomas showed besides their typical alterations - deletions of 1p36 (9/12) and the deletions of 19q (12/12) - also a very high proportion of tumors with gains of #7 (11/12) and losses of #10 (12/12). Statistical analyses showed no correlation between tumor entity, genetic alterations and prognosis. However, Kaplan Meier analysis showed a tendency for a better prognosis in oligodendrogliomas, compared with oligoastrocytomas and astrocytomas.
Our study revealed that all investigated chromosomal regions seem to play an important role in these three anaplastic tumor types and even if we observed a trend for better survival of pure oligodendroglial tumors, no correlation with the genetic alterations were detectable in our group of patients.

P144

Compound heterozygosity for two MSH6 mutations in a patient with early onset colorectal cancers, vitiligo and systemic lupus erythematosus

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Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC) is an autosomal dominant condition caused by heterozygous germline mutations in the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2. Rare cases of inherited bi-allelic deficiency of mismatch repair (MMR) genes associated with multiple café-au-lait spots, early onset CNS tumors, haematological malignancies, or early onset gastrointestinal neoplasia have been reported.
We report on a patient who developed a systemic lupus erythematosus at age 16, three synchronous colorectal cancers at age 17 years, and presents vitiligo in segments of the integument, respectively. Tumor tissues of the colorectal cancers revealed high microsatellite instability (MSI-H) and an exclusive loss of expression of the MSH6 protein. Immunohistochemical analysis of normal colon tissue revealed loss of MSH6 as well, pointing to a bi-allelic MSH6 mutation. Sequencing of the MSH6 gene revealed the two germline mutations c.1806_1809delAAAG;p.Glu604LeufsX5 and c.3226C>T;p.Arg1076Cys. To our knowledge both parents are not affected; they do not want to be tested for the mutations identified in their daughter.
Our data suggest that biallelic mutations of one of the MMR genes should be considered for patients who develop multiple HNPCC-associated tumors at young age and autoimmune disorders even in absence of haematological or brain malignancies.

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P145

Identifying conserved domains of thyroid adenoma associated protein (THADA) through characterization of homologous genes in selected vertebrates

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THADA, a gene which is involved in the development of thyroid benign tumours has been identified and characterized. The gene is localized within the chromosomal region 2p21 and rearranged in thyroid adenomas. Although the 2p21 chromosomal aberration accounts only for 10 % of the thyroid adenomas this rearrangement belongs to the most common rearrangements in benign epithelial tumours. Despite the relevance of this gene in tumour development almost nothing is known about the function of the gene product. No apparent homology to other human proteins has been identified so far. Therefore we have analyzed the genetic structure of THADA homologous genes in selected vertebrates (Cercopithecus aethiops, Mus musculus, Rattus norvegicus, Canis familiaris and Gallus gallus) , which are not characterized up to now. The coding sequences of the mRNA of all mentioned organisms have been sequenced. The genomic structures have been determined, except for Cercopithecus aethiops where no genomic sequence of this region is available. The gene is spanning 190, 000 bp in Gallus gallus and 410, 000 bp in Canis familiaris. The structure is conserved in all organisms and the reading frame is organized in 37 Exons. The lengths of the predicted proteins are all in the same range (1930 amino acids Gallus gallus and 1953 amino acids Homo sapiens). Using multiple alignments we identified the most conserved part of the protein (amino acids 1140 – 1380 Homo sapiens) with an identity of 73 % between the most different organisms indicating a putative important functional domain. We will use this region for producing a recombinant protein as antigen for polyclonal antibody production to have the possibility to gather more information regarding the function of this protein and its deregulation in thyroid adenomas.

P146

Four novel BRCA1 germline mutations in high risk breast/ovarian cancer families

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Familial aggregation of breast/ovarian cancer cases is frequently associated with genetic alterations in the BRCA1/2 cancer susceptibility genes. However, unless described in the BIC mutation database, the interpretation of some of these mutations as truly pathogenic mutations is sometimes difficult. Here we report four novel germline mutations in the BRCA1 gene, not previously reported in the BIC mutation database.
Methods: Prescreening of BRCA1 and BRCA2 was by denaturing high performance liquid chromatography (dHPLC) followed by direct sequence analysis of genomic DNA in families of German origin, fulfilling the criteria for familial breast and/or ovary carcinoma.
Results: We detected two novel frameshift and two novel splice mutations in the BRCA1 gene, not yet reported in the BIC mutation database: In exon 11 of the BRCA1 gene, we identified a heterozygous deletion of two base pairs at nucleotide 3472 (3472_3473delAG, Gln1118fsX1120) and a deletion of 17 bp at nucleotide 4193 (4193_4209del17, Glu1358fsX1361) leading to a premature stop codon. The third patient had a novel splice mutation resulting from a 10 bp deletion which includes the splice donor sequence of exon 13 (IVS13+1_+10delGTGTGTATTG). Another cancer prone family also displayed an intronic mutation (IVS7-7T>A) possibly affecting splicing. Functional consequences of the intronic mutations were demonstrated by analysis of mRNA, with RT-PCR products revealing heterozygous skipping of exon 13 (IVS13+1_+10delGTGTGTATTG). The second intronic mutation (IVS7-7T>A) could be shown to cause the creation a new splice acceptor site leading to a premature stop codon after 16 newly coded amino acids.
Conclusion: These four novel genetic alterations in the BRCA1 gene were identified as likely causal mutations in patients with familial of breast and ovarian cancer.

P147

Frequency of monoallelic MUTYH mutations in carriers of a MSH6 germline mutation

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Biallelic mutations in the base excision repair (BER) gene MUTYH are associated with multiple adenomas and an increased risk of colorectal cancer. The carrier frequency in the general population is about 1-2 %. Gu et al. (2002) reported on an interaction of MUTYH and mismatch repair (MMR) proteins. They showed that MUTYH enhances the binding affinity to the mismatched DNA substrate and the glycosylase activities of the MSH2/MSH6 complex. Mutations in MMR genes are known to cause hereditary non-polyposis colorectal cancer (HNPCC/ Lynch syndrome), an autosomal dominant tumor predisposition syndrome. Niessen et al. (2005) tested 76 carriers of germline mutations in MMR genes (25 in MLH1, 26 in MSH2 and 25 in MSH6) for additional mutations in the MUTYH gene and found a significantly higher rate of heterozygote MUTYH mutations among carriers of a MSH6 muta-

tion. They concluded that interaction of mutations in MSH6 and MUTYH leads to an increased risk for colorectal cancer.
To test this hypothesis we examined 74 MSH6 mutation carriers (42 pathogenic mutations and 32 variants of unknown pathogenic relevance) of the German HNPCC Consortium for MUTYH mutations. We sequenced the complete coding region of the MUTYH gene and identified the following variants each in one patient: the truncating MUTYH mutation c.247C>T;p.Arg83X and the missense mutation c.502C>T;p.Arg168Cys. No patient was found to have a biallelic MUTYH mutation. The frequency of MUTYH mutations among carriers of MSH6 mutations in our patient group was not significantly higher than in the general population. Our results do not confirm the findings reported by Niessen et al.

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P148

Repression of the human androgen receptor (AR) by corepressors (CoR) and new strategies to inhibit AR in prostate cancer cells using peptide aptamers

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The androgen receptor (AR) plays an important role in male development and prostate cell proliferation. AR is involved in various medical conditions like the androgen insensitivity syndrome, Kennedys disease, and also prostate cancer. Prostate cancer is one of the most often diagnosed malignancies in men and therefore a significant health problem. AR is activated by androgens and regulates the proliferation of prostate cancer cells. Prostate cancer is treated through the ablation of androgens and application of anti-androgens. These therapies are at first successful, but eventually prostate cancer proliferation becomes independent of androgens. Our group is investigating the molecular mechanism of the interaction between corepressors (CoR) with AR. Here we showed that the overexpression of CoR in LNCaP cells, a prostate cancer cell line, leads to a synergistic inhibition of cell growth in the presence of CPA, pointing towards an essential role of CoR in prostate cancer therapy. Crucially, AR can also be activated in a ligand-independent manner through the signal transduction machinery. We show that treatment of cells with 8-Bromo-cAMP, an activator of PKA, and expression of PKA leads to the release of CoR from AR. These activation pathways may contribute to androgen refractory prostate cancer proliferation. In this project we aimed to develop new strategies to permanently inactivate AR by identification of new binding motifs that interact with the anti-hormone-bound AR, that are independent of the signal transduction machinery. Fusion of these AR-interacting peptides to a potent silencing domain leads to novel CoR. In order to discover such binding motifs we have screened a peptide aptamer library in yeast. Subsequently, identified peptide aptamers were tested for their interaction with AR in mammalian cells. Finally the interacting peptide aptamers were fused to a potent silencers and one was identified to repress AR transactivation and prostate cancer cell growth.

P149

PLAG1 up-regulation in lipoblastoma through low-level amplification of a derivative chromosome 8 with a deletion del(8)(q13q22)

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Lipoblastoma is a rare benign tumour of adipose tissue affecting mainly children under three years of life. In lipoblastoma, the typical cytogenetic features are clonal rearrangements involving chromosomal region 8q11 – 13. The oncogene PLAG1 (pleomorphic adenoma gene 1) is located within this chromosomal region, on band 8q12. In recent reports it could be demonstrated that in lipoblastomas the PLAG1 gene is activated by "promoter-swapping". Herein, we demonstrate that in lipoblastoma the PLAG1 gene may also be activated by a low-level amplification. We report on a lipoblastoma with the karyotype
48~50,XX,del(8)(q13q22),+del(8)(q13q22)x3[cp12]. Subsequent FISH evaluation on uncultured tumour cells confirmed this result and demonstrated a low-level amplification of 8pter to 8q13 and 8q22 to 8qter. A partial monosomy was seen for the chromosomal region 8q13 to 8q22. CGH analysis revealed also a low-level amplification of partial regions of the chromosome 8. Furthermore, no other gains or losses were observed by CGH analysis. RT-PCR analysis showed that the PLAG1 gene is up-regulated in the tumour sample of the analysed lipoblastoma in contrast to normal fatty tissue with no PLAG1 expression. In conclusion our results demonstrate that low-level amplification is a further mechanism of PLAG1 activation in lipoblastomas.

P150

NBBS isolated from Pygeum africanum bark exhibits androgen antagonistic activity and prostate cancer cell growth

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Extracts from Pygeum africanum, Serenoa repens and Cucurbita pepo are used in the treatment of benign prostatic hyperplasia (BPH) and prostate cancer (PCa). The activity of the androgen receptor (AR) is known to control growth of the prostate. Here, we examined extracts of these plants for their antiandrogenic activity using an AR responsive reporter gene assay for drug discovery. Bioactivity-directed fractionation of this extract led to the isolation of N-butylbenzenesulfonamide (NBBS) indicating that extracts of the stem bark of P. africanum harbours androgen antagonistic activity. In many cases, mutations of the AR gene have a potent growth promoting role in PCa allowing the activation of AR by various ligands. We show here that the hot spot mutant of AR T877A can be inhibited by NBBS. Furthermore, the growth of PCa cells is strongly reduced by NBBS treatment, suggesting that NBBS can overcome PCa resistance based on AR mutations. Thus, this compound may provide a novel approach for the prevention and treatment of BPH and human PCa.

P151

Variant Ph-producing translocations and their consequences

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In the past few years Imatinib (Gleevec) became the drug of choice for patients with chronic myeloid leukemia (CML) and BCR-ABL tyrosine kinase activation. Almost all chronic phase CML patients show this single cytogenetic abnormality seen as the Philadelphia chromosome (Ph) in conventional cytogenetic karyotyping. The Ph translocation t(9;22)(q34;q11) results in the fusion of the BCR-ABL gene, leading to a chimeric protein with a constitutivly activated enzyme and the effect of a deregulated signal transduction pathway in the cells. Imatinib has the potency to inhibit these cells and to entail a cytogenetic and hematologic remission. Most of the patients in chronic phase under Imatinib therapy do not have severe side effects, do not reach blast crisis as fast as with alternative therapies, escape from high-risk bone marrow transplantation and show a longer overall survival.
But 2-10% of CML patients carry variant Ph translocations. There are simple and complex variants, the complex ones are of interest because of BCR-ABL involvement. The question arises: Do these patients also respond to the new targeted therapy with Imatinib? Or do the cells escape the treatment because of the chromosomal complexity?
In our Institute we collected samples of CML patients for 10 years and in cooperation with the Department of Haematology we have access to data about therapy and overall survival. Today the diagnosis of these chromosomal rearrangements and the involvement of BCR-ABL can be simply verified by commercial FISH probes. Here we would like to illustrate the interesting cases and compare the characteristics of the older samples and data before Imatinib with the current ones. For example in one case with a translocation between chromosomes 9, 22 and 13 and a coexistent BCR-ABL amplification up to six times, we would expect a poor response. Another patient attracted attention because he carries a variant t(2;9;22) for three years now.

P152

Clonal analysis of recurrent oligodendrogliomas and oligoastrocytomas

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We investigated the dynamics of genetic changes associated with oligodendroglioma (12 cases) and oligoastrocytoma (6 cases) recurrence. Tumors were analyzed for allelic loss 1p/19q (LOH 1p/19q) and for TP53 mutations. Oligoastrocytoma are mixed glial tumors with morphological and molecular features of oligodendrogliomas and astrocytomas. Oligodenrogliomas and astrocytomas differ in molecular alterations. While LOH 1p/19q is a frequent finding in oligodendrogliomas associated with a more favorable prognosis due to a better response to chemotherapy, TP53 mutations are more common in astrocytic tumors. Microdissection was used to elucidate clonal origin of oligodendroglial and astrocytic tumor components. The majority of oligoastrocytomas investigated were of clonal origin and genetic changes did not alter with recurrence. However, two cases of biphasic oligoastrocytomas revealed different genetic alterations in oligodendroglial and astrocytic tumor components, suggesting multiclonal origin in these tumors.
The pure oligodendrogliomas predominantly showed identical genetic alterations in primary and their recurrent tumors. Two tumors were significant for divertive molecular features. One oligodendroglioma WHO II with LOH 1p/19q in its primary lesion, revealed retained alleles for 1p/19q in its malignant recurrence after chemotherapy. Another case with LOH 1p/19q which recurred at a higher grade, displayed an additionally TP53 mutation in its recurrence only. In conclusion, our results suggest that oligoastrocytoma are predominantly of monoclonal origin. Although, a small subset of tumors may harbor morphologically and genetically distinct tumor cell clones. The loss of LOH 1p/19 after treatment with chemotherapy may be suggestive for selectively eliminating the subclone sensitive to treatment.

P153

Novel BMPR1A gene mutations in Polish JPS patients

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The Juvenile Polyposis Syndrome (JPS), OMIM 174900 is an autosomal dominant hamartoma polyposis syndrome predisposing to gastrointestinal cancer. Autosomal dominant inheritance of the disease is linked with the mutations in one of the tumor suppressor genes MADH4 (mothers against decapentaplegic, drosophila, homolog of, 4) or BMPR1A (bone morphogenetic protein receptor, type 1A). The JPS is characterized by the occurrence of juvenile polyps in colon and upper parts of the gastrointestinal tract, the average frequency of JPS is 1:100000. The polyps occurring in childhood in most are the hamartoma polyps. The solitary or not numerous and self-limited polyps are not associated with the genetic predisposition. The characteristic feature of the occurrence juvenile polyps is rectal bleeding what is an indication for further diagnostics. The JPS encompasses the cases of numerous polyps and/or a familial component. The characteristic feature of the juvenile polyps are a markedly expanded lamina propria containing dilated cystic glands, inflammatory cells, and prominent stroma with a normal overlying epithelium.
The five juvenile polyposis patients were diagnosed in specialized clinics. The recognition of the JPS syndrome in 3 cases was based on observations over 100 juvenile polyps in colon and in two cases on observation over 5 juvenile polyps in colon. The entire coding sequences of the MADH4 and BMPR1A genes were sequenced by direct PCR product sequencing. The novel BMPR1A gene mutations were detected in three JPS families.
The study was financed by the Ministry of Education and Science, Poland, grant number 2PO5E02630

P154

A closer look at Warthin's tumors in respect to the t(11;19)(q21;p13) translocation

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The translocation t(11;19)(q21;p13) first has been described in our laboratory and is known to occur in both mucoepidermoid carcinomas (MEC) and rarely for Warthin's tumours (WT), both tumours occurring in the salivary glands. Recently, the breakpoint was cloned and the fusion gene TORC1 (also known as MECT1)-MAML2 resulting from this translocation was identified.
Generally, WTs and MECs are two salivary gland tumors that do not share clinicopathologic features, and as widely accepted their histogenesis is also different. However, metaplastic WTs are characterized by replacement of a large amount of the formerl epithelium by metaplastic squamous cells, along with regressive changes in the stroma areas. Therefore, misinterpretation of metaplastic WTs for malignancy like squamous epithelial carcinoma is more likely.
We have screened a total of 48 WTs for the occurrence of the translocation t(11;19)(q21;p13) by using FISH and RT-PCR. A MECT1-MAML2 fusion transcript was detected only in two WTs, both metaplastic variants.
Indeed, our experience and published observations of the t(11;19)(q21;p13) translocation in WTs reveal that only a small subset of WTs are positive, and that these tumors are often classified as infarcted/metaplastic WTs

P155

AML with overexpression of C8FW and MYC

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In the present study a case with AML with 8q24/MYC amplification in the form of an additional ring chromosome was analyzed in detail. Beside the additional ring chromosome no other chromosome aberration was found. FISH using a LSI MYC probe showed high amplification on the additional ring chromosome (>20-fold) and signals on both chromosome 8 homologues. RQ-PCR analysis revealed that MYC and C8FW, a gene localized also on 8q24 close to MYC, were clearly overexpressed when compared with the expression level in 3 healthy donors, 3 AML patients without MYC amplification, 3 patients with CML and 3 patients with ALL. These results may indicate an important role of MYC and C8FW overexpression in AML. The observation of C8FW overexpression is in agreement with a description of 2 cases published by Storlazzi et al. (Hum Mol Genet 2004,13:1479-1485) and with a high-resolution genome-wide screening approach of genomic imbalances by Rücker et al. (J Clin Oncol 2006,24:3887-3894). In contrast, in another publication by Storlazzi et al. (Hum Mol Genet 2006,15:933-942) the authors suggest that neither MYC nor C8FW may be the critical gene, since MYC was overexpressed in 0 of 9 investigated cases and C8FW was overexpressed in only 2 of 9 cases. We suggest, that the discrepancy in this study might be explained by different reasons. First, RNA seemed to be of very low quality in 5 of 9 investigated cases. Second, northern blot is less sensitive then RQ-PCR. Third, 5 of 9 cases showed highly complex chromosome aberrations indicating advanced disease. Therefore, to our opinion, a potential role for C8FW and even MYC at tumor initiation cannot be excluded. In conclusion, we present a case with AML and high amplification of 8q24/MYC as sole detected genetic anomaly resulting in overexpression of C8FW and MYC. Therefore we suggest that overexpression of C8FW and even MYC may have an important role in the pathogenesis of certain subtypes of AML.

P156

Search for common ancestry of the MLH1 c.2141G>A mutation: a haplotype study of 5 Swiss HNPCC families

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Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common inherited cancer syndromes, accounting for 3-5% of all cases of colorectal cancer. Most HNPCC families

result from germline mutations in the DNA mismatch repair genes MLH1 and MSH2. In some populations founder mutations explain a substantial fraction of HNPCC, although this situation has rarely been reported. In Switzerland, the MLH1 c.2141G>A (p.W714X) mutation has been initially identified in one large Swiss kindred in 1996.
We report here a comprehensive clinical and molecular study of five Swiss HNPCC families harbouring this truncating mutation. All these families originated from the same Swiss alpine region and no common ancestor has been identified over 8 generations by genealogical study. Within the 5 families, 41 individuals carrying the MLH1 c.2141G>A mutation and 15 obligate carriers have been identified. Thirty individuals are affected (20 males, 10 females) and 21 are asymptomatic; 5 of the obligate carriers have no recorded medical history. Colon cancer was diagnosed in 25 individuals, and 5 affected persons had extracolonic primary tumours (2 endometrium, 1 stomach, 1 ureter, 1 leukaemia). Ten patients showed metachronous tumours other than colorectal cancer. One family seems to demonstrate an overall lower penetrance.
In order to document the common ancestry of this MLH1 alteration we performed a haplotype analysis in 2 to 4 individuals per family using polymorphic microsatellite markers within and near the MLH1 gene. First results show a shared three-marker haplotype in the 5 families spanning 0,58Mb (D3S1561, MLH1+250, D3S1611). At least one recombination event appears to be located at a maximum distance of 1,8Mb telomeric and 0,37Mb centromeric from the shared haplotype.
We will present a refined haplotype analysis in order to infer the age of this founder mutation.

P157

Familial adenomatous polyposis (FAP) in Poland –present state of research

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Familial adenomatous polyposis (FAP) is an autosomal dominant, predisposed disorder that results in the development of numerous polyps in the colon and rectum, usually beginning in childhood or adolescence. Other extracolonic features may include polyps in the upper parts of the gastroenterological tract, desmoid tumours, ocular lesions, osteomas, dental abnormalities, and malignancies in other organs. FAP incidence is estimated at 1:10,000.
FAP arises due to germ line mutations in the adenomatous polyposis coli (APC) gene, which was first described in 1991. This gene consists of 8.529 bp open reading frame and is predicted to encode a 2.843 amino acid protein. The APC protein acts as an inhibitor of cell proliferation in the Wnt signal cascade via the degradation of β-catenin, a transcription-activating protein that promotes transcription of the protooncogenes c-myc and cyclin D1. Most APC gene mutations are categorized as small deletions or insertions, and single base substitutions constitute 26-38% of detected mutations. A total of 92% of APC gene mutations lead to translation termination of the APC protein.
Seven hundreds DNA samples from persons from 280 FAP Polish families were collected. 380 patients were diagnosed with FAP, 215 persons belong to risk group and 67 persons excluded from the risk group during this study. The entire APC gene was screened for mutations in 240 families. The APC gene point mutations were identified in 115 FAP families. Thirty six of them have not been described before. Seven mutation types recurred two or more times. The recurrent mutations were detected in 52% of diagnosed families. The large rearrangements of the APC gene were studied in 95 FAP families without the point mutation. In this group we identified 24 large APC gene rearrangements with two cases of whole APC gene deletions.

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P158

Characterization of a constitutional translocation t(6;9)(p12;p24) in a patient with B-CLL

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Several gene loci have been described to be constitutionally altered in patients with hematologic neoplasms. Mutation of tyrosine kinase genes like Janus Kinase 2 (JAK2) located in 9p24 is a frequent event in such cases. We report on a 71 years-old man with B-CLL and a translocation t(6;9) in his diagnostic bone marrow. Subsequent chromosome analysis of his blood lymphocytes revealed a constitutional translocation 46,XY,t(6;9)(p12;p24) that has not been previously reported. The patient refuses examination of other family members. His daughter and granddaughter are notified with an apparently inconspicuous phenotype. Seeking for potential gene disruption correlating with the B-CLL we mapped both breakpoints of this translocation. For FISH analysis we used 15 BACs specific for 6p12 and 12 BACs for 9p24. Split signals indicating a breakpoint spanning clone were observed with RP11-399A15 and RP11-147I11. Thus the revised karyotype is: 46,XY.ish t(6;9)(p12.1;p24.3)(RP11-399A15sp;RP11-147I11sp). To narrow down both breakpoint regions (BPs) 4 subfragments of the BP-spanning clones were generated by Long Range PCR (Roche Expand Long Template PCR System). An 11kb PCR fragment derived from RP11-399A15 was found to span the 6p12.1 BP. FISH analysis with a 12kb long range PCR fragment from RP11-147I11 which overlaps with RP11-110M16 as well as with a cDNA clone for DMRT2 (doublesex and mab-3 related transcription factor) maps the 9p24.3 BP max. 10kb upstream from DMRT2. According to our in silico analysis JAK2 is located ~ 4Mb away from the 9p24 BP and, like other known genes, appears to be not disrupted by the translocation. Long range effects on JAK2 expression can not be excluded so far. In the area of interest we identified few putative candidate genes, gene predictions as well as several ESTs. Further studies will focus on sequencing both BPs and flanking regions as well as on functional analysis of candidate genes such as e.g. DMRT2.

P159

A novel germline mutation (p.Arg552Gln) of the RET protooncogene in a patient with MTC

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Introduction: Familial medullary thyroid carcinoma (FMTC) and multiple endocrine neoplasia type 2 (MEN2) are autosomal-dominant inherited diseases caused by germline mutations within the RET protooncogene. Until now genetic testing for mutations of exon 10, 11, 13-16 was recommended for these patients, because the majority of the patients showed mutations in these “hot-spots”. Furthermore in some cases mutations in exon 8 are known as the cause of the disease.
Patient: The index case is a 69-year-old patient from Germany, who was suffering from struma nodosa. His medical history showed that a hemithyroidectomy was performed three years ago and postoperative elevated levels of calcitonin.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of exons including corresponding exon-intron boundaries, known as hot-spots for mutations within the RET protoonkogene (exons 10, 11, 13-16) and exon 8 that seems to be a novel hot-spot for MTC. PCR products were sequenced directly.
Results: Sequence analysis of exon 10, 11 and 13-16 of the RET protooncogene did not show any disease-causing mutation. Additional analysis of exon 8 revealed a novel heterozygous germline mutation, p.Arg552Gln. Further investigations were performed to clarify the clinical relevance of the mutation and we were able to exclude this mutation in a healthy control group of 100 individuals.
Conclusion: Genetic counseling and careful genetic testing of the RET protooncogene including exon 8 should be done in families of “sporadic” MTC cases as well as in families where FMTC is known, to confirm the diagnosis and define presymptomatic gene carriers.

P160

Cytogenetic and molecular biological characterization of an adult medulloblastoma

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Medulloblastoma (WHO grade IV) is a malignant, invasive embryonal tumor which mainly occurs in children and represents less than 1% of all adult brain tumors. Therefore comprehensive cytogenetic and molecular biological data on adult medulloblastomas are very limited. Since conventional therapies provide disappointing long-term disease control new therapeutic options are being tested.

We performed comprehensive cytogenetic analyses of an adult medulloblastoma, WHO grade IV, using trypsin-Giemsa staining (GTG-banding), multicolor fluorescence in situ hybridization (M-FISH), and locus-specific FISH complemented by molecular karyotyping using high density SNP-arrays. GTG-banding of 25 metaphases revealed 31 structural chromosomal aberrations, predominantly located on 4q, 9q, 10q, 11p, and 20q, which were confirmed by M-FISH analysis. Interestingly, we found two novel, so far not described translocations, t(4;11)(q25;p15) and t(9;20)(p23;p12). Using GTG-banding, locus-specific FISH, and M-FISH we detected frequent numerical changes of chromosomes 8, 14, 18, 19, 20, 21, and 22. Molecular karyotyping by SNP-array confirmed the chromosomal changes +4q, +9q, -10q, and –11p, and revealed, for medulloblastoma, de novo partial uniparental disomy (UPD) 1q and 9q. Primary medulloblastoma cells were resistant to TRAIL, a novel anti-cancer cytokine, but could be efficiently sensitized by co-treatment with the proteasome inhibitor Bortezomib.

Applying complementary methods for cytogenetic analysis we detected novel chromosomal aberrations in medulloblastoma. Bortezomib/TRAIL co-treatment may serve as a powerful therapeutic option for medulloblastoma patients.

P161

Double minutes with C-MYC amplification in a case of AML-M4

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Among the acquired molecular genetic abnormalities occurring in, and characterizing, hematologic malignancies, most studies have focused on translocation-generated fusion genes, activating point mutations in oncogenes and inactivated tumor suppressor genes. Less is known about neoplasia-associated genes that may be amplified. Cytogenetic hallmarks of genomic amplification are homogeneously staining regions and double minutes (dmin), latter being small, paired chromatin bodies without a centromere and represent a form of extrachromosomal gene amplification. Although dmin have been found in a variety of solid tumors, their presence in hematological malignancies, especially acute myeloid leukemia (AML), is quite rare (≤ 1%). Here we present the results of cytogenetic and molecular cytogenetic characterization of clonal aberration present in peripheral blood cells of a patient with AML-M4. GTG-banding showed a normal karyotype with 15-30 dmin in almost 90% of the metaphases. To further characterize the dmin, FISH studies using probes for 8q24, 11q23 and 14q32 were performed. FISH results revealed normal signal pattern for the used probes but demonstrated the dmin to be C-MYC amplicons. Comparison of signal intensity on both chromosomes 8 and the dmin supposed a high amplification rate of C-MYC on the dmin. Due to the rarity of dmin in malignant hematologic disorders, their clinical and prognostic impact has not been well elucidated. In fact, AML patients with complex karyotypes harboring dmin have a dismal outcome, whereas those with an otherwise normal karyotype or with a single chromosomal aberration in addition to numerous dmin seem to fare better. It has been suggested that dmin in AML are associated with reduced responsiveness to chemotherapy and hence a poor prognosis. However, to prove if there is a prognostic impact, it is necessary to perform conventional cytogenetic investigations as well as FISH analyses in all AML patients prior to chemotherapy.

P162

Analysis of ATM gene mutation in brain tumors

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ATM is a recessive gene, defected in Ataxia telangictasia patients. Affected patients face with many different solid tumors such as Brain tumors; but in this case few reports showed ATM involvement in Brain tumors of patients who are not affected with Ataxia telangictasia. DNA was extracted from 50 patients affected with brain tumors without any clinical features of A-T disease. PCR was performed for two hot spot exons of ATM gene (Theses two exons was showed polymorphic in one study for involvement of ATM in medulloblastoma.). SSCP method and sequencing was carried out for these products .Our current study includes 50 patients affected with Brain tumors ; 46% (23) of patients were female and 54% (27) were male. About the types of the tumors 38%(19) affected with meningioma, 12% (6) GBM, 18%(9) astrocytoma ,8%(4) schwannoma, 4% (2) oligodendroglioma, 2% (1) hemangioblastom, 4% (2) medulloblastoma, 2%(1) chordoma, 2%(1) optic gliom , 2%(1) neurofibrom, 2% (1)Craniopharyngioma, 2%(1)esthesioneuroblastoma. The mean age of the patients is 42 years, 20% (10) of patients have family history of other cancers and 80% (40) of them don't have any family history of cancer. 18% (9) of patients have positive parent consanguinity and 82% (41) have negative parent consanguinity. In the case of metastasis from other tumors just 4% (2) of patients have Brain tumors as the metastatic form of other tumors and 14% (7) of these patients had recurrence of Brain tumors. We could detect two polymorphisms (D1853N &F858L) in 6 patients affected both exons includes meningioma and chordoma. Few reports showed ATM gene involvement in Brain Tumours of non affected A-T patients. In one study researches found theses two polymorphisms (D1853N &F858L) in medulloblastoma but this study showed these two polymorphisms in two types of Brain tumors not related to medulloblastoma. These results may show more effects of these polymorphisms in pathogenesis and therapy of Brain Tumors.

P163

A new complex translocation event described in a case with chronic myelogenous leukemia involving BCR/ABL: t(6;9;22)(q11;q34;q11)

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A translocation t(9;22)(q34;q11) is evident for more than 95% of patients with chronic myelogenous leukemia (CML) and gives rise to the Philadelphia chromosome. Approximately 5%-10% of CML patients show variant translocations involving other chromosomes in addition to chromosomes 9 and 22. Here we report a CML case showing a complex translocation event t(6;9;22). FISH analysis using chromosome specific multicolor banding (MCB) probe sets precisely characterized chromosomal breakpoints as t(6;9;22)(q11;q34;q11). The presence of the BCR/ABL fusion gene was confirmed on the der(22) using M-BCR/ABL probe (Q-Biogene). In addition to this presumably primary complex translocation event, secondary chromosomal aberrations were found: using M-FISH a trisomy 8 and an isochromosome (17)(q10) were verified. To the best of our knowledge the t(6;9;22)(q11;q34;q11) is not yet described in literature, previously. The nature of the chromosomal breakpoint in 6q11, involved in this rearrangement, remains unknown. According to the literature variant rearrangements of Ph chromosome do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph chromosome, but revealed additional aberrations in the case have a poor prognosis.

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Fluorescence in situ hybridization (FISH) analysis of the high mobility group gene HMGA2 on formalin-fixed, paraffin-embedded thyroid neoplasias

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Epithelial tumors of the thyroid are cytogenetically well-investigated tumors. Follicular thyroid adenomas (FTAs) are characterized by cytogenetic subgroups including trisomy 7 as well as rearrangements either of chromosomal band 19q13.4 or chromosomal band 2p21. In subsets of follicular thyroid carcinomas (FTCs) a translocation t(2;3)(q13;p25) results in the fusion of the nuclear receptor domains of peroxisome proliferator-activated receptor gamma (PPARγ) and the DNA binding domains of the paired-box gene 8 (PAX8). Beneath the FTAs and FTCs chromosomal aberrations are also described for papillary thyroid tumors (PTCs). The most common chromosomal aberrations found in PTCs are rearrangements involving 10q11.2. The rearrangements including inversions as well as translocations leading to the fusion of the tyrosin kinase domain of proto-oncogene RET located within 10q11.2 and the 5' portion of different genes.

Chromosomal rearrangements of 12q13-15, involving the high mobility group AT hook 2 gene (HMGA2), were observed in benign human mesenchymal tumors. For another member of the HMGA proteins, HMGA1, a re-expression was observed in malign human tumors, i.e. thyroid carcinomas. Re-expression of HMGA2 due to amplifications and/or rearrangements was analyzed in human prolactinomas.

To observe whether HMGA2 is involved in thyroid neoplasias due to amplifications and/or rearrangements we performed fluorescence in situ hybridization (FISH) analysis on 12 formalin-fixed, paraffin-embedded thyroid tissues involving FTAs, FTCs, PTCs and anaplastic thyroid carcinomas (ATCs). The hybridization mixture contains a HMGA2 specific probe and a chromosome 12 alpha-satellite probe. For each case at least 200 nuclei were analysed. Although we did not find rearrangements of HMGA2 a polysomy 12 was observed in 5 of 12 analysed cases (2 ATCs and 3 FTCs).

P165

A rare MLL-rearrangement t(11;17)(q23;q11.1) with deletion of the terminal part of MLL-locus in acut myeloid leukemia

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Translocations involving the mixed lineage leukemia (MLL) gene at 11q23 have been implicated in acute myeloid leukemia (AML), as well as acute lymphoblastic leukemia (ALL). Such translocations result in gain of function fusion proteins that drive cell proliferation . Rearrangement of MLL gene have been associated with a poor prognosis. We report a case of de novo AML-M4 (FAB). Using both conventional and molecular cytogenetic methodes , we found a new case of MLL rearrangement t(11;17)(q23; q11.1) with deletion of the terminal part of MLL locus. The partner gene of the fusion is still unknown. Further studies will be necessary to identify the fusions gene of the translocation. Clinical and cytogenetic data will be presented and discussed.

Molecular and Biochemical Basis of Disease, Developmental Genetics, Neurogenetics

P166

Digenic inheritance in a genetically homogeneous disease and a new mutational mechanism with mutations in a transcription factor and its regulated gene?

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Autosomal recessive polycystic kidney disease (ARPKD) is genetically homogeneous with mutations in PKHD1 on 6p12. We present a patient heterozygote for the novel PKHD1 1-bp deletion c.1151delA (p.P383fs), while a second PKHD1 change was not identified.

The transcription factor HNF1ß has been recently shown to be crucial for kidney development, as demonstrated by in vivo experiments where Xenopus embryos microinjected with mutant HNF1ß RNA transcripts had impaired pronephros development. HNF1ß binds specifically as homo- or heterodimer in concert with HNF1α to the proximal PKHD1 promoter and stimulates gene transcription. Recent studies revealed that mutations of the HNF1 binding site, expression of a dominant-negative HNF1ß mutant and mice with renal-specific inactivation of Hnf1ß exhibit a drastic defect in PKHD1 expression.

Thus, we hypothesized that the second disease allele in our patient may reside in the HNF1ß gene. Mutation analysis revealed the novel, non-conservative missense change c.244G>A (p.D82N) in exon 1 of the HNF1ß gene that affects an evolutionarily highly conserved aspartic acid residue and considerably disturbs the protein's DNA binding domain. This change was not present among 500 tested chromosomes. Another argument of its pathogenic character is the conservation of p.D82 in its counterpart HNF1α. Reporter gene analysis is currently underway to further corroborate the functional significance of this change.

Beyond the first description of digenic inheritance in ARPKD, our study is of general interest as it demonstrates an intriguing new regulatory mutational mechanism with heterozygous mutations in a transcription factor and its activated gene.

P167

Revertant mosaicism in Fanconi anemia: natural gene therapy at work

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The restoration to normal (or close to normal) function of a gene previously inactivated by a constitutional mutation is refered to as "reversion". The coexistence of reverted and wildtype cells within otherwise isogenic organisms is refered to as "revertant mosaicism." This phenomenon, also refered to as "natural gene therapy" has been observed in a number of inherited diseases, including epidermolysis bullosa, tyrosinemia type I,Wiskott-Aldrich syndrome, X-linked severe combined immunodeficiency, ADA-deficiency, Bloom syndrome, and Fanconi anemia (FA). We estimate that between 15 and 25% of FA patients will develop revertant mosaicism during their lifetime. Depending on the developmental stage and the cell lineages affected by the reversion event the haematological situation of mosaic FA patients may improve over time. Molecular mechanisms of reversion include back mutation, intragenic recombination, gene conversion, and second site (compensatory) mutations. Since reverted peripheral blood cells no longer show FA-typical chromosomal instability or cell cycle alterations diagnosis of revertant mosaicism requires proof of elevated sensitivity of skin fibroblasts towards bifunctional alkylating agents such as DEB or MMC. We present the natural history, diagnostic assessment, and molecular analysis of a cohort of mosaic FA patients that have been followed by our laboratory for a number of years. Revertant mosaicism was only noted in patients of subtypes FA-A, FA-C, FA-D2 and FA-L. Most mosaic patients were compound heterozygotes.

Compensatory second site mutations and back mutation or gene conversion were the most frequent molecular mechanisms of reversion. Improvement of blood counts over a time course of 3 to 6 years was noted only in patients with proven or presumptive multi-lineage reversions, whereas reversions limited to precursor cells of the lymphoid cell lineage did not change the natural history of the disease.

P168

The intracellular localization of ataxin-3, the affected protein in spinocerebellar ataxia type 3, is controlled by localization signals

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Spinocerebellar Ataxia Type 3 (SCA3), also known as Machado-Joseph disease (MJD), is an

autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene encoding a polyglutamine repeat in the respective ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases.
Ataxin-3 is mainly localized in the cytoplasm. However, one hallmark of SCA3 is the formation of ataxin-3 containing aggregates in the nucleus of neurons. In addition, we recently demonstrated in vivo using transgenic mouse models that nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3 and that cytoplasmically localized ataxin-3 is not able to induce a phenotype even with a very high number of polyglutamine repeats.
Up to now it is not known why and how ataxin-3 leaves the cytoplasm and translocates into the nucleus. The identification of nuclear localization (NLS) or nuclear export signals (NES) within ataxin-3 would make an important contribution to the answer of this question. Recently, using computerized comparison with known sequence profiles, ataxin-3 was proposed to contain both one NLS and one NES. We screened ataxin-3 in silico for additional signals and performed intracellular localization studies of putative localization signals in tissue culture. Using different assays, we could identify two NES and one NLS within ataxin-3. Mutating crucial amino acids inactivated the functionality of these signals. In addition, we could demonstrate that the inactivation of these signals influence the formation of intranuclear inclusion bodies: Inactivating the NES increased the number of aggregates whereas the inactivation of the NLS reduced their number. This data demonstrates the importance of the identified signals for the pathogenesis in SCA3, helps to understand the intracellular localization of ataxin-3 and might lead to novel possibilities for therapeutically intervention.

P169

Timp3-deficient and Timp3-S156C knock-in mice as models to study the role of TIMP3 in VEGF-mediated angiogenesis

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Introduction: TIMP3 is an extracellular matrix protein known to inhibit various proteases (e.g. MMPs, ADAMs). In addition, it competes with VEGF ligand binding to VEGFR2. TIMP3 mutations are associated with Sorsby fundus dystrophy (SFD), a late-onset macula disease characterized by drusen and choroidal neovascularisation. Here we studied the effect of TIMP3 deficiency and mutated TIMP3 on VEGF-mediated angiogenesis using two gene-targeted mouse models.
Methods: Mice were analyzed by scanning laser ophthalmoscopy, light and electron microscopy. Angiogenesis was monitored ex vivo by aortic ring and fibrin bead assays using aortic explants and purified primary aortic endothelial cells (EC), respectively. Competitive ELISAs were performed to study TIMP3/VEGFR2 binding. Intracellular VEGFR2 pathways were analyzed by Western blots.
Results: TIMP3 null but not TIMP3 knock-in mice revealed severe vessel dilatation in the retinal choroid. A strong angiogenic response was found in TIMP3-deficient aortic explants and ECs while only mild effects were observed in Timp3-S156C. This effect could be reversed to a level comparable to wt by the addition of rTIMP3. Conversely, addition of rVEGF to wt explants led to an angiogenic response similar to the null situation. ZM323881, a synthetic VEGFR2 inhibitor suppressed angiogenesis in null and VEGF stimulated wt explants. Enhanced angiogenesis was shown to be mediated by downstream VEGFR2 signalling via the ERK1/2 MAPK pathway.
Conclusion: TIMP3 ablation leads to an enhanced VEGF-mediated angiogenesis resulting in abnormally enlarged choroidal blood vessels. The moderate pro-angiogenic effect of missense mutation S156C is not sufficient to cause vessel abnormalities in the retina.

P170

Foundermutation in GNE (UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase) causes autosomal rezessive distal myopathy in Romani (Gypsies)

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Mendelian disorders are common in Romani (Gypsies) due to frequent Founder mutations; we present a new Founder mutation causing an autosomal recessive distal myopathy with age of onset in the early adulthood at the anterior compartment of lower legs in 27 patients (15 families). Twelve patients had also cardiac involvement mostly by cardiac rhythm disorder, leading to sudden death in 4 cases. This feature seems to co segregating with the myopathy. Immunohistochemistry showed normal expression of laminin $\alpha 2$,$\beta 1$, $\beta 2$, $\gamma 1$, α-DG, β-DG, α-β-γ-δ-SG, dysferlin and collagen VI. We identified a locus of 2.95 cM between D9S1788 and D9S50, with a Multipoint Lodscore Zmax= 11.3. In all pedigrees the affected individuals were homozygous for a founder haplotype. The Xoroxane Romani belong genetically to an isolated founder population, due to extreme inbreeding. A homozygous missense mutation I587T in the kinase domain of GNE (UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase) was found in all affected individuals. Astonishingly mutations in the allosteric domain of GNE cause Sialuria, a totally different metabolic disorder. Mutations in different domains in GNE cause different disorders. GNE is the bifunctional key enzyme of the sialic acid synthesis. The Carrier frequency in the Romani population is about 2%. The age of mutation using the size of the founderhaplotype was estimated about 850 years. The I587T mutation has been found in an Italian and an US-american pedigree with hereditary inclusion body myopathy (HIBM), investigation of the origin of these mutations by haplotype analysis is in progress. Current results demonstrated that this distal myopathy and the HIBM are not only allelic, they are the same disorder! The remaining enzymatic activity of the recombinant mutant protein for epimerase domain was 55% and for the kinase domain 35%. Also the activities of other GNE mutants were high in the same range, except for G576E and N519S. The CD Spectroscopy didn't any structural changes of the enzyme by the mutations. The molecular path physiology of the distal myopathy is not sufficiently explained by simple loss of enzyme activity, further investigations are necessary.

P171

Long-range conserved non-coding SHOX sequences regulate expression in developing chicken limb and are associated with short stature phenotypes in human patients

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Defects in long-range regulatory elements have recently emerged as previously underestimated factors in the genesis of human congenital disorders. Léri-Weill dyschondrosteosis is a dominant skeletal malformation syndrome caused by mutations in the short stature homeobox gene SHOX. We have analyzed four families with Léri-Weill dyschondrosteosis with deletions in the pseudoautosomal region but still with an intact SHOX coding region. Using FISH and SNP studies, we identified an interval of ~200 kb that was deleted in all tested affected family members but retained in the unaffected members and in 100 control individuals. Comparative genomic analysis of this interval revealed eight highly conserved non-genic elements between 48 kb and 215 kb downstream of the SHOX gene. As mice do not have a SHOX gene, we analyzed their enhancer potential in chicken embryos using a

GFP reporter construct driven by the ß-globin promoter, by in ovo electroporation of the limb bud. We observed cis-regulatory activity in 3 of the 8 non-genic elements in the developing limbs argueing for an extensive control region of this gene. These findings are consistent with the idea that the deleted region in the affected families contains several distinct elements that regulate Shox expression in the developing limb. Furthermore, the deletion of these elements in humans generates a phenotype apparently undistinguishable to those patients identified with mutations in the SHOX coding region and, for the first time, demonstrates the potential of an in vivo assay in chicken to monitor putative enhancer activity in relation to human disease.

P172

Complex regulation of LMX1B expression: a possible autoregulatory mechanism may involve a novel ncRNA gene adjacent to human LMX1B

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Haploinsufficiency of the transcription factor LMX1B resulting from heterozygous loss-of-function mutations causes Nail-Patella Syndrome (NPS), a rare autosomal-dominant disorder characterized by dysplastic nails, skeletal changes and a variable renal phenotype with nephropathy in about 50 % of the cases. Recently, we investigated Lmx1b expression in a knockout mouse model of Lmx1b established by Chen et al. (1998). When we crossed the Lmx1b+/- knockout mice into different genetic backgrounds we were able to show strain specific differences in the expression level of the residual functional Lmx1b allele, indicating that regulation of Lmx1b expression depends on the genetic background. We therefore investigated the human and murine LMX1B promoter in more detail. Despite several evolutionary conserved promoter elements, in silico analysis revealed a binding site (FLAT-F-element) for LMX1B itself. This element indeed binds LMX1B, as demonstrated by gel shift assays. Although transient reporter gene assays showed a slight transactivation of the LMX1B promoter by LMX1B itself, this transactivation was not dependent on binding of LMX1B to the FLAT-F element. We therefore suggest another function of this element. Interestingly, we identified an antisense transcript of LMX1B which overlaps with LMX1B transcripts. Reverse transcription PCR experiments on RNA isolated from HEK293 cells demonstrated that a stable RNA-RNA duplex molecule forms between the two transcripts in vivo, which includes the LMX1B binding site. We are currently trying to demonstrate binding of LMX1B to these RNA-RNA duplexes.
In conclusion, the data indicate a complex regulation of LMX1B expression with possible autoregulation. It may be suggested that differences in the efficiency of this autoregulation in different genetic backgrounds may cause transcriptional variance, which may also play a role in the variability of renal insufficiency in NPS patients.

P173

Genome-wide DNA methylation analysis of imprinted genes in the family of a patient with transient neonatal diabetes mellitus caused by an imprinting defect at the PLAGL1 locus in 6q24

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In a previous study we investigated 12 patients with transient neonatal diabetes mellitus (TNDM) due to a loss of maternal methylation at the PLAGL1 locus in 6q24 (Mackay et al., 2006). Besides PLAGL1, the methylation status of 7 additional imprinted genes was studied. Interestingly, 6/12 patients displayed a complete and/or partial loss of imprinting of 4 additional maternally methylated genes (GRB10, PEG1, PEG3 and KvDMR), which suggests the presence of a maternal hypomethylation syndrome. However, only PLAGL1 was hypomethylated in the remaining 6 TNDM patients, and the possible presence of additional hypomethylated genes was suspected. Therefore, we have here investigated DNA from peripheral blood samples of one of those TNDM patients, in which only PLAGL1 was hypomethylated, and her parents with the aim of detecting additional TNDM-related hypomethylated genes. To that purpose, a novel high-throughput DNA methylation approach based on the BeadArray technology (Ilumina Inc.) was applied. This array allows the detection of methylation changes in 1536 individual CpGs located in regulatory regions from over 500 genes selected by their known involvement in human disease, including 56 imprinted gene loci. Samples were analyzed in duplicate and evaluated with the BeadStudio software. The microarray data confirmed that the PLAGL1-specific CpGs were hypomethylated in the DNA of the patient but not in that of the parents. Promoter regions of the other 55 imprinted genes did not show any methylation change. With regard to non-imprinted genes, only HLA-DQA1 seemed to be differentially hypomethylated in the patient but the significance of this finding in the context of TNDM requires further research. In conclusion, our studies suggest that TNDM can be associated with a maternal hypomethylation syndrome, with epigenetic defects in several imprinted genes, or with hypomethylation of only the PLAGL1 locus.

P174

Normal axoneme ultrastructure and perturbed ciliary beating in primary ciliary dyskinesia and Kartagener syndrome due to C-terminal mutations in the dynein axonemal heavy chain DNAH11

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Primary Ciliary Dyskinesia (PCD) is a disorder characterized by perturbed or absent beating of motile cilia associated with random organ lateralization. We present a German family with six individuals affected by PCD or Kartagener Syndrome (KS) and characteristic chronic respiratory infections from early childhood. Ciliary beating was reduced or absent in native respiratory cilia of affected individuals. Surprisingly, analysis of cultured cilia, using high-speed video microscopy, revealed an abnormal hyperkinetic beating of respiratory cilia. Electron micrcoscopy displayed a normal ultrastructure of axonemes, and outer dynein arms were normal, as shown by anti-DNAH5 and anti-DNAH9 immunohistochemistry. Microsatellite analysis indicated linkage to the dynein heavy chain DNAH11 on chromosome 7p15. All the affected were found to be compound heterozygotes for mutations c.12415C>G and c.13583_13639del. Both mutations are located in the C-terminal domain and result in a truncated DNAH11 protein (p.4128Y>X, p.A4518_A4523del). Our findings indicate that mutations in the DNAH11 gene indeed cause PCD and KS. We conclude, that DNAH11 nonsense mutations may be associated with a normal axoneme, but abnormal beating pattern of respiratory cilia, while do not affect male fertility.

P175

Renal phenotype in heterozygous Lmx1b knockout mice (Lmx1b+/-) after unilateral nephrectomy

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The Nail-Patella Syndrome (NPS) is a rare autosomal-dominant disorder which is caused by loss-of-function mutations in the transcription factor LMX1B. NPS is characterized by dysplastic nails, absent or hypoplastic patellae, minor skeletal abnormalities and, in 20 - 40 % of the cases, nephropathy, which is the most severe aspect of the disorder. The current data suggest that genetic modifiers in the outbred human genetic background are responsible for this variable phenotype. Preliminary work on the function of Lmx1b in the kidney has been performed

using Lmx1b knockout mice (Lmx1b-/-). Although early lethal, Lmx1b-/- mice exhibit the characteristic NPS features including the renal abnormalities. But in contrast to the situation in human, no phenotype could so far been detected in heterozygous Lmx1b+/- mice. This indicates that not only our understanding of the intra- and interfamilial variability as well as the exact pathomechanism underlying the nephropathy is still very limited. In an attempt to identify modifying factors of LMX1B activity and to evaluate the mechanism resulting in nephropathy, we tried to induce a renal phenotype in Lmx1b+/- mice, and thus model the human (NPS) situation. We applied unilateral nephrectomy (Unx) as a model to induce nephron loss and were able to detected a significant ($p = 0.02$) reduction in compensatory renal growth (CRG) in heterozygous knockout animals (Lmx1b+/-) compared to Lmx1b+/+ animals which was correlated with a significantly reduced increase in glomerular volume ($p = 0.0034$) and an increase in glomerulosclerosis ($p = 0.085$). Thus, Lmx1b deficiency in heterozygous Lmx1b (Lmx1b+/-) knockout mice profoundly affects the compensatory response to nephron loss. This is the first report of a phenotype in Lmx1b+/- knockout animals, and the first report of a connection between CRG and Lmx1b action

P176

Primary Congenital Glaucoma: SNP haplotypes of cytochrome P450 1B1 (CYP1B1) determine the basic enzyme activity whereas mutations act either via loss-of-function or protein integrity

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CYP1B1 is located in the GLC3A locus on chromosome 2p21 and mutations therein cause primary congenital glaucoma. We have identified a healthy compound heterozygous carrier of the E229K and 1064-1076del CYP1B1 variants, raising the question of the pathogenicity of E229K. Furthermore E229K in the heterozygous state has been discussed as a risk factor for (adult onset) autosomal dominant primary open angle glaucoma. We therefore decided to functionally investigate E229K as well as other CYP1B1 variants. We first characterised the 4 common wild type haplotypes: 5'-RAVDN-3', 5'-GSLDN-3', 5'-RALDS-3' and 5'-RALDN-3'; and subsequently variants G61E, N203S, L343del, E229K and Y81N on their respective founder haplotype. All variants were introduced by in vitro mutagenesis and functional analysis was based on protein extracts derived from heterologous expression in yeast (INVSc1). The molar concentration of CYP1B1 was determined by UV spectroscopy under CO saturation. The enzyme activity of each haplotype/variant was determined by bioluminescence. The 5'-RAVDN-3' haplotype showed the highest molar activity at 0.32 (100%), while the other 3 haplotypes were in the range of 25% to 59% of this value. Variants G61E, N203S and L343del each revealed a clear decrease in activity to 5%, 11% and 6% of their respective haplotype, indicating a loss-of-function. Y81N and E229K have molar activities similar to the wild type. However, the relative amount of CYP1B1 protein for these variants is decreased resulting in a reduced relative activity per µg total protein of 41% and 77%, respectively. Molecular modelling of Y81N and E229K indicated an influence on protein stability rather than enzyme activity. Thus CYP1B1 mutations lead to either reduction of molar activity or reduction of the relative protein amount. The latter could be explained by either altered translation efficiency or degradation, resulting in a classification as risk factor depending on yet unknown modifiers.

P177

Induction of macrophage chemotaxis by aortic extracts of the mgR Marfan mouse model and a GxxPG-containing fibrillin-1 fragment

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The primary cause of early death in untreated Marfan syndrome (MFS) patients is aortic dilatation and dissection. Mutations in the gene for fibrillin-1 (FBN1) cause MFS, but the pathophysiology of this disorder is only partially understood, with abnormalities in microfibrillar assembly, increased susceptibility to proteolysis of mutation fibrillin-1, abnormalities in TGFbeta signalling all being postulated to contribute to the development of disease. In this work, we investigated whether ascending aortic samples from the fibrillin-1-underexpressing mgR mouse model for MFS or a recombinant fibrillin-1 fragment containing an elastin-binding protein (EBP) recognition sequence can act as chemotactic stimuli for macrophages. Both the aortic extracts from the mgR/mgR mice and the fibrillin-1 fragment significantly increased macrophage chemotaxis compared with extracts from wild-type mice or buffer controls. The chemotactic response was significantly diminished by pretreatment of macrophages with lactose or with the elastin-derived peptide VGVAPG and by pretreatment of samples with a monoclonal antibody directed against an EBP recognition sequence. Mutation of the EBP recognition sequence in the fibrillin-1 fragment also abolished the chemotactic response. These results indicate the involvement of EBP in mediating the effects. Additionally, investigation of macrophages in aortic specimens of MFS patients demonstrated macrophage infiltration in the tunica media. Our findings demonstrate that aortic extracts from mgR/mgR mice can stimulate macrophage chemotaxis by interaction with EBP and show that a fibrillin-1 fragment possesses chemotactic stimulatory activity similar to that of elastin degradation peptides. They provide a plausible molecular mechanism for the inflammatory infiltrates observed in the mgR mouse model and suggest that inflammation may represent a component of the complex pathogenesis of MFS.

P178

C15orf2 from the Prader-Willi / Angelman syndrome region shows monoallelic expression in fetal brain

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Prader-Willi syndrome (PWS) is a neurogenetic disorder which results from the loss of paternal contribution for a 1.5 Mb imprinted region on the proximal long arm of chromosome 15. We have previously identified a testis-specific gene in this region, C15orf2, which maps between Necdin and SNURF-SNRPN. It encodes an 1156-amino-acid protein of unknown function and is biallelically expressed in adult testis. Based on these findings we had suggested that C15orf2 might play a role in spermatogenesis. This notion has recently been substantiated by the finding that C15orf2 harbours several genes for PIWI interacting RNAs (piRNAs), which are believed to regulate spermatogenesis. Recently, we identified another transcript, NCT1, which maps 25 kb upstream of C15orf2. Since NCT1 showed most abundant expression in testis, but low level expression in other tissues, we reinvestigated the tissue distribution of C15orf2 transcripts. By RT-PCR we detected C15orf2 expression in a number of tissues including fetal brain. The finding of mononallelic expression of NCT1 in fetal brain also raised the question whether C15orf2 is monoallelically expressed in this tissue. To clarify this question we made use of expressed fragment length polymorphisms. In three heterozygous individuals, we observed by RT-PCR only one allele, indicating that C15orf2 is monoallelically expressed in fetal brain. Previously, methylation-sensitive Southern blot analysis had revealed that a SacII restriction site inside the CpG island of C15orf2 is unmethylated in testis and sperm. Using bisulfite sequencing and cloning of fetal brain DNA, we obtained several sequences with an unmethylated SacII site. Since we have now found that C15orf2 is expressed in fetal brain and may be subject to genomic imprinting, we cannot any longer exclude a role of this gene in PWS.

P179

SMN2 gene promoter methylation correlates with the spinal muscular atrophy (SMA) disease severity

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Spinal muscular atrophy (SMA) is an autosomal recessively inherited alpha-motoneuron disorder. The disease determining survival motor neuron gene 1 (SMN1) is homozygously deleted in approximately 96% of SMA patients. Within the SMA region on chromosome 5q, the SMN genes exist in two almost identical copies, SMN1 and SMN2, which are ubiquitously expressed and encode identical proteins. Since all SMA patients lacking SMN1 carry at least one SMN2 copy, transcriptional activation of the disease-modifying SMN2 gene is likely to be clinically beneficial. We have shown that histone deacetylase (HDAC) inhibitors such as valproic acid,

SAHA (Vorinostat™) and M344 increase SMN2 expression in fibroblasts derived from SMA patients as well as in several neuroectodermal tissues. Here, we show that the DNA demethylating agent 5-aza-2´-deoxycytidine increases SMN2 transcript- and protein levels in SMN1-deleted fibroblasts. This finding indicates that both, histone acetylation and DNA methylation, regulate SMN2 gene activity. SMN2 promoter analyses indicated that the SMN2 gene contains 4 putative CpG islands (CpG1-4). Bisulfite treatment of DNA derived from SMN1-deleted fibroblasts followed by PCR, cloning of PCR products and sequencing revealed methylation of cytosine residues within and adjacent to the predicted SMN2 CpG-1, 2 and 4, while CpG-3 appears to be hypomethylated. The SMN2 methylation was subsequently analyzed by pyrosequencing using DNA isolated from blood samples of 10 type I and 8 type III SMA patients carrying 2 SMN2 copies. The comparison of the methylation patterns revealed significant differences in cytosine methylation ($p<0.05$, t-test), which are located in putative transcription factor binding sites. These results improve the understanding of how the SMN2 gene expression is regulated. We suggest that SMN2 promoter methylation modulates the disease severity which may in part explain the variable phenotypes in the presence of identical SMN2 copy numbers.

P180

Double PKHD1 mutations in cis in patients with autosomal recessive polycystic kidney disease (ARPKD)

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Autosomal recessive polycystic kidney disease (ARPKD) is an important cause of childhood morbidity and mortality. ARPKD is caused by mutations in the exceptionally large PKHD1 (Polycystic Kidney and Hepatic Disease 1) gene (470 kb of genomic DNA) with a longest open reading frame transcript of 67 exons predicted to encode a 4,074 aa protein. Except for a few population-specific founder alleles and the common c.107C>T (p.Thr36Met) missense change, PKHD1 is characterized by considerable allelic diversity. So far, a total of about 350 different PKHD1 mutations (approximately 1000 mutated alleles) are included in the locus-specific database (URL: http://www.humgen.rwth-aachen.de). Whilst curating this database, we observed that in several non-related families (n = 9) certain PKHD1 missense changes occurred tandemly in cis on the same parental allele. Mere polymorphisms were excluded by screening each change in more than 500 control chromosomes.

Intriguingly, independent of the mutation on the other parental chromosome, most patients harbouring the specific P805L/I3177T allele died perinatally allowing the careful categorization as a rather severe change. As ARPKD patients have been described so far carrying I3177T alone, clarification of the consequences of the non-conservative, evolutionarily highly conserved missense change P805L awaits identification of a patient bearing P805L on one or both disease alleles alone. Alternatively, it is conceivable that the consequences of a certain sequence variant also depend on the combination of changes or alleles in which this alteration occurs/is integrated. Allele sharing analyses based on intragenic microsatellite markers indicate common founder haplotypes bearing two mutations as the most plausible explanation. This study clearly depicts that some alertness is warranted in the evaluation of mutational data to avoid possible pitfalls, e.g. with respect to prenatal diagnosis.

P181

Targeted disruption of the murine retinal dehydrogenase gene Rdh12 does not limit visual cycle function

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RDH12 codes for a member of the family of short-chain alcohol dehydrogenases/reductases proposed to function in the visual cycle that supplies the chromophore 11-cis retinal to photoreceptor cells. Mutations in RDH12 cause a severe and progressive form of childhood-onset autosomal-recessive retinal dystrophy (arRD), including Leber congenital amaurosis (LCA). We generated Rdh12 knockout mice, which exhibited grossly normal retinal histology at 10 months of age. Levels of all-trans and 11-cis retinoids in dark- and light-adapted animals, and scotopic and photopic electroretinogram (ERG) responses were similar to wild type, as was recovery of the ERG response following bleaching in animals matched for an Rpe65 polymorphism (p.L450M). Lipid peroxidation products and other measures of oxidative stress did not appear to be elevated in Rdh12-/- animals. RDH12 was localized to photoreceptor inner segments and the outer nuclear layer both in mouse and human retina using immunohistochemistry. The present findings, together with those of earlier studies showing only minor functional deficits in mice deficient for Rdh5, Rdh8, or Rdh11, suggest that the activity of any one isoform is not rate limiting in the visual response.

P182

BSCL2 missense mutations provoke ER stress and ensue apoptosis

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The Silver syndrome, a complicated hereditary spastic paraplegia, is caused by two heterozygous missense mutations in the Berardinelli-Seip congenital lipodystrophy gene, BSCL2. Phenotype shows amyotrophy of the small hand muscles and spasticity of the lower extremities. BSCL2 encodes Seipin, a transmembrane protein that is localised in the ER. The missense mutations N88S and S90L affect the N-glycosylation site resulting in an accumulation of the unfolded protein in the ER lumen. That is known to initiate unfolded protein response (UPR) to restore normal ER function. If the ER stress is prolonged apoptotic cell death ensues and results in cell or neurodegeneration. This has been suggested to be involved in some human neuronal diseases.

Grp78 is a central regulator and indicator for ER stress due to its role as a major ER chaperone. Induction of ER stress was determined in transiently transfected cell culture (either N88S or WT construct) by use of real-time PCR. Levels of GRP78 mRNA were elevated after one day and showed a 20-fold increase after two days in HUVEC (human umbilical vein endothelial cells) misexpressing mutant N88S. Cells expressing WT construct showed no significant elevation of Grp78. In addition to ER chaperon induction an increase of SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase) pumping capacity in stressed cells has been described. To investigate changes of Ca2+ homeostasis we used FURA-2 technique. Results show an upregulation of SERCA activity in HUVEC misexpressing N88S in contrast to controls (WT construct or non-transfected). Furthermore annexin V tests and bisbenzimide stainings were conducted in transfected HUVEC and AtT20 cells (mouse pituitary tumour cells) to determine induction of apoptosis. Both tests give evidence to apoptosis due to N88S misexpression. We are trying to elucidate events leading to neurodegeneration in Silver syndrome and therefore further investigations concerning apoptosis will be carried out.

P183

Isolation and characterization of the male germ cell specific expressed gene Pxt1, encoding for a peroxisomal protein

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Spermatogenesis is a complex process of cell differentiation, including the reduction of genetic material and a remodeling of cell structure. Genes reported to be crucial for this process are often exclusively expressed in the testis. We have identified a novel testis-specific gene, named Pxt1 with expression starting at the spermatocyte stage during mouse spermatogenesis. The analysis of the Pxt1 gene sequence revealed that in the potential amino acid sequence a conserved NHL motif is present, which is characteristic for peroxisomal proteins. The cellular localization of Pxt1-GFP fusion protein supports the hypothesis that the Pxt1 gene encodes for a peroxisomal protein. Furthermore we have analyzed if the NHL motif plays a specific role in the cytoplasmic localization of the Pxt1 gene product by introducing point mutations in the conserved NHL sequence. We could demonstrate that this motif is indeed responsible for the correct subcellular localization of the Pxt1 protein. In addition, our co-localization experiment of the Pxt1 protein with known peroxisomal markers

confirmed that the Pxt1 protein is localized within peroxisomes. Our results are in agreement with recently published reports about the presence of peroxisomes in male germ cells. Moreover our findings strongly indicate that peroxisomes are present not only in early spermatogonia, but also in later stages of sparmatogensis.

P184

Truncating mutation of the DFNB59 gene causes cochlear hearing impairment and central vestibular dysfunction

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We have identified a consanguineous family from Morocco segregating autosomal recessive congenital progressive hearing loss (ARNSHL) and retinal degeneration. Detailed clinical investigation of the six siblings revealed combined severe cone-rod dystrophy (CORD) and severe/profound hearing impairment in two of them, while there is isolated CORD in three and non-syndromic profound hearing loss in one. We therefore assumed a partial overlap of two non-syndromic autosomal recessive conditions instead of a monogenic syndrome and performed genome-wide linkage analysis. The disease loci were mapped to chromosome 2q31.1-q32.1 for ARNSHL and to 2q13-q14.1 for CORD, respectively. The retinal phenotype was shown to be due to homozygosity for a novel splice site mutation, c.2189+1G>T, in the retinitis pigmentosa gene MERTK. The ARNSHL interval comprised the DFNB59 locus. The DFNB59 gene has been identified recently, and two missense mutations (p.R183W and p.T54I) have been shown to cause auditory neuropathy in both humans and transgenic mice. Mutation screening in the DFNB59 gene in our family revealed homozygosity for a 1-bp-insertion in exon 2 (c.113_114insT), predicting a truncated protein of 47 amino acids, in all three hearing impaired subjects. This is the first description of biallelic putative loss-of-function of the DFNB59 gene. Detailed audiological investigation clearly indicated hair cell dysfunction and, in contrast to cases reported previously, excluded auditory neuropathy. We show that besides otoferlin (OTOF), DFNB59 is the second known gene in which mutations can result in these two distinct forms of hearing impairment. Moreover, all patients in our family with homozygosity for the DFNB59 mutation display central vestibular dysfunction.

P185

The potassium-channel gene Kcnk9 is paternally imprinted in mice and humans

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Genomic imprinting is the epigenetic marking of a subset of genes which results in monoallelic or predominant expression of one of the two parental alleles according to their parental origin. We have established Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) to systematically analyze and verify the imprinted expression of promising candidate imprinted genes predicted by sequence-based bioinformatic analyses. Using this approach, maternal-specific imprinted expression of the Kcnk9 gene was discovered in E11.5 (C57BL/6 × Cast/Ei)F1 and informative (C57BL/6 × Cast/Ei) × C57BL/6 backcross mouse embryos. Expression of Kcnk9 in adult mouse tissues is restricted to the brain and also maternal-specific. QUASEP analysis of informative human fetal brain samples further demonstrated maternal-specific imprinted expression of the KCNK9 gene in humans. The CpG island associated with the mouse Kcnk9 gene is not differentially methylated but strongly hypomethylated. We speculate that Kcnk9 imprinting may be regulated by the differentially methylated region in Peg13, an imprinted non-coding RNA gene in close proximity to Kcnk9 on distal mouse chromosome 15. Kcnk9 is a member of the two-pore (2P)-domain K+ channel family and encodes for the potassium channel protein TASK-3. This family of K+ channels was already shown to play a critical role in apoptosis and tumorigenesis. Furthermore, a Kcnk9 mutation in a rat model of absence epilepsy along with the highest expression in brain indicates that Kcnk9 plays an important role in the central nervous system. In summary, the identified imprinted expression of the Kcnk9 gene raises important questions about the role of this gene in fetal and placental growth, neurodevelopment as well as postnatal adaptations and behaviour.

P186

TLR4- and Egr1-dependent regulatory networks control retinal microglia activation in the mouse model for X-linked juvenile retinoschsis

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Objectives: Genetic and biochemical evidence suggests that innate immunity plays a pivotal role in retinal degenerative disorders. Our previous work in the mouse model for X-linked juvenile retinoschisis (RS1h-/Y) has identified microglia activation preceding photoreceptor apoptosis and retinal degeneration. In this study, our aim was to further characterize underlying early signaling events, transcriptional markers, and effector mechanisms in microglia.
Methods: Early postnatal retinae and ex vivo microglia cells from retinoschisin-deficient and wild type mice were subjected to DNA-microarray and realtime qRT-PCR analysis. Cells were functionally characterized and lineage markers and molecular activation patterns were examined. RAW264.7 and BV-2 cells were used as model system to study TLR4-dependent activation of microglia-specific genes and to characterize TLR4-responsive Egr1 promoter elements.
Results: After isolation, purification and culture, phase contrast micrographs and latex bead phagocytosis assays demonstrate a highly activated state of retinoschisin-deficient microglia cells compared to controls. Starting from the induced transcripts in retinal samples of RS1h(-/Y) mice, a transcriptional network of TLR4-activated genes composed of Casp11, Spp1, Clec7a, Fcer1g, as well as a group of highly expressed constitutive markers (Tyrobp, CCl6, Cd68, and S100A6) could be specifically allocated to the microglia population in the diseased retina. Furthermore, LPS-stimulation of RAW264.7 and BV-2 cells mimicks endogenous microglia activation, up-regulates the TLR4-dependent gene cluster and targets a specific region in the proximal Egr1 promoter.
Conclusions: TLR4 signaling and up-regulation of Egr1 are early events during microglia activation in Rs1h-deficiency leading to transcriptional induction of pro-inflammatory and activation-induced apoptosis pathways. Targeting of these pathways may point to novel therapeutic options in neurodegenerative retinal disease.

P187

Spinocerebellar ataxia type 3 (SCA3): Analysis of a conditional mouse model

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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. In order to study the course of the disease we generated an inducible transgenic mouse model using the inducible "Tet-Off-System". This system is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest.

The expression can be blocked by the addition of Tetracycline which allosterically inhibits the tTA protein.
For the ataxin-3-responder mouse lines a full length constructs containing an expanded repeat with the pathological length of 77 glutamine repeats was used. The use of two different promoter mouse lines with known expression in the brain (Prion protein (Prp) promoter, Ca2+/Calmoduline-dependent protein kinase II (CamKII) promoter) allows us to target the transgene expression to different brain regions. In preceding promoter analyses we identified and characterized these brain regions in detail. Double transgenic SCA3 mice were analysed at different levels: We first analysed the phenotype in behavioural studies. Afterwards we performed immunohistochemical analyses of brain sections to study the expression of the transgene in the brain. In addition the Tet-Off-System allows us to turn the expression of ataxin-3 on or off at different developmental stages. Using this model we therefore will be enabled to study whether or not the disease phenotype is reversible.

P188

Sox9 is necessary for the maintenance of spermatogenesis in mice

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Heterozygous loss-of-function mutations of the transcription factor gene SOX9 result in the human skeletal malformation syndrome campomelic dysplasia with XY sex reversal. Inactivation of both Sox9 alleles before the sex determination stage leads to complete XY sex reversal in mice, while ectopic expression of Sox9 in XX mice causes testis development in the absence of Sry. Murine Sox9 is expressed in the bipotential gonadal anlagen of both sexes before the sex determination stage, becoming up-regulated at embryonic day 12.5 (E12.5) in the male gonad and down-regulated in the female gonad. Sox9 continues to be expressed in a male-specific pattern in the Sertoli cells, the epithelial cells of the testis cords.
To see whether Sox9 is required for testis development after the intitial steps of sex determination, we have homozygously inactivated Sox9 at E13.5, shortly after the sex determination stage. To achieve this, we crossed mice carrying a conditional Sox9 allele with an AMH-Cre mouse line where the Cre recombinase is expressed under the control of the human anti-Müllerian hormone (AMH) promoter region. Conditional mutants show normal embryonic testis development, and molecular markers for testicular Sertoli cells (Amh, Sox8), Leydig cells (Scc) and endothelial cells (Pecam-1) are expressed normally. Early postnatal development of Sox9 mutant mice is also completely normal, and these mice are initially fertile. However, starting at 2 months, there is a progressive impairment of the spermatogenic cycle. Around 5 months, mutant males became sterile due to a complete meiotic arrest. Immunohistochemistry for gamma-H2AX, a marker for meiotic prophase I, shows that spermatogenesis can not progress any more beyond prophase I. These results reveal that Sox9 is not only essential for testis induction, but is also necessary for the maintenance of spermatogenesis.

P189

Paraxial Protocadherin is a component of the planar cell polarity pathway

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In the vertebrate embryo morphogenetic movements which establish the basic body plan are regulated by ß-catenin-independent Wnt-signalling. Perturbed morphogenetic movements and cell behaviors can cause severe developmental defects. When the establishment of planar cell polarity (PCP) is perturbed in the human embryo, neural tube closure is impaired and spina bifia-type phenotypes develop. A growing number of proteins contributing to these morphogenetic processes have been identified and characterised recently. Among them is Paraxial Protocadherin (PAPC) which can modulate C-cadherin mediated cell adhesion and is involved in cell sorting. We have identified PAPC as a component of the PCP pathway using Xenopus and Drosophila embryos as experimental models. PAPC modulates the activities of the Rho GTPase and c-jun N-terminal kinase, which are effectors of the planar cell polarity (PCP) pathway. We demonstrated that PAPC interacts with the Sprouty 1 protein, which is an inhibitor of PCP signalling in vertebrates. We provide evidence that sprouty links the Protocadherins to β-catenin-independent Wnt-signalling.

P190

Characterisation of three overlapping microdeletions including SALL4 renders 5 neighbouring genes responsible for severe developmental delay in a patient with Okihiro syndrome

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Okihiro syndrome results from point mutations and larger deletions of the SALL4 locus on the chromosome 20q13.13-13.2. The phenotype of Okihiro syndrome does not include developmental delay, suggesting such patients may have larger deletions, which include both SALL4 and neighboring genes affecting cognitive development. Here we report three novel overlapping heterozygous microdeletions including SALL4. All deletions were identified by quantitative real time PCR and in two families confirmed by high resolution oligo-based array CGH. The first patient carried a deletion of approximately 2.01-2.05 Mb including SALL4 and 9 other functional genes. In the second patient, a 2.16-2.18 Mb deletion was found to include 5 genes in addition to SALL4. A third case carried a deletion of 1.3-2.8 Mb including up to 7 additional genes. Only the first patient presented with severe developmental delay in addition to Okihiro syndrome features. Comparison of the three deletions showed that only MOCS3, DPM1, ADNP, TMSL6 and BCAS4 were not deleted in the other patients, suggesting that heterozygous deletion of one or more of these genes is responsible for the severe developmental delay. This patient did not have Molybdenum cofactor deficiency, and she had normal carbohydrate-deficient transferrin studies, suggesting that she does not have a congenital disorder of glycosylation.

P191

Autoactivated PDGFRα leads to craniosynostosis

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Craniosynostosis is one of the most common congenital craniofacial deformities affecting one in 2.500 individuals, resulting in cranial dysmorphism that can be familial or sporadic in origin. Although linkage analysis has shown that mutations in Fgfr, Msx2 and Twist genes are associated with craniosynostosis, mechanisms regulating cranial suture development remain elusive. Hence, the identification of genetic components controlling cranial development is critical for deciphering the pathology of craniosynostosis. PDGFRα is a receptor tyrosine kinase, which has been described to be expressed in cranial neural crest cells that give rise to the nasal and frontal bones of the skull and the surrounding suture tissue. PDGF signaling is known to play a significant role in osteogenic precursor cells by keeping the cells in an undifferentiated and proliferative stage. To investigate the role of PDGFRα in cranial skeletogenesis, a conditional mouse model has been generated expressing autoactivated PDGFRα in cranial neural crest cells. Histological analyses show that the mutated PDGFRα induces premature closure of the frontal suture and fusion of the coronal suture after birth, suggesting that PDGFRα regulates intramembraneous ossification during skull development. Therefore primary osteoblasts were isolated and the effects of PDGFRα on proliferation and differentiation were examined in long-term mineralizing cultures. While there is no increase of proliferation, detectable osteoblast differentiation is dramatically suppressed. We show further, that PLCγ downstream signalling is upregulated through PDGFRα activation. Additional studies will address the relationship between the upregulated PLCγ pathway and suppression of differentiation in order to elucidate the mechanisms of intramembraneous ossification regulated by PDGFRα. This mouse model might be a useful system for the study of craniosynostosis.

P192

The third dimension of congenital ichthyosis: Gene knock-down in organotypic cell systems

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In the last four years several new genes for autosomal recessive congenital ichthyosis (ARCI) have been identified. However, still only little is known about the pathophysiology of this genetically and clinically heterogeneous group of severe disorders of keratinisation with a prevalence of 1 in 100,000 to 200,000 persons in the European population. Although up to date five gene loci with the genes TGM1, ALOX12B, ALOXE3, ABCA12, Ichthyin, and FLJ39501 have been mapped, more loci must exist, as 30-40 % of all ARCI patients do not have mutations in one of these genes. We have mainly focussed on the investigation of the epidermal lipoxygenase genes ALOX12B and ALOXE3 on chromosome 17p13 and Ichthyin on 5q33. In order to investigate the role of these genes and their products in the pathophysiology of ARCI, we established 3D skin models with primary keratinocytes and fibroblasts from voluntary donors and from patients with ARCI. Here we were able to analyze immunohistochemically the structure of the epidermis equivalent, especially the suprabasal layers including the stratum corneum. We found the typical hyperproliferation and impaired differentiation in patient samples. To stratify the effects of inactivation of different genes involved in ARCI, primary keratinocytes from healthy volunteers were transfected with siRNA to knock down expression of single genes. This is now done for ALOX12B, ALOXE3, ABCA12, Ichthyin, and various combinations. Knock down efficiency is checked by quantitative RT-PCR and Western analysis, and keratinocytes are used for 3D skin models 48h after siRNA transfection. In addition to the detailed characterisation of these ichthyosis skin models we will further investigate the stratum corneum in 3D skin models of ARCI patient keratinocytes after transfection with the wild type gene that is inactive in the patient. These reconstitution experiments will give us important hints at the ARCI pathways and can be first options for future therapy.

P193

New locus for an axonal type of Charcot-Marie-Tooth disease (CMT2) on chromosome 10p11.22-q21.1

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Charcot- Marie-Tooth type 2 disorder is an inherited motor and sensory neuropathy with primary axonal lesion. It is characterized by severely decreased amplitudes of nerve action potentials and only slightly decreased motor nerve conduction velocities (NCV).
The Czech family CZ-CMT-B originates from northern Moravia and was recruited at the Charles University in Prague. The family comprises 11 members, among them are 9 affected. The CMT2 neuropathy in this family follows an autosomal dominant pattern of inheritance. The age of onset is between 12 and 50 years. The lower extremities are much more affected than the upper limbs in most of the patients. Earlier onset is associated with a more severe phenotype. Some of the patients show severe muscle atrophies below their knees, affecting also N. tibialis innervated muscles. However, nobody in the family is wheel chair bound. Tendon reflexes were absent on the legs and decreased on the arms. No other associated features as deafness, cranial nerve involvement or scoliosis etc. were observed. Electrophysiology showed amplitudes of nerve action potentials of 0.1 mV to 0.5 mV (N. tibialis) and NCVs of 30-36 m/s.
Initially a CMT1A duplication/deletion was excluded by microsatellite analysis. Furthermore, gene mutations in MFN2 and MPZ have been excluded by sequencing. The GJB1/Cx32 gene is excluded by the autosomal inheritance. Subsequently all known dominant CMT loci were excluded by linkage analysis using a panel of two microsatellite markers for each locus.
The genome scan was performed with the 10 k microarray of Affymetrix and revealed a lod score of 2.4 in a 24 Mb region on chromosome 10p11.22-q21.1. This locus does neither overlap with the DI-CMTA locus on chromosome 10q24.1-q25.1 nor has been associated with other types of hereditary neuropathies. Overall 169 genes are located in the critical interval. Most probable candidate genes are currently under investigation.

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P194

From eye to brain: Congenital, hereditary eye disorders as indicators for brain dysfunction?

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A broad variety of mouse mutants and human families suffering from ocular disorders have been characterized with respect to the underlying mutations. In some cases, the expression pattern and functional analysis of the affected genes pointed to additional functions outside the eye. This holds true at least for genes encoding the transcription factors Pax6 and Pitx3 and for the structural protein beta-B2-crystallin.
Mutations in Pax6 lead to microphthalmia in mice and aniridia in man. Moreover, Pax6 is expressed also in the developing forebrain of the mouse and involved in the regulation of neurogenesis, cell proliferation and patterning. A Pax6 mutant line shows a reduced exploratory behaviour in the modified hole board test.
Mutations in Pitx3 lead to aphakia in mice, but to cataracs and anterior segment dysgenesis in humans. Pitx3 is part of a signalling cascade including the transcription factors Pax6 and AP2-alpha. Outside the eye, Pitx3 is expressed in the mesencephalic dopaminergic system. Behavioral tests in male mutants suggest a reduced motor activity. The aphakia mouse mutant might be considered as a model for Parkinson´s disease.
Mutations in Crybb2 lead to dominant cataracts in mice and humans, because a classical structural protein of the eye lens is affected, beta-B2-crystallin. However, additional studies showed expression in the brain cortex, the olfactory bulb, the hippocampus and the cerebellum. Concerning behavioural consequences, homozygous mutants exhibit a clear prepulse inhibition deficit indicating altered sensorimotor gating. Using a large group of schizophrenic patients we demonstrated that some endophenotypes of schizophrenia are significantly associated with regulatory regions of the CRYBB2 gene.
These studies demonstrate mutations in some genes, whose pathogenic potential becomes first obvious in the eye, but having also some consequences for the central nervous system.

P195

TAGL1- a novel gene involved in Chondrocytes differentiation and a specific marker of distal chondrocytes in the growth plate

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In the context of an EST-project aimed at identifying genes and pathways involved in bone growth and differentiation, novel transcripts were isolated from a human fetal growth plate cartilage cDNA library. One of these transcript (which we called Tagl1), corresponds to a novel gene located on chromosome 10p13 in human and on 2qA1 in mouse was selected for further analysis because of its restricted representation in EST databases. We determined 1154 bp of cDNA sequence, which comprise an open reading frame of 414 bp. The predicted protein has a length of 138 amino acids with a signal peptide, a coil-coiled domain and is evolutionary high conserved throughout the eukaryotic kingdom. Northern blot analysis revealed a main transcript of 1.2 kb strongly exclusively expressed in skeletal elements of the mouse. Moreover, we could demonstrate high expression levels by in situ-hybridisation exclusively to distal (resting) chondrocytes. To confirm the specific expression of Tagl1 we analysed the expression in limb sections of knockout mouse models for different well known “skeletal” mutant mice. The data verified the distinct expression of Tagl1 in distal chondrocytes. To gain insight into the function of Tagl1 we analyzed its expression during the insulin induced differentiation of ATDC5 cells into a chondrocytes phenotype. Tagl1 mRNA levels increased gradually during the ATDC5 dif-

ferentiation till day 24 and decreased after day 24 with a simultaneous increased of Collagen X. First studies with an antibody against Tagl1 confirmed the results. In conclusion the present data strongly suggest an important function of Tagl1 in the early phase of chondrocytes differentiation. Thus, Tagl1 represents a novel putative candidate gene for skeletal dysplasias.

P196

Nuclear interaction of SALL4 with Cyclin D1 enhances the transcriptional repressor activity of SALL4

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The SALL4 gene is located on chromosome 20q13.13-13.2 and encodes a zinc finger transcription factor. Loss of function mutations in SALL4 cause Okihiro syndrome, an autosomal dominant disorder characterized by radial ray malformations associated with Duane anomaly. Studies in zebrafish and mouse showed that Sall4 interacts with TBX5 during limb and heart development and plays a crucial role for embryonic stem (ES) cell pluripotency. In this study we report the nuclear interaction of the murine orthologue Sall4 with Cyclin D1, one of the main regulators of the transition from G1 to S phase in cell cycle. Both Sall4 and Cyclin D1 were previously shown to be downstream targets of the canonical WNT signaling pathway and to be overexpressed in diverse cancers. Furthermore, we demonstrate that Sall4 operates as a transcriptional repressor located to heterochromatin and that this activity is modulated by Cyclin D1.

P197

Hspa4l-deficient mice display increased incidence of male infertility and hydronephrosis development

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The Hspa4l gene, also known as Apg1 or Osp94, belongs to the HSP110 heat shock gene family, which includes three genes encoding highly conserved proteins. This study shows that Hspa4l is expressed ubiquitously and predominantly in the testis. The protein is highly expressed in spermatogenic cells, from late pachytene spermatocytes to postmeiotic spermatids. In the kidney, the protein is restricted to cortical segments of distal tubules. To study the physiological role of this gene in vivo, we generated mice deficient in Hspa4l by gene targeting. Hspa4l-deficient mice were born at expected ratios and appeared healthy. However, approximately 42% of Hspa4l-/- male mice suffered from fertility defects. Whereas the seminiferous tubules of Hspa4l-/- testes contained all stages of germ cells, the number of mature sperm in the epididymis and sperm motility were drastically reduced. The reduction of the sperm count was due to the elimination of a significant number of developing germ cells via apoptosis. No defects in fertility were observed in female mutants. In addition, 12% of null mutant mice developed hydronephrosis. Concentrations of plasma and urine electrolytes in Hspa4l-/- mice were similar to wild-type values, suggesting that the renal function was not impaired. However, Hspa4l-/- animals were preferentially susceptible to osmotic stress. These results provide evidence that Hspa4l is required for normal spermatogenesis and suggest that Hspa4l plays a role in osmotolerance.

P198

Inactivation of the mouse Machado-Joseph-Disease gene (Mjd) alters protein ubiquitination

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Spinocerebellar ataxia type 3 (or Machado-Joseph-disease; SCA3/MJD), the most common autosomal dominantly inherited ataxia, is a neurodegenerative disease caused by expansion of a polyglutamine domain in the protein ataxin-3 (AT3). Physiological functions of normal AT3 presumably include ubiquitin protease and transcriptional corepressor activity. Furthermore normal AT3 may have a protective role as it has been found in protein aggregates in SCA3 and other polyQ diseases. To gain insight into the function of AT3 and to test the hypothesis that loss of AT3 contributes to the pathology in SCA3 we generated Mjd knockout (ko) mice by targeted mutagenesis.
Loss of AT3 had no influence on viability and fertility and the mice displayed no visible abnormalities. On the accelerating Rotarod Mjd ko mice performed as well as wildtype (wt) animals, but behavior in the open field suggested a sense of heightened anxiety. While no overt defects were apparent upon morphological examination, we found increased levels of ubiquitin in Mjd ko mice especially in cells expressing high levels of AT3 in wt animals. Mjd ko mice provide the first in vivo confirmation for the deubiquitinating activity of AT3 and may help to identify putative physiological substrates of AT3.

P199

The Fanconi anemia gene family: structure, function, mutations, and their consequences

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Fanconi anemia (FA) is a rare recessive multisystem disease with a high risk of bone marrow failure and neoplasia. The disease is caused by biallelic or hemizygous mutations in any of at least 12 genes. Three FA genes are identical to known cancer susceptibility genes, highlighting the close connection between the FA and BRCA pathways for maintenance of genomic stability. We show that FA genes are part of a protective network that functions in the response to and in the repair of DNA damage. FA genes display variability of size and mutational load which partly reflect their evolutionary history, genomic composition and functional constraints. Biallelic mutations in three recently emerged FA genes (FANCA, FANCC and FANCG) account for over 80% of all FA patients. We show that viability of patients with biallelic mutations in the highly conserved FANCD2 gene depends on the presence of hypomorphic mutations and residual protein. Although generally rare, mutation frequencies in excess of 1:100 have been observed for FANCA and FANCC in isolated populations with defined founder mutations. FANCD1/BRCA2 and FANCN/PALB2 patients consistently present with early childhood malignancies whereas most other FA genes lack strict genotype-phenotype correlations. Rather, the particular type of mutation in a given gene appears to determine the severity of congenital malformations, the age of onset of bone marrow failure, the probability to develop certain types of leukemia, the length of survival, and the propensity to develop revertant mosaicism – although striking differences among siblings have been observed and are unexplained. We also illustrate that FA patients reaching adulthood may go undiagnosed until they present with adverse reactions during treatment for malignancies. We conclude that the FA family of genes plays an important role in the maintenance of genomic stability, thereby preventing the premature occurrence of cancer and aging.

P200

Evidence for extensive locus heterogeneity in Meckel Gruber syndrome and that MKS1 is mainly caused by aberrant splicing and not restricted to the Caucasian gene pool

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Meckel-Gruber syndrome (MKS) is an autosomal recessive, usually lethal multisystemic disorder characterized by anomalies of the central nerv-

ous system, cystic kidneys, and postaxial polydactyly. Three MKS loci have been mapped and recently, two genes were identified: MKS1 in Finnish/Caucasian kindreds and MKS3 in Omani/Pakistani families, putting MKS on the growing list of ciliary disorders ("ciliopathies").
We performed linkage analysis for MKS1-3 in 15 consanguineous (multiplex) families of different ethnic origins with histologic diagnosis of MKS and typical CNS malformations.
Unexpectedly, only four families were linked to any of the known MKS loci, clearly indicating further locus heterogeneity. These four families showed homozygous haplotypes for MKS1 and, intriguingly, were of non-Caucasian, Turkish and Kuwaiti origin. MKS1 sequencing revealed different splicing defects in all four families and a sporadic German patient.
In one family we found the deletion c.262-37_179del that disrupts the IVS3 branching site. To investigate its effect on mRNA level, we analysed RT-PCR products from parental fibroblasts that showed two smaller transcripts not present among controls. The smallest band corresponded to skipping of exon 4 predicting a protein that lacks aa 88-139. The other transcript resulted from exon 4-skipping and insertion of the final 60 bp of intron 4 by usage of an alternative acceptor splice site. This complex alteration will lead to a truncated protein given that the 8th of the 20 newly inserted residues represents a new stop codon. Given that all of our MKS1 mutations and two of the three MKS1 mutations reported so far in the literature caused aberrant splicing, we hypothesize splicing defects as the most common mutational mechanism in MKS1 that is obviously not restricted to the Caucasian gene pool. Moreover, our results suggest extensive locus heterogeneity in MKS. This knowledge has immediate implications for genetic testing in MKS.

P201

Novel functional and structural implications of a 3-bp duplication in HRAS in a patient with Costello syndrome

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Costello syndrome (CS) is a rare congenital disorder characterized by postnatal growth deficiency, macrocephaly, coarse face, loose skin, heart abnormalities, and mental retardation. Heterozygous germline mutations in HRAS have been described to cause CS. H-Ras belongs to the family of small GTPases, which cycle between an inactive, GDP-bound and an active, GTP-bound state. All HRAS sequence alterations yet identified in patients with CS are missense mutations and almost exclusively cause a change of glycine at position 12 or 13. Recently, we demonstrated that mutations affecting G12 or G13 of H-Ras lead to a significant increase in the GTP-bound H-Ras form in fibroblast cells of CS patients. In a patient with typical characteristics of CS, we identified an undescribed de novo mutation, a duplication of three bps leading to a duplicated glutamic acid at position 37 (p.E37dup).
E37 plays an important role in stabilization of the switch regions, which mediate the conformational change depending on the bound nucleotide. In addition, E37 is essential for H-Ras interaction with its various binding partners, including effectors. Computational analysis of H-Ras E37dup indicated that the active form is most likely favored. We followed up this prediction by using the H-Ras effector protein RAF1 to specifically pull-down the active form of H-Ras from cells overexpressing E37dup, wild type, constitutive active, or dominant negative variants of H-Ras. Remarkably, we detected decreased levels of active H-Ras when H-Ras-E37dup was expressed. These data suggest that although H-Ras-E37dup is supposed to prefer the GTP-bound state, this mutation might result in weakening of RAF1 binding, probably followed by a decrease in RAF1-dependent downstream signaling. Nonetheless, similar H-Ras-dependent signaling pathways may be deregulated in the CS patient with p.E37dup as well as in others carrying G12 or G13 alterations as the clinical phenotypes are remarkably similar.

P202

Identification of a nonsense mutation in the very low density receptor (VLDLR) gene in patients with dysequilibrium syndrome from a large Iranian family

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We have investigated an Iranian consanguinous family with eight mentally retarded individuals. The patients also show disturbed equilibrium; walking disability and most of them have strabismus and short stature. By genome wide linkage analysis with 10,204 SNPs (Affymetrix Human Mapping 10K Array Version 2) we identified three regions of shared homozygosity with a LOD score >3 on chromosomes 6, 9 and 10 in all affected individuals.
The interval on chromosome 9 (p24.2-24.3) contains the VLDLR (very low-density lipoprotein) gene. VLDLR has 19 exons and spans ~40kb of genomic DNA. Its gene product is part of the reelin signaling pathway, which is involved in neuroblast migration in the cerebral cortex and cerebellum.
A homozygous deletion of this gene has been previously found to cause a syndrome of cerebellar ataxia and mental retardation associated with cerebellar hypoplasia in the Hutterite population (Boycott et al. 2005; Am.J.Hum.Genet. 77). In view of this finding we screened the coding region of VLDLR for mutations and found a homozygous 1342C>T nucleotide substitution, which leads to a premature stop codon in exon 10 of this gene in our patients. This is the second VLDLR mutation to be described, confirming the role of this gene in mental retardation.

P203

SALL4 is directly activated by TCF/LEF in the canonical Wnt signaling pathway

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Mutations in SALL4, a member of the SALL family of zinc finger transcription factors, cause Okihiro/ Duane radial ray syndrome characterized by a combination of Duane anomaly and radial ray defects of the upper limbs. Animal studies in zebrafish and mouse showed that SALL4 orthologues are downstream of and interact with TBX5 during limb and heart development, but the promoter of SALL4 has not yet been characterized. For other SAL-like genes, regulation within the Shh, Wnt and Fgf pathways has been reported. Here we describe the cloning and functional analysis of the SALL4 promoter region. In transient transfection assays, a minimal promoter region of 31 bp was identified containing a consensus binding site for members of the TCF/LEF family. The SALL4 promoter was strongly activated not only by endogenous or exogenously added LEF1 protein but also by TCF4E. Mutation of the TCF/LEF binding site results in a significant decrease in promoter activation. Our results demonstrate for the first time the direct regulation of a SALL gene by the canonical Wnt signaling pathway.

P204

Epigenetic regulation of cellular senescence by p33ING1

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Cellular Senescence is an interesting phenomenon of primary cells, which arrest irreversibly their cell cycle and change completely their morphology and gene expression. p33ING1 was identified as an inhibitor of growth and has been described as a tumor suppressor. The expression of p33ING1 is induced in senescent cells and antisense p33ING1 extends the proliferative life span of primary human fibroblasts.
Here, we show that p33ING1 is a potent transcriptional silencer associated with histone deacetylase (HDAC) and histone methyltransferase (HMT) activity. Interestingly, p33ING1 expression in primary human diploid fibroblasts induces premature cellular senescence. Using deletion mutants two potent autonomous and transferable silencing domains were identified but no evidence of an activation domain was found. The amino-(N-) terminal silencing domain is sensitive to the histone deacetylase inhibitor trichostatin A (TSA) while the carboxy-terminal silencing function is resistant to TSA, suggesting that p33ING1 confers gene silencing through both HDAC-dependent and -independent mech-

anisms. Interestingly, the oncogenic Ras, which is able to induce premature senescence, increases the p33ING1-mediated silencing function. Moreover, ING1-mediated silencing was reduced by co-expressing dominant negative Ras or by treatment with the MAPK inhibitor PD98059, but not by SB203580 an inhibitor of the p38 pathway. In addition, we show that both silencing domains of ING1 are involved in cell cycle control measured by inhibition of colony formation of immortalized cells and by thymidine incorporation of primary human diploid fibroblasts. Notably p33ING1 was shown to bind to trimethylated K9 of histone H3 suggesting that cellular senescence is controlled at epigenetic level.

P205

Broad phenotypic spectrum of neuromuscular disorders associated with defective O-glycosylation of alpha-dystroglycan

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Defects in O-glycosylation of alpha-dystroglycan result in a distinct group of autosomal recessive disorders with congenital muscular dystrophy (CMD) and associated brain and/or eye abnormalities. Patients with Muscle-eye-brain disease (MEB, OMIM 253280) present with early onset muscular hypotonia, severely compromised motor development and mental retardation. MR imaging reveals a lissencephaly type II with hypoplasia of the brain stem and cerebellum. MEB is associated with mutations in the POMGnT1 gene. We report about 9 MEB patients with POMGnT1 mutations from 8 families, including one patient with additional severe autistic features and one patient with an unusually mild phenotype, initially diagnosed as CMD. A severe hydrocephalus had been reported in a previous pregnancy of two families, pronouncing the phenotypic overlap with Walker-Warburg syndrome (WWS; OMIM 236670).

The classical WWS phenotype is caused by mutations in the POMT1 (OMIM 607423) and POMT2 (OMIM 607439) gene; mutations in Fukutin (607440) or FKRP (OMIM 606596) have been reported occasionally. We demonstrate the severe WWS phenotype of three cases with homozygous truncating POMT1 mutations including severe hydrocephalus. MR imaging of two patients in addition confirmed a cortical malformation with cobblestone lissencephaly and hypoplasia of brain stem as well as cerebellum.
Furthermore, we could recently identify a specific POMT1 missense mutation A200P in 5 Turkish families, resulting in limb-girdle muscular dystrophy, mild microcephaly and mental retardation (LGMD2K, OMIM 609308). Muscle weakness started after achievement of the first motor milestones, serum CK levels were increased more than 20fold, the IQ ranged between 50 and 65. For four patients a normal brain CT/MRI without any structural abnormalities could be obtained.
Comparison of the current clinical and molecular data suggests a clear genotype-phenotype correlation for disorders associated with POMT1, but not POMGnT1 mutations.

P206

Extended pedigree with multiple cases of XX sex reversal in the absence of SRY and of a mutation at the SOX9 locus

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It is well established that testicular differentiation of the human embryonic gonad depends on the action of the Y-chromosomal gene SRY. However, exceptional cases such as SRY-negative cases of 46,XX testicular disorder of sexual development (DSD) (previously known as 46,XX males) and of 46,XX ovotesticular DSD (previously known as 46,XX true hermaphrodites) document that testicular tissue can develop in the absence of the SRY gene. These SRY-negative XX sex reversal cases are very rare and usually sporadic, but a few familial cases have been reported. We present a large, consanguineous family with nine affected individuals with phenotypes ranging from 46,XX testicular DSD to 46,XX ovotesticular DSD, with predominance of male characteristics. Absence of SRY in peripheral blood was documented by fluoresence in situ hybridization (FISH) and PCR analysis in all nine affected individuals, and by FISH analysis on gonadal sections with testicular tissue in four affected individuals. By quantitative PCR, a duplication of the SOX9 gene was excluded. In addition, as linkage analysis showed that the nine affected members of the family do not share a common SOX9 haplotype, any mutation at the SOX9 locus could be ruled out. Together, these findings implicate a mutation at a sex-determining locus other than SRY and SOX9 as the cause for the XX sex reversal trait in this family.

P207

Townes-Brocks syndrome: 20 novel SALL1 mutations in sporadic and familial cases and refinement of the SALL1 hot spot region

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Townes-Brocks syndrome (TBS, OMIM#104780) is an autosomal dominant malformation syndrome characterized by renal, anal, ear and thumb anomalies caused by SALL1 mutations. To date 36 SALL1 mutations have been described in TBS patients. All but three of those, namely p.R276X, p.S372X, and c.1404dupG, have been found only in single families thereby preventing phenotype-genotype correlations. Here we present 20 novel mutations (12 short deletions, 5 short duplications, 3 nonsense mutations) in 20 unrelated families. We delineate the phenotypes and report previously unknown ocular manifestations, i.e. congenital cataracts with unilateral microphthalmia. We show that 46 out of the now 56 SALL1 mutations are located between the coding regions for the glutamine-rich domain mediating SALL protein interactions and 65 bp 3' of the coding region for the first double zinc finger domain, narrowing the SALL1 mutational hotspot region to a stretch of 802 bp within exon 2. Of note, only two SALL1 mutations would result in truncated proteins without the glutamine-rich domain, one of which is reported here. The latter is associated with anal, ear, hand and renal manifestations, indicating that the glutamine-rich domain is not required for a severe outcome.

P208

Functional analysis of Pelota during the cell cycle

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Mutation in either the Drosophila Pelota (Pelo) or the S. cerevisiae homologue, dom34 cause defects of spermatogenesis and oogenesis in Drosophila, and delay of growth and failure of sporulation in yeast. Both phenotypes are suggestive a requirement of Pelo for normal progression of the mitotic and meiotic cell cycle. To explore the function of Pelo in mammals, we have disrupted the mouse Pelo gene and shown that the gene is essential for normal mouse embryonic development. Development of homozygous embryos arrests about 6.5-7.5 days after conception. The failure of mitotic active inner cell mass (ICM) of the Pelo-/- blastocysts to expand in growth after 4 days in culture and survival of mitotic inactive trophoblast indicate that the lethality of Pelo null embryos is due to defect in cell proliferation. Increase of percentage of cells exhibiting polyploidy at E7.5 can be directly responsible for the arrested development and suggests that the Pelo is required for the maintenance of the genomic stability. To establish Pelo-/- cell lines and determine the consequence of Pelo deficiency on the cell cycle, we have generated a contitional knockout mice by using Cre/loxP recombination system. Using

Pelo- specific antibody, we found that Pelo is associated with cytoskeleton. Western blot analysis revealed the presence of Pelo in the cytoskeleton and membrane-fractions but not in nuclear and cytoplasmic fractions. These results suggest a possible role of Pelo in cytoskeleton organization and cell motility. Using yeast two hybrid system, we isolated several putative interaction partners of Pelota, which are associated with the cytoskeleton. To prove the interaction of the Pelota with our candidate genes, we have done immunoprecipitations, GST Pelota pull-down assay and live cells studies.

P209

One gene is not enough – dissecting the transcriptional network in Maturity Onset Diabetes of the Young (MODY), urogenital abnormalities and thyroid cysts

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The transcription factors HNF1α and HNF1β bind to DNA as homo- or heterodimers and usually display activating functions. HNF1α mutations are the most frequent cause of Maturity Onset Diabetes of the Young (MODY), extrapancreatic manifestations are usually not seen. HNF1β mutations result in Renal Cysts and Diabetes/MODY. However, neither gene is known to result in thyroid disorders. We present a MODY family with thyroid cysts and widely variable expressivity. Extensive urogenital abnormalities were exclusively seen in the proposita. HNF1α sequencing revealed the novel mutation c.526+1delGTAA that affects the canonic IVS2 donor splice site and segregates with the diabetic phenotype in this family. HNF1β mutation analysis showed the novel, non-conservative exchange c.1006C>G (p.His336Asp) that affects an evolutionarily highly conserved residue and disturbs the protein's transactivation domain. This change was detected in 1/400 tested chromosomes and is supposed to be pathogenic too. Interestingly, this mutation was found in the proposita and her younger sister, but not in the milder affected family members. We hypothesized further alleles to be involved to explain the thyroid phenotype of our family. In the HNF6 promoter, three variants were found in patients, but also in the unaffected mother and controls. Thyroid follicular cell development and expression of thyroid-specific genes are dependent on PAX8, mutations in which cause thyroid dysgenesis and follicular carcinomas. Close interdependencies have been shown with HNF1β. We identified in all patients and controls the non-conservative PAX8 missense change c.490G>A (p.Ala164Thr) that affects an evolutionarily highly conserved residue. In conclusion, we dissected the complex transcriptional network that most likely underlies phenotypic variability in this interesting family. To corroborate transcriptional interdependencies and the functional significance of changes, reporter gene assays are underway.

P210

Alternative splicing variants of the human MCPH1 gene

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Primary microcephaly (MCPH) is a heterogeneous autosomal recessive disorder characterized by a pronounced reduction of brain size and variable mental retardation without additional neurological deficits. Four genes have been identified to date whose biallelic mutations cause MCPH. A hallmark of patients with mutations in the MCPH1 gene is a cellular phenotype of premature chromosome condensation in the G2 phase of the cell cycle and delayed decondensation in the subsequent G1 phase. MCPH1 is located on human chromosome 8p23.1, consists of 14 exons and encodes the BRCT (BRCA1 C-terminus)-domain containing protein microcephalin. We performed RT-PCR with MCPH1-specific primers and analysed the products by agarose-gel-electrophoresis. Two prominent bands with different sizes were detectable - 2500 bp and 1500 bp, respectively. Sequencing revealed that the 2500 bp-band corresponds to a full length MCPH1-transcript while 1500 bp-product results from an MCPH1-variant lacking exon 8 which encodes a NLS. The skipping of exon 8 produces a new ORF which remains in frame with the ORF in full length MCPH1. The resulting polypeptide has a predicted molecular weight of approximately 50 kDa. These findings are consistent with earlier immunoblotting experiments which indicated the existence of more than one MCPH1-translational product. We cloned these two alternative splicing variants into retroviral vectors in order to analyse the complementation ability of MCPH1-deficient cells. Chromatin fractionation assays indicated the localization of microcephalin in the nucleic-acid-binding protein fraction. The absence of a NLS in the variant lacking exon 8 may point to a novel non-nuclear function of microcephalin. This as yet unknown function may involve the centrosome, since all other MCPH genes known to date encode proteins associated with the centrosome or spindle apparatus.

P211

A novel locus for autosomal dominant hereditary motor neuropathy on chromosome 4p11-p15.1

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The family CZ-HMN-M was recruited at the Charles University in Prague. The family comprises 12 members, among them 8 patients affected with autosomal dominant HMN (dHMN II). The age of onset is between 15 to 26 years initially with foot drop, distal weakness in the legs. Despite the severe distal leg weakness patients stay self ambulant without wheelchair even after the age of 50 years. The index patient is now 30 years old and shows pronounced steppage gait, patellar reflexes are increased, Achilles tendon reflexes are absent and Babinski sign is negative, sensation is normal. His mother, brother, sister, maternal uncle and his son and daughter are similarly affected. Electromyography at age of 29 years showed normal SNAPs and only motor lesion, a tibial CMAP amplitude of 0,1 mV, the nerve conduction velocity (NCV) was 32 m/s, the peroneal CMAP amplitude was at 3,4 mV, NCV 37 m/s, N. medianus CMAP amplitude at 10.5 mV and NCV at 41 m/s. The needle EMG revealed chronic neurogenic lesion, a lot of spontaneous activity and decreased number of motor units all being consistent with pure motor axonal polyneuropathy. Initially a CMT1A duplication or HNPP deletion was excluded by microsatellite analysis using 10 markers within the CMT1A region. Furthermore gene mutations in Cx32 (GJB1), myelin protein zero (MPZ, P0), heat shock protein 22 (HSP22) and HSP27 have been excluded prior to a genome wide analysis. The genome scan was performed with the 10 k microarray of Affymetrix and revealed a lod score of 2.4 in a 10 cM region on chromosome 4p11-p15.1. This locus was not yet associated with HMN or other types of hereditary motor and sensory neuropathies. Overall 97 genes are located in the critical interval, among them neurological important genes like GABA receptors. Sequence analysis is ongoing.

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P212

Cellular and genetic characterisation of a new chromosomal instability disorder

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A family with members showing an unusual profile of chromosomal instability was discovered during routine diagnosis of Nijmegen Breakage Syndrome (NBS [MIM 251260]). The index patient presented with microcephaly, growth retardation and recurrent infections. She, and a similarly affected brother, died of a malignancy. There is a further affected family member, a cousin of the index patient. Examination of cells after ionising irradiation or treatment with mitomycin C revealed only moderately increased rates of chromosome breakage. Spontaneous chromosome breakage, however, was consistently increased and manifested particularly as dicentric chromosomes. This is an unusual pattern for a chromosome breakage syndrome. Im-

munoblot examination of ATM, CHK2, SMC1 and p53 confirmed the impression of a DNA-repair-competent but chronically stressed cellular phenotype. Since the parents of the index case were consanguineous we were able to conduct a genome wide scan for homozygosity and thus identified a region on chromosome 12 as the most likely location of the underlying gene. Candidate genes in this region are currently being examined.

P213

Deficient expression of trefoil peptides in knock-out mice causes hearing loss

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Introduction: In mammals trefoil factor family (TFF) consists of three members: TFF1, TFF2 and TFF3. They are major secretory products of mucous epithelia and are usually expressed in association with mucins. They play a multifunctional role in cytoprotection, apoptosis, and immune response. They are predominantly expressed in the gastrointestinal tract but also in other tissues like respiratory tract, salivary glands, uterus, conjuctiva and brain. We show for the first time the expression of TFF2 and TFF3 proteins in the mouse inner ear and present data from hearing measurements of Tff3 and Tff2 knock-out animals.
Methods: We used quantitative real time PCR (qPCR) with primers specific for mouse Tff2 and Tff3 genes to analyze their expression in the inner ear. To show the localization of both proteins immunohistochemistry using antibody specific for mouse TFF2 and TFF3 proteins was performed. Auditory evoked brainstem responses (ABR) were measured in wild-type and Tff3 ko animals aged 3 weeks up to 1 year and on 3 months old Tff2 ko animals to assess possible functional changes.
Results: Data obtained from qPCR pointed to a specific expression pattern. Immunohistochemical experiments demonstrated TFF2 in the spiral ligament and TFF3 in the stria vascularis of the inner ear. ABR measurements revealed that young Tff3 ko mice showed a flat hearing loss of about 15 dB below 10 kHz when compared to age matched wild-type animals. At one year of age ko mice showed a flat hearing loss of about 30 dB below 10 kHz and an increasing loss towards higher frequencies (50 dB at 32 kHz). At this age wild-type animals had a high-frequency loss of about 25 dB. Tff2 ko mice showed normal hearing at 3 month of age.
Conclusions: Beside secreting epithelia of various organs, trefoil factor 2 and 3 are also expressed in the inner ear. Tff3 ko mice show an accelerated presbyacusis and a flat hearing loss below 10 kHz. The situation in Tff2 ko mice still needs to be evaluated.

P214

ICR1 epimutations in 11p15 are restricted to patients with Silver-Russell syndrome features

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(Epi)mutations affecting chromosome 11p15 are well known to be associated with growth disturbances. The finding of 11p15 mutations in the overgrowth disease Beckwith-Wiedemann syndrome leads to the identification of imprinted growth-promoting genes which are expressed paternally and of imprinted growth-suppressing genes in the same region that are expressed maternally. An opposite epimutation in the same region is associated with Silver-Russell syndrome (SRS), a growth retardation disorder characterised by a typical facial gestalt, clinodactyly V and asymmetry. In more than 30% of SRS patients, hypomethylation of the telomeric 11p15 imprinting domain (ICR1) can be detected. However, the general significance of this epimutation for human growth retardation was unclear. In a previous study1 we showed that the ICR1 epimutation is not present in growth retarded patients with dysmorphisms not typical for SRS, but its role in the development of isolated growth restriction needed to be further elucidated.
We therefore screened 30 patients with isolated pre- and postnatal growth retardation (IUGR/PNGR) and 65 patients diagnosed as SRS by external clinicians for ICR1 epimutations. These 65 SRS patients were additionally analysed for maternal uniparental disomy 7 (matUPD7).
We excluded the ICR1 hypomethylation in all 30 patients with isolated growth retardation. In the SRS group, we detected 4 cases with ICR1 epimutation and 3 with matUPD7.
By combining our data with those from our previous study we could show that the hypomethylation of ICR1 in 11p15 is indeed restricted to patients with SRS features and can be neglected in isolated IUGR/PNGR. Thus, testing for the epimutation is indicated only in patients clinically diagnosed as SRS. The low detection rate of the ICR1 epimutation in our SRS group might be influenced by inclusion of cases with suspected SRS only referred by external institutions.

P215

RNA analysis improves the quality of genetic testing in patients with retinoblastoma

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The biological interpretation of mutational consequences are based on assumptions regarding the effect on the transcript level. To test the validity of these assumptions we have studied 7 point mutations in 3'-intronic sequences, 10 point mutations in splice donor regions, 5 point mutations in exons, and 6 gross alterations using RT-PCR and sequence analysis on RNA obtained after inhibition of nonsense meditated decay. Furthermore, to find out if RNA analysis may aid in mutation detection we analyzed 9 patients in whom no alteration had been identified in DNA based tests. Most mutations at proximal splice sites resulted in skipping of the nearest exon. However, the effect was leaky for some mutations and this was associated with incomplete penetrance. Remote 3'-intronic mutations caused intron inclusion (at -12, -13, -14) or exon skipping (-26A>T). A new germline mutation at –27 (T>C) showed no effect on transcript composition. None of 4 exonic missense and inframe mutations tested showed an effect on splicing. A recurrent silent mutation created a new donor site with exon exclusion. In two patients with mutational mosaicism for gross alterations, RNA analyses verified the presence of the alteration in peripheral blood. RNA based mutation screening showed inclusion of cryptic exons in 2 of 9 patients. Sequencing of the flanking genomic DNA revealed new splice donor sites caused by deep intronic mutations (IVS23-1401A>G and IVS6–3416A>G). Our data point out that qualitative (transcript composition) and quantitative (relative abundance) analyses of RNA are important for a valid biologic interpretation. Moreover, gross alterations, which can be difficult to verify by quantitative methods (e.g. MLPA), are reliably verified if they result in structurally aberrant transcripts. Finally, transcript analysis helps to identify mutations remote from the regions covered by conventional surveys and, therefore, reduces the number of missed mutations.

P216

Hydroxysteroid dehydrogenase type 10 (HSD10) in embryonic development and neurodegeneration

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Hydroxysteroid dehydrogenase type 10 (HSD10, also denoted ERAB, ABAD or MHBD) is mutated in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, a neurodegenerative childhood disorder. It is also implicated in the pathogenesis of Alzheimer`s Disease where it is thought to mediate neurotoxic effects of amyloid- β. HSD10 is involved in isoleucine metabolism but may have additional, as yet undefined functions. It was previously shown that HSD10 mutants (Scully) in Drosophila are embryonic lethal. For further characterization of HSD10 function in early embryonic development, we chose Xenopus as an animal model and identified HSD10 orthologues in Xenopus laevis and Xenopus tropicalis. HSD10 mRNA is provided maternally and expressed throughout embryonic development. Antisense Morpholino oligonucleotides which block translation of Xenopus HSD10 mRNA in early embryos resulted in defects of neural tissue. Morpholino knock-down in the neuroectoderm caused enhanced apoptosis at tadpole stages. Rescue experiments in which mutated human HSD proteins are expressed in HSD10 depleted Xenopus embryos will help to separate the distinct functions of this protein and may hopefully help to clarify the pathogenesis of neurodegeneration both in

MHBD deficiency and possibly in Alzheimer's disease.

P217

Novel variants in VKORC1 - the target protein of cumarin-type anticoagulants - in rodents from warfarin-resistance areas in Europe, Asia and America

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Cumarin derivatives, e.g. warfarin, are in worldwide use as effective anticoagulants for therapy of thrombo-embolic diseases in humans as well as for rodent pest control since they effectively inhibit blood coagulation. However, patients under anticoagulant therapy as well as rodent populations have developed resistance to cumarins soon after the introduction of these compounds. Today, in many countries, effective rodent pest control is hampered by the rapid spreading of warfarin-resistant populations.

Recently, the target enzyme for cumarins has been identified: VKORC1 (vitamin K epoxide reductase complex subunit 1), the key component of the vitamin K cycle, is capable of reducing vitamin K epoxide and is inhibited by warfarin. Mutations in VKORC1 have been shown to confer resistance to anticoagulants of the cumarin-type in humans and rodents. In rats and mice independent mutations have arisen in different warfarin-resistance areas throughout the world and affect different amino acid positions of the VKORC1 protein. Here, we report on the analysis of the VKORC1 gene in more than 100 DNA samples of rats and mice from resistance areas in Europe, Asia, and America. More than 20 different mutations have been found demonstrating a high degree of sequence divergence in both species. Some variants do not alter the amino acid sequence of the protein and are shared by animals from distant populations. Thus, they are likely to represent neutral polymorphisms. Other mutations, however, result in amino acid substitutions and are predicted to alter the protein structure. These variants were investigated in a VKOR activity assay after recombinant expression in a HEK293 cell system. Most of these recombinant proteins showed a functional impairment of VKOR activity and / or warfarin sensitivity.

Mutation studies in the VKORC1 gene are an important basis to understand the structure and function of VKORC1 also in view of an improvement in anticoagulation therapy in humans.

P218

Improvement of SMA diagnostics by use of MLPA

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Infantile spinal muscular atrophy (SMA types I-III) is a common autosomal recessive neuromuscular disorder caused by deletions/mutations of the SMN1 gene. While in the majority of cases homozygous deletions of SMN1 exons 7 and 8 can be detected, the deletion may also be restricted to exon 7. A homozygous deletion of the highly homologous SMN2 gene is not associated with clinical consequences. However, in the absence of SMN1, SMN protein of at least one SMN2 copy is necessary to ensure fetal development. We present two SMA patients where the initial PCR based deletion screening gave misleading results. Further analysis by multiplex ligation-dependent probe amplification (MLPA) revealed more complex underlying genetic mechanisms. In patient 1 with typical SMA type I, initially a homozygous deletion restricted to SMN1 exon 7 was found. However, MLPA revealed a heterozygous deletion of both exons 7 and 8 of the SMN1 gene. Sequencing of the patient`s DNA revealed a new splice site mutation, IVS6-2A>G, within the deletion test primer region. The patient is now considered to be compound heterozygous for a maternal SMN1 deletion and a paternally inherited point mutation. In patient 2, SMN1 deletion screening did not show a homozygous SMN1 deletion but SMN2 exon 8 was homozygously deleted. The diagnosis of SMA5q was thought unlikely but subsequently performed MLPA showed heterozygous deletions of both SMN1 and SMN2 exons 7 while two SMN1 exon 8 copies were retained. There is good evidence to believe that she might carry the typical SMN1 gene deletion on one allele, a yet unknown point mutation on the other allele, and an unusual SMN2 hybrid gene. To conclude, the additional information gained by MLPA prompted us to revisit the genetic diagnosis which had major consequences for the patients and their families. Since MLPA offers the option to determine the SMN1 and SMN2 copy numbers in one assay we think that it will become the method of choice in SMA diagnostics.

P219

Transcriptional and cellular analysis of the Cohen syndrome gene, COH1

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Cohen syndrome is a rare autosomal recessive disorder, clinically highly variable and mainly characterised by mental retardation, microcephaly, facial dysmorphism, pigmentary retinopathy, and intermittent neutropenia. Cohen syndrome is associated with mutations in the gene COH1.

COH1 comprises a genomic region of 846 kb on chromosome 8q22 and up to 62 exons. It was described to encode various splice forms. By detailed analysis of human and mouse tissues we identified one transcript variant of COH1, including exon 28b instead of exon 28. Northern blot analysis of different human tissues confirmed these results. Only kidney, brain, placenta, and small intestine gave a signal at 12-14 kb. However, the tissue-specific COH1 mRNA levels and assumed ubiquitous expression have still to be analysed by qPCR.

For further functional analysis we raised four different peptide antibodies against human COH1. Moreover, we cloned COH1 codons 1-3682 into a mammalian expression vector. Hitherto, three antibodies could be validated by immunoblotting of COH1-FLAG protein lysates. Analysis of membrane preparations from human pons, cerebellum, and platelets revealed a distinct band at ~450 kDa. Additionally, immunofluorescence microscopy showed cytosolic vesicle-like as well as weak plasma membrane staining of COH1 in human cell lines. The same distribution was found after transient expression. Using organelle marker proteins, co-localisation was observed with α-adaptin, a subunit of adaptor-related protein complex 2. However, the fourth, C-terminal antibody showed strong staining of the Golgi-apparatus by co-localisation with γ-adaptin. These findings correspond with the partial homology to Vps13p, a membrane-associated intracellular sorting protein in yeast. In conclusion, our results indicate a role for COH1 in the endosomal protein sorting pathway. Identification of binding partners by yeast two-hybrid analysis and immunoprecipitation will further clarify the molecular nature of COH1.

P220

The Shh regulatory mouse mutant Dsh (short digits) represents a model for Holoprosencephaly

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Holoprosencephaly (HPE) is a congenital malformation of the developing forebrain which is characterized by a failure of the forebrain to separate into two hemispheres and associated craniofacial malformations. SHH mutations cause a subset of genetic HPE cases in humans. During early stages of CNS development Shh is expressed in the notochord, the floor plate and the prechordal plate, where it regulates patterning of neural precursor cells. At later stages Shh expression in the medial ganglionic eminence and the subventricular zone is essential for the induction of ventral neurons, oligodendrocyte precursors and progression of axonal growth cones. Here we present the mouse mutant Short digits (Dsh), which is caused by an 11.7 Mb inversion comprising the Shh locus. The inversion separates Shh from its long-range enhancers BE2, BE3 and BE4. Dsh/Dsh mice display the full HPE spectrum, strongly resembling Shh-/- mice. In Dsh/Dsh mutants Shh expression is severely reduced but increases to about 30% of wt level at E13.5. In contrast to Shh+/- mice, which have an inconspicuous phenotype Dsh/+ mice show a limb reduction phenotype caused by a late ectopic Shh over-expression in the digits. In addition Dsh/+ mutants exhibit a brain phenotype characterized by improper midline formation, a migration defect of callosal axons and a skull defect of frontal bones. Preliminary data indicate

that expression of Shh and its targets is reduced in the prechordal plate at E10.5 in Dsh/+ mutants. In addition, first experiments indicate, that the axon guidance molecule Semaphorin 3C (Sema3C) which is also included in the Dsh inversion is ectopically expressed in the prechordal plate of the Dsh/+ mutant. As Sema3C is a chemorepellent for axon growth cones, its ectopic expression may explain the callosal migration defects of the Dsh/+ mutant.

P221

Genetic heterogeneity of NOTCH2 in patients with ALGS

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Alagille syndrome (ALGS1) is an autosomal dominant multisystemic disorder with chronic cholestasis, congenital heart anomaly, skeletal defects, eye and kidney abnormalities and a characteristic face. Approx. 80% of the cases are associated with mutations of Jagged 1 (JAG1). The disease of the remaining cases remains unexplained so far. McCright et al. (2002) studied the phenotype of transgenic mice double heterozygous for Jag1 and Notch2 knockouts and suggested that Notch2 , a JAG1 receptor of the NOTCH signalling pathway should be a candidate/modifier gene for ALGS. And recently, McDaniells et al. (2006) provided evidence for such a role of NOTCH2 by reporting a splice site mutation and an amino acid substitution, respectively, in 2 ALGS families without a JAG1 mutation. Accordingly, we investigated a sample of 103 patients with the clinical diagnosis of ALGS by screening for NOTCH2 sequence variants (SSCP and sequencing analysis). Of the 51 genomic NOTCH2 variants identified in our study 3 SNPs (c.3980A>G, c.6412C>T, c.7341T>A) had been described already by McDaniells et al. (2002). Of the remaining 48 variants 32 were detected in patients as well as in controls and hence might be considered as SNPs. The 16 unique mutations observed in patients all affected functionally relevant protein domains such as the region of the signal peptide, the EGF-like repeats as well as the intracellular NOTCH2 domain. Altogether, 11 of these 16 novel mutations created an amino acid exchange presumably influencing NOTCH2 receptor function including the interaction with its ligand JAG1. Our findings underline the assumption that NOTCH2 appears to be involved in Alagille syndrome, as the disease causing gene and/or as a modifier of the heterogeneous clinical phenotype.

P222

A novel initiation-codon mutation (M1V) in the GDAP1-gene causes Charcot-Marie-Tooth 4A (CMT4A) disease

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Charcot-Marie-Tooth disease is a genetically heterogeneous group of disorders, characterized by distal muscle wasting and weakness, usually accompanied by skeletal deformities. Genetic heterogeneity of the disease is underlined by 21 genes identified so far, and also by different patterns of inheritance. Here we report on a consanguineous family from Oman with three affected children, initially diagnosed with having progressive muscular dystrophy of unknown origin. To localize the disease gene we performed homozygosity mapping using the 10K Affymetrix SNP chip for a whole genome scan. Significant linkage with a maximum LOD score of 2,5 was found on chromosome 8q12 – 8q21. The most likely candidate gene was the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) mutated in CMT4A disease. Sequencing of the GDAP1 gene revealed a novel mutation affecting the initiation codon methionine - M1V (ATG>GTG). The mutation affects only the isoform 1 of the GDAP1 gene. The second isofom contains an alternate in-frame exon and uses a start codon 68 aminoacids downstream. To determine the functional consequences of the M1V mutation analyses at the protein level are in progress.

P223

Pathogenesis in 2-methyl-3-hydroxybutyryl-CoA-dehydrogenase (MHBD) deficiency is unrelated to enzyme function

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17β-hydroxysteroid dehydrogenase type 10 (HSD10, also denoted ERAB, ABAD or MHBD) is a mitochondrial enzyme involved in the beta-oxidation of isoleucine. It has been implicated in two neurodegenerative disorders: (1) HSD10 binds amyloid-β and has been reported to mediate mitochondrial dysfunction in the pathogenesis of Alzheimer disease; (2) Mutations in the HADH2 gene coding for HSD10 cause 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, an unusual childhood disease with a neurodegenerative course. Most known patients are hemizygous for the same recurrent mutation R130C in the HADH2 gene; this mutation does not involve a hypermutable CpG site and the reason for its high frequency is unknown. Observations in two novel families with MHBD deficiency now indicate that the clinical features are unrelated to the enzymatic function of HSD10 but are mediated through an as yet uncharacterised protein function. One boy presented in the neonatal period with evidence of severe mitochondrial dysfunction; he died with progressive hypertrophic cardiomyopathy at the age of seven months. He was hemizygous for a novel mutation D86G in the HADH2 gene; enzyme studies in two different laboratories showed high residual activity of approx. 30 %. Two cousins in the other family had no residual enzyme activity in fibroblasts and were hemizygous for a novel mutation Q165H in the HADH2 gene. One of them had severe feeding problems but is neurologically normal at age 3 years whilst his cousin is completely asymptomatic at age 6 years. Work is in progress to characterise the role of HSD10 in neurodegeneration both in MHBD deficiency and Alzheimer disease.

P224

Mutation detection rate in female patients with reduced factor VIII activity and negative family history for hemophilia A

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The factor VIII activity (FVIII:C) is often reduced in female carriers of mutations in the F8 gene. Consequently these women are prone to bleeding complications after surgery or trauma. Screening for molecular defects in the factor VIII gene is therefore usually performed in female patients with reduced FVIII:C in order to determine the risk for haemophilia A by male offspring.

Here we report on the analysis in 20 women with FVIII:C $\leq$50% (10-50%) and a negative family history for haemophilia A. All patients were screened for the intron 22 and intron 1 inversion, as well as for gross rearrangements in the F8 gene. Finally, sequencing analysis of the coding region including the intron/exon boundaries was performed.

In three of the patients we identified pathogenic mutations. Two amino-acid changes - c.1636C>T (R527W, exon 11, A2 domain) and c.5899G>T (G1948C, exon 18, A3 domain) - were detected in women with FVIII:C 27% and 33% respectively. One splicing error, IVS22+5G>A, predicted to destroy the donor splice site of intron 22 was found in an young girl with FVIII:C of 40%. This mutation was present in patients' mother, who had normal FVIII:C. Using semiquantitative analysis of microsatellite markers located on the X-chromosome we were able to show that the paternal X-chromosome is preferentially inactivated in the patient.

Fifteen percent of our patients with reduced FVIII:C and negative family history were shown to be carries for the haemophilia A. Our analysis failed to detect a mutation in 17 individuals (85%). These patients could be heterozygous carriers of mutations in the intronic regions or, most likely, the reduction of the F8 activity is due to other (probably unknown) reasons.

P225

Mutation screening in the IRF6-Gene in central European families with non-syndromic cleft lip with or without cleft palate (CL/P) and/or cleft palate only (CPO)

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Introduction: Orofacial clefts belong to the most common congenital disorders, with a prevalence among Caucasians from 1/500 to 1/1000. Orofacial clefts occur either as part of complex malformation syndromes or as an isolated anomaly. Non-syndromic cases represent about 50-60% of all orofacial clefts and have a multifactorial etiology encompassing both genetic and environmental components. Van-der-Woude syndrome (VWS) is an autosomal dominant disorder caused by mutations in the IRF6-gene. The anomalies seen in individuals with VWS are lower-lip pits and/or CL/P and/or CPO. The overall penetrance was estimated as 92% with a very variable expressivity even within families.
Hypothesis: We hypothesized that some of the cases with apparently non-syndromic CL/P or CPO who have a positive family history suggestive of autosomal-dominant inheritance might be attributed to IRF6-mutations.
Method: A total of 62 families with two or more affected individuals (CL/P or CPO; no other anomalies) affecting at least two consecutive generations were recruited throughout Germany. All participating families were clinically assessed by one of two medical geneticists. One affected per family was screened for mutations in IRF6 by direct sequencing of exons 1-9.
Results: In 2 families pathogenic frameshift-mutations were detected. In Family 1 four individuals in 3 generations are affected with either cleft lip and alveolus or complete cleft lip and palate. In Family 2 there is an affected mother with a submucous cleft of the soft palate and her daughter with a bilateral complete cleft lip and palate. In both families none of the affected persons and none of the relatives had any lip pits.
Conclusion: IRF6 mutation analysis should be discussed in families with two ore more persons affected with CL/P and CPO compatible with autosomal-dominant inheritance.
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Expression profiling of miRNAs in a cell culture model of SCA3 indicates significant changes in the expression levels of several miRNAs upon expansion of polyglutamine repeats in ataxin-3

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Spinocerebellar ataxia type 3 (SCA3) belongs to a group of clinically and genetically heterogeneous autosomal-dominant neurodegenerative diseases. In the responsible gene (MJD1) a CAG trinucleotid expansion has been identified which result in a polyglutamine expansion of the encoded ataxin-3 protein. It was proposed that the sequestration of transcription factors in protein inclusion bodies lead to the perturbation of basal mRNA transcription. However, up to now there is no information available about the expression of microRNAs in the context of SCA3. MicroRNAs are small, between 19-23 nucleotide long non-coding RNA molecules, which are predicted to directly regulate the expression of at least 30% of all human protein-encoding genes and act in the control of important biological process like development, neurogenesis and cell cycle control. We used stably transfected SK-N-AS cells (human neuroblastoma) overexpressing normal and pathogenic ataxin-3 with 15 and 148 CAG repeats, respectively. Changes in the expression levels of 384 miRNAs were measured using custom oligo arrays. The GeneChip® Human Exon 1.0 ST Array (Affymetrix) with its 1.4 million Probe Sets offers the possibility for the detection of around 1 million Exons and indicate a new dimension of expression and alternativ splicing analysis. We monitored the global changes expression and found 188 differential expressed mRNAs (Fold Change $>$ 1.5 and p-value $<$ 0.05). Differential expression of 30 significant mRNAs and 4 miRNAs was verified by qRT-PCR using the Light Cycler 480 system (Roche). We identified several differentially expressed mRNAs that are potential targets of the differentially expressed miRNAs. The correlation of changes in miRNA and target mRNA expression was tested in native SK-N-AS and SH-SY5Y cells by transfection of mature miRNAs and miRNA antagonists. Furthermore, we analyzed protein levels of selected target genes with western blots and immunohistochemistry.

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The two most common alleles of the coding GGN repeat in the androgen receptor gene cause differences in protein function

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Genetic studies have reported association of a polyglycine-encoding GGN repeat in exon 1 of the androgen receptor gene with common human traits. The polyglycine tract is located in the transactivating domain of the androgen receptor protein, suggesting an effect of repeat length on receptor function. Here we compare the functional characteristics of the two most common alleles (23 and 24 repeats) and two extreme alleles (10 and 27 repeats) in a reporter gene assay in HeLa cells. A correlation between the repeat length and AR activity was observed. This is attributable to both a higher protein concentration, determined by ELISA, and a higher per-protein activity of long repeat alleles. Interestingly, protein concentration does not correlate with transcript quantity, determined by real time PCR assays, suggesting an influence of repeat length on protein stability. In conclusion, our data provide evidence of functional differences between the two most common alleles of the AR GGN repeat, supporting its direct role in the development of human traits.

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The SUZ12 sequences are preferred regions of non-allelic mitotic recombination causing NF1 microdeletions

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder caused by defects of the tumor suppressor neurofibromin. While the majority of NF1 patients have intragenic mutations in the NF1 gene, large deletions of the NF1 gene region in 17q11.2 occur in 5% of patients. Most frequently, these deletions encompass 1.4 Mb (type-1) and the breakpoints are located in segmental duplications, the NF1-LCRs. Type-1 deletions are caused by nonallelic homologous recombination (NAHR) between the NF1-LCRs during maternal meiosis. Remarkably, ~70% of the type-1 deletions have breakpoints in a confined 2-kb breakpoint hotspot. The second most frequent type of NF1 deletions (type-2) span 1.2 Mb with breakpoints in the SUZ12 gene and its pseudogene, which map close to the NF1 LCRs. Importantly, 13/14 type-2 deletion patients investigated so far are somatic mosaics. We have characterized the deletion breakpoints in these 14 patients and identified a clustering of breakpoints in SUZ12 intron 5. However a very confined hotspot of NAHR as characteristic for the type-1 deletions which occur during meiosis does not account for the type-2 deletions which are mediated by mitotic NAHR. The reasons for the spatial difference of mitotic versus meiotic NAHR in the NF1 gene region are unknown. Conspicously, all 13 mosaic type-2 deletions

were observed in female patients. This preponderance suggests that the SUZ12 gene is more prone to mitotic aberrant recombination in females than in males. In order to investigate whether hypomethylation is associated with the enhanced non-allelic mitotic recombination frequencies, we investigated the methylation pattern of the SUZ12 gene and pseudogene sequences comparatively in female and male tissues. However, since we did not identify significant sex-specific differences in methylation patterns of the SUZ12 sequences, other factors like differential DNAse hypersensitivity might account for the observed mitotic instability causing type-2 deletions.

P229

Polyalanine repeat expansion in embryonic gene Hoxd13: Molecular basis of synpolydactyly variability

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Hoxd13 belongs to a cluster of homeobox containing embryonic genes and plays a major role during limb development in humans and several other species. Expansion mutations (+7Ala to +14Ala) of the poly-alanine tract (15Ala in wt) in the N-terminal domain of Hoxd13 cause the autosomal dominant hand malformation synpolydactyly (SPD) in humans. While the penetrance and the severity of the synpolydactyly correlates with the expansion length of the poly-alanine tract the underlying molecular basis for phenotype variability is not completely understood yet. We have investigated the synpolydactyly homologue (spdh), which corresponds to a homozygous Hoxd13 +7Ala mutant, as a model for human SPD. Cell experiments revealed that the spdh protein is transported into the nucleus like wt protein, while constructs with longer expansions of the alanine-repeat (+14 and +21) accumulate extranuclear. Interestingly, the protein accumulations increased with rising number of alanines. This could be an explanation for the increase of penetrance and severity of the phenotype in SPD patients. There is strong evidence that phenotype variability of Hoxd13 mutation is influenced by protein interactions depending on poly-alanine length. Our aim is to elucidate the underlying molecular basis of SPD variability and to identify phenotype modifiers.

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Siver-Russell syndrome does not belong to the group of hypomethylation syndromes

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Silver-Russell syndrome (SRS) is a disorder mainly characterised by intrauterine and postnatal growth retardation (<P3), a typical craniofacial gestalt, asymmetry and clinodactyly V. More than 35% of patients show demethylation of the telomeric imprinting centre region (ICR1) in 11p15, a region which is also involved in Beckwith-Wiedemann syndrome. In addition, in ~10% of SRS cases a maternal uniparental disomy 7 (mUPD7) can be detected.
The transient neonatal diabetes mellitus (TNDM) is another disease associated with an imprinting defect: in many cases it is associated with a loss of methylation (LOM) at the ZAC/HYMAI domain on 6q24. Although a close correlation between epigenotype and phenotype was assumed, Mackay et al. (1) recently presented two patients with TNDM who had a LOM at the centromeric imprinting centre region (ICR2) in 11p15 in addition to their imprinting defect in 6q24. In a further study they reported on six patients with TNDM who showed additional hypomethylation of other imprinted regions than 6q24 and ICR2 (2). Mackay et al. therefore postulated the existence of a maternal hypomethylation syndrome. Similar findings were reported by Rossignol et al. (3) for BWS patients who carry imprinting defects additional to 11p15 epimutations.
Due to these observations we analysed the methylation patterns of several imprinted regions in 10 SRS patients with mUPD7 and 22 patients with LOM in the ICR1 on 11p15. In none of the investigated cases a methylation defect of the imprinted regions in 6q24, 14q32 or in the ICR2 in 11p15 could be detected. These results demonstrate that a spectrum of different epigenetic defects does not exist in the groups of SRS with LOM of the ICR1 or mUPD7 and that these subentities do not belong to the diseases with a general hypomethylation such as TNDM.
(1) Mackay et al., Hum Genet (2006) 119:79-184
(2) Mackay et al., Hum Genet (2006) in press
(3) Rossignol et al., J Med Genet (2006) 43: 902-907

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Reduced endocytosis of mannose-6-phosphate receptors in fibroblasts of patients with Lowe syndrome

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Oculocerebrorenal syndrome of Lowe (OCRL) is a rare X-linked disease characterized by bilateral congenital cataract, severe hypotonia, mental retardation, and a proximal renal tubulopathy. Lowe syndrome is caused by mutations in the OCRL gene, encoding a phosphatidylinositol 4,5-bisphosphate [PtdIns(4)P] 5-phosphatase which catalyzes hydrolysis of PtdIns(4,5)P2 to phosphatidylinositol-4-phosphate. OCRL1 localizes to the Golgi apparatus, early endosomes and is also present at the trans Golgi network (TGN). It is supposed to play a role in clathrin-mediated vesicular trafficking between endosomes and the TGN.
Although PtdIns(4,5)P2 is important for formation and transport of plasma membrane derived vesicles, phosphoinositide dephosphorylation is most likely required for endocytic vesicle fission and rapid dissociation of endocytic factors. Thus, ectopic accumulation of PtdIns(4,5)P2 in OCRL1-deficient cells may result in an impaired endocytic process. The aim of the present study was to examine the ability of Lowe fibroblast cells to endocytose mannose-6-phosphat (M6P)-containing lysosomal enzymes. These ligands are internalized by 300 kDa M6P receptors. In five out of six fibroblast cell lines of patients with Lowe syndrome and a defined OCRL mutation, no OCRL1 protein was detectable by western blotting, while a truncated OCRL1 protein was present in one cell line. We identified a reduced endocytosis of M6P receptors in Lowe fibroblast compared with control cells. The decreased capacity to internalize ligands appears to be the result of a reduced number of M6P receptors at the cell surface. Further experiments will focus on investigating total expression and intracellular distribution of M6P receptors as well as to study the endocytic process of other receptors.

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Htra2-beta1 – a splicing regulator of full-length SMN2 transcripts: functional studies in transgenic and knock out mice

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Spinal muscular atrophy is an autosomal recessive disorder caused by homozygous loss of the Survival Motor Neuron gene SMN1, leading to degeneration of α- motor neurons in the spinal cord. Patients retain at least one of a nearly identical copy of the gene - SMN2. Due to alternative splicing most of the transcripts generated from SMN2 lack exon 7, resulting in SMN protein deficiency. The remaining correctly spliced transcripts are not successful to compensate the loss of SMN1 in patients. We have already shown that the SR-like splicing factor Htra2-ß1 restores the correct splicing of SMN2 exon 7 in vitro. The protein is therefore a promising candidate for an in vivo modulation of SMN RNA processing to serve as a therapeutic tool to prevent SMA. Compared to humans, mice possess only one Smn gene whose loss is embryonic lethal. Transgenic Smn-/- mice carrying the human SMN2 show an SMA-like phenotype. We have generated mice overexpressing the Htra2-ß1 neuronal specific, which are phenotypically normal. Our final goal is to create Smn-/-, SMN2+, Htra2-ß1+ animals to investigate if Htra2-ß1 rescues the SMA phenotype in vivo. Further we have developed a knock-out strategy for the murine homologue Sfrs10 via the Cre/loxP system to investigate the function of Tra2-ß1. We have previously excluded Sfrs10 splice variants in tissues of interest. Recently we have succeeded in generating heterozygous knock out mice by cross breeding germ line transmitted mice with a Cre deleter strain. The Sfrs10+/- mice seem to be phenotypically normal. We finally want to generate and analyse Sfrs10-/- mice. The Cre/loxP system offers various possibilities for further investigations. Depending on the phenotype of Sfrs10-/- mice we are planning a motorneuron specific deletion of Sfrs10 via an Hb9-Cre deletion strain. Since we

could show that reduced SMN protein level also leads to reduced Htra2-beta1 protein level the construction of Sfrs10 knock-out mice is of particular interest.

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Functional characterization of CCM1 missense mutations

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Cerebral cavernous malformations (CCM) are prevalent vascular lesions characterized by enlarged immature capillaries. Clinical symptoms include recurrent headaches, seizures, and stroke. Autosomal dominantly inherited forms are caused by loss-of-function mutations in three genes, CCM1/KRIT1, CCM2/OSM, and CCM3/PDCD10. A two-hit model for CCM pathogenesis was thus far only proven in a singular lesion. Another study described two different somatic CCM1 missense mutations of unknown relevance in DNA from CCM tissue of an isolated case. It is striking that both mutations are the only known missense mutations among the more than 100 truncating mutations described for the CCM1 gene to date. These two missense mutations could therefore indicate specific functions for the two amino acid residues. Furthermore, confirmation of the two-hit model is important since the cytoarchitecture of CCMs barely enables molecular analyses.
In order to verify whether the two missense mutations are in fact disease-causing, we have generated and expressed Flag-, HA-, Myc-, and His-tagged CCM1 and CCM2 in HEK293 cells. Co-expression and -immunoprecipitation of CCM1 and CCM2 confirmed a direct interaction of these two proteins in vitro. Mutagenesis of F97 and K569 in CCM1 did not disrupt CCM1/CCM2 association. Thus, the two residues do not appear to be essential for direct interaction of CCM1 and CCM2. However, our results do not rule out other specific functions for phenylalanine at position 97 and lysine at position 569 of CCM1.

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Analysis of the SLC26A4 gene in Czech patients with early non-syndromic hearing loss - a pilot study

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The most common cause of non-syndromic hearing loss (NSHL) is biallelic pathogenic mutations in the GJB2 (about 40% cases). In foreign countries the SLC26A4 mutations were discovered as the second most frequent cause of early NSHL comprising up to 10% cases. The spectrum of phenotypic manifestation of the SLC26A4 mutations vary from NSHL with Enlarged Vestibular Aqueduct (EVA) and/or Mondini dysplasia (MD) on HRCT of the temporal bone (DFNB4) to Pendred syndrome (PS), where the picture of DFNB4 is accompanied by goiter in early adulthood.
Objectives of our pilot study were to determine the presence, frequency and spectrum of the SLC26A4 mutations in Czech patients with early NSHL and test our chosen diagnostic criteria.
From the group of 238 patients with AR NSHL (all GJB2 negative) we chose 13 unrelated patients with highest probability of SLC26A4 mutations according to clinical data and CT/MRI findings. We performed direct sequencing of all 21 exons of the SLC26A4.
Detailed retrospective evaluation of all 108 available CT/MRI scans of NHHL patients revealed 1 MD, 2 Mondini-like pictures, 11 EVA (10%) and 64 EVA/MD negative scans. Among 13 selected patients we detected 8 different mutations in 6 patients (46%). Pathogenic mutations in both gene alleles were found in 5 patients (38.5%) - all compound heterozygotes. Each of these mutations has been already decribed as disease causing. All patients with pathogenic mutations showed bilateral EVA associated with progressive or severe to profound HL. Unilateral EVA, MD or Mondini-like was observed only in association with wild type alleles. Both adult subjects with initially normal thyroid function showed hypofunction goiter recently.
This pilot study shows high incidence of SLC26A4 mutations in Czech patients with NSHL and particularly that the mutation detection rate among patients can be high using our evidence-based clinical selection criteria.

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Large germline deletions and duplication in cerebral cavernous malformation

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Cerebral cavernous malformations (CCM) are frequently caused by autosomal dominant mutations in three genes, CCM1, CCM2, and CCM3. A mutation detection rate of 94% is achieved for familial CCM if DNA- and RNA-based screening techniques are used. At least 57% of isolated cases with multiple lesions also harbour detectable mutations in CCM1, CCM2 or CCM3. One explanation for the lack of detectable mutations in the remaining patients is the existence of large genomic rearrangements which escape exon-by-exon sequencing.
We have applied the multiplex ligation-dependent probe amplification (MLPA) technique which permits the detection of such large genomic rearrangements. In our cohorts, direct sequencing did not reveal a mutation in 2 out of 10 German familial cases and 9 out of 14 isolated Swiss cases with multiple lesions. MLPA allowed the detection of two CCM1 gene deletions that had occurred independently, one multi-exon duplication within the CCM1 gene, a deletion of the entire CCM2 gene and a deletion of exon 1 and part of the 5'-upstream region of CCM2.
Thus, the combination of direct sequencing and MLPA analyses enabled us a mutation detection rate of 90% for inherited cases and 64% for isolated cases with multiple lesions. The deletion of the entire CCM1 gene in one CCM family is associated with the same intrafamilial phenotypic variability that has been observed for patients with small mutations. An up-to-date overview of all our mutations will be presented.

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The novel human serotonin receptor subunits 5-HT3C, 5- HT3D and 5-HT3E modulate 5-HT3 receptor function

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Within the family of serotonin receptors the 5-HT3 receptor, composed of five subunits, is the only ligand-gated ion channel. To date, the 5-HT3A and 5-HT3B receptor subunits are best characterized. But several studies reported on the diversity of native 5-HT3 receptors which cannot solely be explained on the basis of the 5-HT3A and 5-HT3B subunits. Since we have described further putative 5-HT3 serotonin receptor encoding genes, HTR3C, HTR3D and HTR3E, we here investigated if these novel candidates and the isoform 5-HT3Ea are able to form functional 5-HT3 receptor complexes. By immunofluorescence and immunoprecipitation studies of heterologous expressed proteins, we found each of the respective candidates co-assembled with 5-HT3A. To investigate if the novel subunits modulate 5-HT3 receptor function, we perfomed radioligand-binding assays and calcium-influx studies in HEK293 cells. Our study revealed that

the 5-HT3C, D, E and Ea subunit alone cannot form functional receptors. Co-expression with 5-HT3A, however, yielded functional heteromeric complexes with different serotonin efficacies. Potencies of two agonists and antagonists were nearly identical at homomeric 5-HT3A and heteromeric complexes. However, 5-HT showed increased efficacy at 5-HT3A/D and 5-HT3A/E receptors consistent with an increased surface expression compared to 5-HT3A receptors, whereas 5-HT3A/C and 5-HT3A/Ea receptors exhibited decreased 5-HT efficacy. This data show for the first time that the novel 5-HT3 subunits are able to form heteromeric 5-HT3 receptors and represent major modulators of 5-HT3 receptor function.

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Inhibitors of histone deacetylases modulate the processing of the ionotropic glutamate receptor subunit GluR2

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AMPA-type glutamate receptors mediate fast excitatory synaptic transmission in the brain. These ionotropic receptors are composed of four subunits encoded by four different genes (GluR1-4). During the past years, the GluR2 subunit emerged as a therapeutic target for neurodegenerative disorders including sporadic ALS and forebrain ischemia. GluR2 primary transcripts are subject to alternative splicing and the alternative inclusion of two exons (flip and flop) has shown to crucially influence the AMPA receptor desensitization properties. Moreover, GluR2 transcripts contain two RNA editing sites (Q/R and R/G), which modulate the Ca2+ permeability (Q/R editing) and recovery rate of AMPA receptors (R/G editing). In spinal cord samples derived from patients with sporadic ALS, the Q/R editing efficiency as well as the flop inclusion rates have shown to be significantly decreased. We established rat hippocampal brain slice cultures (OHSC) to investigate the effects of histone deacetylase (HDAC) inhibitors on GluR2 processing. HDACs are crucial in epigenetic regulation of transcription and their inhibitors are emerging as neuroprotective agents. Here, we show that HDAC inhibitors such as valproic acid (VPA), SAHA, MS-275 and FK-228 modulate the splicing pattern of the GluR2 subunit in rat OHSCs in a dose-dependent manner, while the overall GluR2 expression was not altered. All HDAC inhibitors tested significantly promoted flop inclusion, while the GluR2 flip expression was unchanged or downregulated. In contrast, the Q/R and R/G editing efficacies remained unchanged following HDAC inhibitor treatment. Since GluR2 flop inclusion has shown to modulate the kinetic and pharmacological properties of AMPA receptors, we hypothesized that HDAC inhibitors are able to counteract AMPA-receptor mediated excitotoxicity. In vivo studies are currently performed to evaluate whether systemic administration of HDAC inhibitors counteracts neurodegeneration in a rat ischemia model.

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Genetic alterations of FANCE, a rare Fanconi anemia gene with multiple functions

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FANCE is one of at least eight proteins forming the Fanconi anemia (FA) nuclear core complex that are required for monoubiquitination of the central FA protein FANCD2 during S phase or in response to DNA damage. FANCE is a 58 kDa protein of 536 aa with a bipartite nuclear localisation signal (NLS) as the only recognizable motif. Together with FANCC and FANCD2, FANCE forms a ternary complex that seems to be essential for the function of the FA/BRCA DNA damage response pathway, possibly by providing a bridge function between the FA core complex and the central FANCD2 protein. In evolutionary terms, FANCE belongs to the group of recently emerged FA genes with homologs limited to the vertebrate lineage. FANCE was identified in the year 2000 by expression cloning and mutation analysis in three families assigned to complementation group FA-E via cell fusion studies. There is only very limited information on the pattern of genetic alterations causing the FA-E subtype. Therefore we started an international collaboration for the identification and molecular analysis of FA-E patients. Using complementation studies with retroviral vectors, we were able to assign 10 so far unclassified FA patients to subtype FA-E. Our preliminary results suggest that, in contrast to most other FA genes, there is a rather narrow spectrum of FANCE mutations, some of which are recurrently found across diverse ethnical backgrounds and occur in a homozygous state. The mutations identified to date cluster in exons 2 and 5, framing the NLS region. The 5' mutations detected so far are exclusively nonsense mutations, whereas those 3' to the NLS region include both frameshift and missense mutations. The definition of the pattern of genetic alterations in this worldwide largest cohort of FA-E patients is expected to improve our understanding of the multiple functions of this rare but obviously important FA gene.

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Osteopathia striata with cranial sclerosis (OSCS): mutation analysis of candidate genes on chromosome Xp11.4-p11.22

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Osteopathia striata with cranial sclerosis (OSCS) refers to the radiological appearance of longitudinal striations of the tubular bones and fan-shaped configurations of the ilia. The skull is abnormally dense, and the clavicles are not striated. Based on various family observations OSCS was initially considered as an autosomal dominant condition with complete penetrance and high clinical variability. However, we and others suggested X-linked segregation due to the observation of female preponderance and of severely affected sons from OSCS mothers. Based on recombination breakpoint and linkage analysis in two families we disclosed a 15cM critical region between markers DXS993 and DXS6800, containing 153 annotated genes.
In our efforts to reveal underlying disease gene we prioritized all genes from critical region by expression profiles and putative function from databases. We selected 30 genes due to their expression pattern and functional role in bone development for mutation analysis by high throughput sequencing in 6 individuals from the two families. No disease causing mutation was identified in the coding regions in any of these genes. In parallel the expression level of 94 out of the 153 genes was determined in an affected boy compared to his healthy uncles using a Low Density Array (384 Well TaqMan Micro Fluidic Card, Applied Biosystems). 6 genes, which were primary not prioritized due to their expression pattern, displayed a reduced expression level in the affected boy versus his healthy uncles. Up to now no mutation could be found in the coding region of these genes. Further analysis of regulatory elements is in progress.

P240

Differential regulation of COX1, a mitochondrial encoded subunit of cytochrome c oxidase, in fibroblasts: a novel biomarker for sporadic Alzheimer disease?

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Alzheimer´s disease (AD) is a progressive neurodegenerative disorder which leads to neuronal cell death in the cerebral cortex and hippocampus. Accumulating evidence suggests a mitochondrial dysfunction in AD resulting from molecular defects in the oxidative phosphorylation (OXPHOS) chain. Alterations in mRNA expression of mitochondrial genes that are involved in the OXPHOS chain were found in both APP transgenic mouse models of AD and in postmortem brain samples of AD patients. In order to identify novel biomarkers for AD, we compared gene expression profiles in postmortem fibroblasts of sporadic AD patients of Braak stages 1 (n=3), 2 (n=4), and 3 (n=7) and of age-matched controls (n=18). To this end, we constructed a specialized cDNA microarray containing more than 600 genes which are known to play a role in brain development and function or AD pathogenesis. Microarray studies revealed transcriptional upregulation of COX1, an mito-

chondrial encoded subunit of cytochrome c oxidase, whereas the nuclear encoded subunit COX6C was not differentially regulated. Expression analysis of further mitochondrial and nuclear genes for all thirteen subunits of the cytochrome c oxidase complex using quantitative real-time PCR are underway. Changes in the stoichiometry of different subunits may interfere with cytochrome c oxidase function.

P241

Functional characterization of Zfyve27, the gene mutated in hereditary spastic paraplegia (SPG33)

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Hereditary spastic paraplegia (HSP) is a neurodegenerative disease characterized by the progressive degeneration of corticospinal tract axons resulting in the spasticity of the lower limbs. ZFYVE27 is a novel spastin interacting protein and we have identified mutation in ZFYVE27 in a German family with autosomal dominant form of HSP. ZFYVE27 contains FYVE domain (Fab1P, YOTB, Vac1P and EEA1) and proteins with FYVE domain are suggested to play key role in membrane trafficking, signal transduction and cytoskeleton regulation.
A comprehensive expression of Zfyve27 was evaluated in mouse by Western blot analysis and high levels of Zfyve27 was detected primarily in HSP affected tissues such as brain, cerebellum and spinal cord. Endogenous expression of ZFYVE27 was attributed to the perinuclear as well as punctated vesicles by means of immunocytochemistry in NIH3T3 cells. Over-expression of disease associated mutation in mouse embryonic fibroblasts showed an abnormal enlargement of endosome like structures signifying the defects in the intracellular membrane trafficking events. Immunohistochemical analysis of brain is being carried out to elucidate the expression of Zfyve27 in a variety of neurons. To gain mechanistic insights on the Zfyve27 function, currently generation of loss of function mouse model is in progress.

P242

Microarray expression analysis of human dopaminergic neuroblastoma cells after RNA interference treatment of LRRK2 - a key player in the pathogenesis of Parkinson´s Disease

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The protein Leucin rich repeat kinase 2 (LRRK2) is a key player in the pathogenesis of Parkinson´s Disease (PD). Furthermore, mutations in the LRRK2 gene lead to autosomal dominant forms of familiar PD. So far the exact biological role of LRRK2 in unaffected brain is elusive. To gain more insights about the biological function of this protein we monitored the changes in the expression profiles of SH-SY5Y cells, a dopaminergic neuroblastoma cell line, induced by a depletion of LRRK2 levels by RNA interference (RNAi). The RNAi resulted in a LRRK2 mRNA downregulation of 70%.
RNA was isolated from cells treated with either control siRNA or siSNCA in triplicates and used to hybridize Affymetrix U133plus2.0 microarrays. The chip analysis was performed using the Array Assist 4.0 software selecting all transcripts with a minimum change in expression level of 1.5 fold with a p-value less than 0.05 (t-Test without multiple testing correction). From the approx. 54.000 transcripts represented on the chip 94 and 93 transcripts were up- and downregulated respectively.
Gene regulation networks were generated by the Ingenuity Pathway Analysis 3.1 software based on the list of 187 differentially expressed transcripts.
To undermine the results of the microarray analysis we choose 9 key players of the interaction network for independent verification by qRT-PCR in the Light Cycler 480 System (Roche) with QuantiTect SYBR Green Assays (Qiagen). For data analysis the best out of 2 reference genes (SDHA and PDHB) was identified by the GeNorm algorithm implemented in the qBASE application (Goossens al., 2005; Hellemans et al., in preparation) and subsequently used for normalization and calculation of the relative expression with REST-XL version 2.0 (Pfaffl, 2001).

P243

Fragile X-associated tremor/ataxia syndrome among patients with differential diagnosis SCA and Parkinson disease

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a multiple-system neurological disorder caused by an expansion of a polymorphic CCG repeat within the FMR1 (fragile site mental retardation 1) gene. Clinical features of FXTAS include the presence of, both, hyperkinecis and hypokineticis such as ataxia, tremor and parkinsonism.
The usual FMR1 allele contains a CGG repeat of $\leq$40 units. The intermediate or "grey zone" length comprises alleles of 50-58 repeats while the range of premutation repeats is 59-200 CCGs. Carriers of the latter alleles typically have children with highly expanded alleles (>200) who develop fragile X syndrome mental retardation syndrome (FRAXA). FXTAS occurs in some individuals with moderately expanded alleles (within the mutation range) of the FMR1 gene. We have investigated 390 males with the clinical diagnosis of spinocerebellar ataxia (SCA without repeat expansion in the major SCA genes) and 140 patients diagnosed with Parkinson' disease (PD). The FMR1 gene was screened for repeat expansions, wherever necessary also by Southern blot hybridization.
In 2 of 390 males diagnosed with cerebellar ataxia we genotyped premutated alleles of ~120 CGG repeats. Three of these patients had alleles in the "grey zone" range. Among PD patients we identified no premutated or mutated FMR1 genes but "grey zone" alleles in 2 patients.
Our findings show a slightly increased frequency of premutation alleles among patients with cerebellar ataxia in comparison to those suffering from PD. An obvious explanation includes clinical overlaps between SCA and FXTAS phenotypes.

P244

Novel mutations in NTRK1 in patients with hereditary sensory and autonomic neuropathy type IV (HSAN type IV/CIPA)

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Background: Hereditary peripheral neuropathies are both clinically and genetically heterogenous. Hereditary sensory and autonomic neuropathy type IV (HSAN type IV), also named congenital insensitivity to pain with anhidrosis (CIPA) presents as congenital loss of pain and sensation combined with anhidrosis, self-mutilation and unexplained fever. Further features are mental retardation, bone fractures, bad wound healing, corneal ulcerations and other abnormalities. Histologically, small unmyelinated nerve fibers are absent and small myelinated fibres are reduced. Inheritance of HSAN type IV is autosomal recessive, and mutations in the NTRK1 gene on 1q21-q22 are responsible for HSAN type IV. NTRK1 encodes a receptor tyrosine kinase for the nerve growth factor (NGF). HSAN type IV is exceedingly rare, ~35 families have been described, mostly from Japan and isolated Bedouin populations.
Purpose: We established mutation screening for HSAN type IV by PCR and subsequent sequencing of the entire coding region of NTRK1 in 12 fragments to search for mutations in NTRK1 in European HSAN patients.
Results: We detected NTRK1 mutations in five different families of European origin: c.145C>T (R49X), c.1878_1879insA (Gln626fs), IVS5+1G>A,c.2089G>A (E697K) and c.899delG (Cys300fs). All patients were homozygous for the mutation inherited in their family. Parents were either consanguineous or at least from an isolated geographical background. The mutation spectrum we detected indicates that the mutations are leading to loss of function in the majority of cases.
Conclusions: Mutations in NTRK1 are a relevant cause of hereditary sensory and autonomic neuropathies. In paediatric patients with loss of pain and sensation, self-mutilation, anhidrosis and mental retardation, the diagnosis of HSAN type IV should be suspected. NTRK1 mutation analysis can be helpful in order to confirm the diagnosis and to offer prenatal diagnostics to families affected by this severe congenital disease.

P245

Expression levels of a truncated protein and clinical phenotype in Nijmegen Breakage Syndrome

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Patients affected by the autosomal recessive Nijmegen Breakage Syndrome (NBS [MIM 251260])

have possibly the highest risk for developing a malignancy of all the chromosomal instability syndromes. This reflects the profound disturbance to genomic integrity and cellular homeostasis that is caused by the mutation of the essential mammalian gene, NBS1. Indeed, null-mutation of NBS1 is lethal in the mouse and NBS patients survive only due to the fact that the common human founder mutation, found in over 90% of patients, is in fact hypomorphic and leads, by alternative translation, to a partially functional carboxyterminal protein fragment. This 555 amino acid protein migrates on SDS-polyacrylamide gels as 70kDa and is termed here, p70-nibrin. p70-nibrin is clearly able to sustain vital cellular functions of the full-length protein. Examining a panel of patient cell lines has indicated that the considerable variation in the expression level of p70-nibrin correlates with the incidence of malignancy. Using real-time PCR we have now found that the variation in p70-nibrin expression is not due differences in mRNA quantity. Thus, transcription rates and nonsense mediated mRNA decay are not likely to be responsible for the observed variation. It seems the alternative translation process itself is vulnerable to other cellular influences. Determining the factors contributing to efficient alternative translation of the NBS1 mRNA is now an important goal.

P246

Functional chracterization of mutations in the HADH2 gene causing 2-methyl-3-hydroxybutyryl-CoA-dehydrogenase (MHBD) deficiency

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2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency is an organic aciduria with an unusual neurodegenerative disease course. It is caused by a deficiency of hydroxysteroid dehydrogenase type 10 (HSD10) due to mutations in the HADH2 gene on chromosome Xp11.2. So far 7 different disease-causing mutations have been identified, all of which are missense mutations. There is no correlation between enzyme activity measured in patient cells and disease severity. In order to further elucidate the pathogenesis of the disease we functionally characterised known mutations in the HADH2 gene. Photometrically measured in vitro enzyme activity of recombinant HSD10 wildtype and mutants showed residual activity with 2-methyl-3-hydroxybutyryl-CoA of <5 % for 3 mutations and a maximum 25 % for 3 other mutations. Activity measurements with other substrates such as 3-hydroybutyryl-CoA produced a similar pattern. Data of thermal stability measurements measured in presence and absence of various substrates and cofactors using a fluorescence-based approach revealed significant increase of stability through cofactor binding for active mutants and wildtype, in contrast to mutants with low enzyme activity. Analysis of the subcellular localization of transfected mutant HSD10 by immunostaining and fluorescence microscopy showed that all mutant proteins investigated remained stable and were imported into mitochondria. These results, in combination with clinical observations, support the notion that pathogenesis in MHBD deficiency is not linked to residual mitochondrial activity of HSD10 but to an as yet uncharacterised protein function.

P247

CDC4, cyclin E, and chromosomal instability

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Several recent studies described a correlation between deregulated cyclin E and chromosomal instability (CIN). Cyclin E levels are regulated by hCDC4, an evolutionary conserved E3 ubiquitin ligase likely involved in regulating the G1-S cell-cycle checkpoint. CDC4 mutant cells show persistently elevated levels of cyclin E protein (Moberg et al. 2001) and constitutive overexpression of cyclin E was shown to cause CIN (Spruck et al. 1999). Indeed, the targeted disruption of both hCDC4 alleles by homologous recombination in the karyotypically stable colorectal carcinoma cell lines HCT116 and DLD1 resulted in both increased cyclin E levels and CIN (Rajagopalan et al. 2004).
We have investigated the chromosomal stability in several cell lines with CDC4 mutations. Our data clearly confirm that CDC4 mutations indeed result in elevated cyclin E levels. We determined in the respective cell lines chromosomal stability using a number of different approaches such as interphase FISH, percentage of cells with micronuclei, and chromosome number in metaphase spreads. However, this plethora of methods failed to show any evidence for CIN, which questions the presumed absolute correlation between cyclin E and CIN. In contrast to other studies, our results suggest that constitutional cyclin E increase alone may not be sufficient to cause CIN. This would correlate very well with in vivo observations as reported by Kemp and co-workers (2005). In addition, our data has far reaching consequences for drawing conclusions from cell lines after gene disruption.

P248

Analysis of gross deletions in patients with autosomal dominant hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous disorder characterized by progressive spasticity, weakness and hyperreflexia of the lower limbs. It is divided into a 'pure' form with progressive spasticity as an isolated symptom and a 'complicated' form accompanied by other neurological abnormalities. ≥ 33 different loci were found to be involved in the pathogenesis of HSP forms which are transmitted in autosomal dominant, recessive or X-linked recessive manner.
In ~40% of families with the 'pure' autosomal dominant form of HSP, sequence analysis identifies a mutation in the SPG4 gene (spastin) and in ~9% a mutation in the SPG3 gene (atlastin). Yet, for several families, no point mutation or small insertion/deletion can be detected in these two loci. We determined the frequency of exon deletions or large rearrangements in the SPG4 and SPG3 genes in patients with the pure autosomal dominant form of HSP. We analysed blood samples from 48 patients with an anamnestically positive family history (symptoms of 'pure' HSP present in ≥ 2 generations) concerning gross deletions in the SPG3 and SPG4 genes using multiplex ligation-dependent probe amplification. Positive findings were confirmed by real-time PCR. We found a deletion of one or more exons of the SPG4 gene in ~7% of cases. - Thus, in addition to missense, nonsense-mutations and small insertions/deletions, gross deletions represent an important aspect of mutation analyses in autosomal dominantly transmitted HSP.

P249

Human TSPY is specifically expressed in fetal germ cells in TSPY transgenic mice

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The TSPY gene encodes the testis-specific protein, Y encoded. Expression of TSPY within the testis is restricted to germ cells. The topology and timing of TSPY expression, and also its homology to members of the TTSN-family indicate that TSPY functions as a proliferation factor of germ cells. Strong TSPY expression in human germ cell tumors and their precursor stages points to an oncogenic role of TSPY in early germ cell tumorigenesis. In contrast to bovines and primates, where TSPY is organized as a repetitive gene family, the situation in rodents is peculiar. While Tspy is still functional in the rat, the laboratory mouse harbours a single-copy pseudogene that is unable to generate a functional transcript. We constructed a TSPY transgenic mouse line harbouring a complete human TSPY gene in approximately 50 copies on the mouse Y chromosome. The tissue specific expression pattern, the expressing cell type and the topology of the testicular expression of the TSPY transgene within this line are nearly in total accordance with the human situation. In order to elucidate TSPY expression during embryogenesis and to investigate the role of TSPY in early germ cell development we analyzed the TSPY expression pattern in transgenic mouse embryos of the stages 12.5-17.5 days post coitum (dpc) by immunohistochemistry. Our results demonstrate that TSPY is expressed in gonocytes and prespermatogonia in fetal testes at the stages 12.5-17.5 dpc. The immunohistochemical staining localizes the TSPY epitope mainly to the cytoplasm of gonocytes and prespermatogonia. During embryogenesis the human TSPY transgene is correctly spliced according to the pattern of the predominant human transcript TSPYmajor. Besides the main transcript, we were able to isolate 6 embryonic splice variants of the human transgene. Our data point to

a role of human TSPY in fetal germ cell development.

P250

Recessive ataxias in German patients with early age of onset

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The major forms of recessive ataxias can be distinguished on clinical signs and age of onset. Rare forms often due to clustering may be discriminable by biochemical parameters. While FRDA is a well known disease in Germany, only single patients with AOA1, AOA2, MIRAS, or IOSCA have been diagnosed. In the context of pilot screening projects and routine diagnostic tasks we looked for mutations in the frataxin (FXN), aprataxin (APTX), senataxin (SETX), mitochondrial DNA polymerase gamma (POLG), and Twinkle (C10orf2) genes.
Diagnostic analyses between 1996 and 2006 detected 134 unrelated FRDA patients. Homozygosity for the intronic GAA repeat expansion was present in 129 cases, five were compound heterozygous for this mutation and a second DNA variation: the start codon mutation Met1Ile in two unrelated patients, the missense exchanges Asn146Lys, Leu186Arg, or the deletion of exon 5 in one patient, respectively. In the AOA1 study, we screened a group of 165 early onset ataxia patients for APTX mutations and detected two non-related cases homozygous for the Trp293X nonsense mutation. One patient was heterozygous for the nonsense mutations Trp221X and Gln240X. Regarding AOA2, in 100 DNA samples seven different point mutations and a four-nucleotide deletion were found (Abstract Bernard et al.). Two of the eight affected patients carry two SETX mutations, and may be affected by AOA2. Six patients are heterozygous for one of four novel missense variations or the stop mutation Arg1606X. Apart from two brothers with Norwegian roots, no case of MIRAS was found. The common missense mutation Trp748Ser was not detected in 203 North European ataxia patients with childhood or juvenile onset. The IOSCA causing founder missense exchange Tyr508Cys, frequent in Finnish ataxia patients, was not present in 203 unrelated samples tested for this genotype. Our results highlight the clinical impact for the growing number of inherited ataxias that can be investigated by genetic assays.

P251

(C/G/T)CTG tetranucleotide arrays in Myotonic Dystrophy 2: On the make-up of disease alleles

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The myotonic dystrophies, Curschmann-Steinert Disease (DM1) and Proximal Myotonic Myopathy (DM2), are clinically similar. Both are genetically caused by large expansion of untranslated sequences, a CTG repeat in the 3'-untranslated region of the DM1 gene (DMPK), and a CCTG repeat in the first intron of the DM2 gene (ZNF9). Also there are striking parallels in the molecular pathophysiology. The repeat expansions expressed at the RNA level alter RNA processing by interfering with alternative splicing of other genes, thereby causing complex multisystemic phenotypes. In the DMPK gene, normal alleles harbour pure CTG arrays only varying in length, and the expanded alleles most likely do as well. In contrast, the variable CCTG arrays of normal ZNF9 alleles show interruptions by one or more GCTG and/or TCTG tetranucleotides, normally situated between a proximal and a distal CCTG stretch. Nevertheless, authors believed that the expanded CCTG arrays of DM2 patients lost their interruptions whereby they became unstable, as is the case in other repeat expansion diseases. But it is not known whether the proximal, the distal, or both CCTG stretches can expand to a disease allele.
We discovered a first example of an interrupted CCTG array in a disease allele of a 59 year-old female who presented with myotonia and proximal myopathy. Her mother had a similar phenotype. The expanded CCTG array was interrupted near the 3' end by about 30 consecutive TCTGs. This finding may suggest that only the proximal CCTG stretch was expanded. As this must then have occurred in a precursor array with a very unusual interruption, we would favour a different explanation, i.e., that other CTG containing tetranucleotides in the CCTG arrays, GCTG and TCTG, are also subject to expansion. If so, all CTGs in these arrays should contribute to the RNA pathogenesis of the disease, and there should be many more interrupted CCTG arrays to be found in the expanded alleles of DM2 patients.

P252

Segregation of two independent dystrophin mutations in one pedigree: A compound heterozygous female patient

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The X-linked inheritance of Duchenne/Becker muscular dystrophies is sometimes obscured by manifesting carriers. Heterozygous carrier women may show clinical signs mostly dependent on the degree of Lyonisation of their X-chromosomes in muscle tissue. In rare cases, X - autosome translocations are the cause of a 100%-activation of the translocated X-chromosome. To our knowledge, an affected woman with a mutation on both of her dystrophin alleles was never reported.
A large consanguineous Turkish family with an unusual pattern of inheritance of a musclar dystrophy was referred to us for dystrophin gene analysis. In one branch of the family, a severe form of muscular dystrophy (Duchenne-like) appeared to be transmitted like an autosomal trait, while in another branch a Becker-like phenotype was inherited in a typical X-linked recessive manner. Two affected cousins in the third generation had only traces of dystrophin in their muscle tissue, though their clinical affection differed markedly. After sequencing of the whole dystrophin gene, both were found to harbour a truncating point mutation, p.Gln17Stop in exon 2. Their maternal uncle, however, who had died at the age of 24 years from a Duchenne-like dystrophy, showed a duplication of exon 59 as detected by MLPA analysis. Surprisingly, the stop mutation was found also in the grandfather's blood DNA whereas the grandmother was a heterozygous carrier of the exon 59 duplication. Both grandparents were said to be „quite healthy". This coincidence of two dystrophin mutations made one of their daughters (the mother of the severely affected cousin) a compound heterozygous female patient. Her phenotype was reported as BMD-like and immunoblotting showed a low amount of dystrophin of normal size.
This very rare constellation adds a note of caution to the recessive mode of inheritance which is usually presumed in consanguineous families. Comprehensive pedigree analysis is required to avoid pitfalls in genetic councelling.

P253

Cellular consequences of missense mutations in the Nijmegen breakage syndrome gene, NBS1

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Nijmegen breakage syndrome (NBS [MIM 251260]) is an autosomal recessive chromosomal instability disorder with hypersensitivity to ionizing radiation. The clinical phenotype is characterized by congenital microcephaly, mild dysmorphic facial appearance, growth retardation, immunodeficiency, and a highly increased risk for lymphoreticular malignancy. Most NBS patients are of Slavic origin and homozygous for the founder mutation 657del5. All other NBS patients also have truncating mutations. Two missense NBS1 mutations, 643C>T(R215W) and 511A>G (I171V), have been reported to have a higher frequency among tumour patients than in the control population. Analysis of amino acid substitutions can be problematical, particularly when the endpoint of interest, malignancy, is late-manifesting and multifactorial in origin. Furthermore, complementation studies after transfer of mutant cDNAs into NBS patient cells can be difficult to interpret since these cells all contain partially functional truncated proteins. We have taken advantage of our conditional null-mutant mice and mouse cells to examine the above mentioned mutations. We find clear evidence for disturbed cellular functions in cell cycle checkpoints leading to long term effect on cell proliferation and survival which may be of relevance for tumourigenesis.

P254

Functional analysis of Mecp2 using transgenic mouse models expressing Mecp2 tagged with EGFP

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The MeCP2 protein is a transcriptional repressor binding to methylated cytosines and modifying the chromatin structure. MECP2 gene was eventually found to be the cause of 95% of Rett Syndrome (RTT) cases. Patients with RTT show symptoms of neuronal dysfunction of the autonomic system. In spite of its ubiquitous expression in the tissues, it is believed to have some unique functions in the neurons. But till now, very less is known about the role of MeCP2 in neuronal cells and the mechanism by which it brings about the deteriorating disorder. The aim of the study is to produce two different transgenic mouse models expressing Mecp2 tagged with EGFP to obtain an appropriate functional analysis. The transgenic constructs have been generated by engineering a BAC clone (B22804) containing the entire Mecp2 gene of the mouse and flanking sequences, by GET recombination. First mouse model over-expresses the wild-type Mecp2 labeled at c-terminus with EGFP. The second transgenic model over-expresses an early truncated (human mutation) Mecp2, lacking the nuclear localization signal, also labeled at the c-terminus with the EGFP. The investigation of the transgenic mouse lines shall allow the spatio-temporal analysis of the Mecp2 by tracing the EGFP. Moreover, the second transgenic mouse model will help to understand the role of the mutated protein in the pathogenesis of the RTT. Although Mecp2 is predominantly expressed in brain, it doesn´t seem to express in all the cells including glial cells. So, we are isolating the Mecp2+ / EGFP+ specific brain cells by FACS using our unique transgenic mice and to characterize them using different markers by immuno-staining. The current analysis includes live cell imaging of Mecp2 at the synapses and determining the size of the neurons expressing the transgenic protein. Also a transcriptomics using the EGFP+ cell population between the two mouse models would validate the regulation of already found or determine new target genes specifically.

P255

Rubinstein syndrome: the first year of CREBBP gene analysis at Mainz identified nine novel mutations in 22 study patients

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Rubinstein-Taybi syndrome (RSTS) is a well-characterized human genetic disorder including facial anomalies, broad thumbs and great toes, compromised growth, and developmental delay. Heterozygous mutations (45-56%) or deletions (10-14%, including exon and gene deletions) of the CREBBP gene can be identified in patients with RSTS. A few patients (~3%) demonstrate mutations in another gene, EP300 (see related abstract by Bartsch et al.). Since the relocation of the RSTS study group to Mainz, we have completed gene testing in 22 patients who were clinically diagnosed with either RSTS (n=10) or possible (or incomplete) RSTS (n=12). In addition to the well-established CREBBP FISH diagnosis (for the detection of gene deletions) and genomic DNA sequencing (for the detection of small molecular mutations), we have established two new techniques for the identification of exon deletions, quantitative PCR (using a ABI 7500 Real-Time PCR Instrument) and MLPA (ServiceXS Company, Leiden, Holland). So far we identified 9 causative mutations in CREBBP, including 7 small molecular mutations and 2 deletions of the 5'-end of CREBBP (one including neighboring genes). All small molecular mutations (3 nonsense mutations and 4 small deletions or insertions from 1 to 26 bp) have not yet been described in the literature, providing further evidence against the existence of mutational hot spots in the CREBBP gene. Although full CREBBP analysis requires only 2-3 weeks now, the duration for individual cases may have taken longer (up to 4-5 months) because of numerous diagnostic requests. The clinical distinction between possible (or incomplete) RSTS and clear RSTS is useful, because different mutation rates, 17% (2 of 12) and 70% (7 of 10), respectively, were identified in these two groups. Our diagnostics includes EP300 gene analysis (using similar methods as for CREBBP) in RSTS patients without detectable CREBBP mutation. EP300 results in the patients described here are still pending.

P256

Autosomal recessive postlingual hearing loss (DFNB8): Compound heterozygosity for two novel TMPRSS3-mutations in German siblings

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Mutations in TMPRSS3 encoding a transmembrane serine protease cause autosomal recessive deafness with childhood (DFNB8) or congenital onset (DFNB10). TMPRSS3 mutations have been identified in Pakistani, Palestinian, Tunisian and Turkish families as well as in sporadic Caucasian patients from Mediterranean countries but not in Northern-Europeans or Northern-Americans so far. The estimated frequency of TMPRSS3 mutations in deaf Caucasian patients is less than 1 %, but hitherto no other locus causing a larger fraction of non-syndromic, postlingual deafness has been identified. We describe the identification of two novel TMPRSS3 mutations in siblings of German origin. Two sisters and two brothers with disease onset of about six years and progression to deafness until about 20 were recruited from the department of ORL, Aachen University. The parents and six siblings of the patients were unaffected. Haplotype analysis of polymorphic markers flanking TMPRSS3 supported linkage to the DFNB8/10 locus on 21.q22.3. All coding exons of TMPRSS3 were amplified and sequenced. Two novel mutations, located on different parental chromosomes and segregating with the phenotype, were detected. The first mutation in exon 8 (c.646C>T) results in an arginine to cysteine substitution at codon 216 (R216C) and affects the same codon as the known pathogenic mutation c.647G>T (R216L). The second mutation was found in exon 9 (c.916G>A) and leads to an alanine to threonine substitution. Both identified missense mutations change evolutionary highly conserved amino acids and were not found in 200 control chromosomes. The identification of novel TMPRSS3 mutations may help to shed light on the genotype-phenotype correlation. Our patients benefit much from early bilateral cochlea implantation.

P257

Production of transgenic pigs expressing α1,2-fucosyltransferase reducing humoral xenograft rejection

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The use of animals as a source of organs and tissues for xenotransplantation can overcome the growing shortage of human organ donors. However, the presence of xenoreactive antibodies in humans directed against swine Gal antigen present on the surface of xenograft donor cells leads to the complement activation and immediate xenograft rejection as a consequence of hyperacute immunological reaction. To prevent hyperacute rejection it is possible to modify swine genome by human gene modifying the set of donor's cell surface proteins. For this purpose gene construct pCMVFut containing the human gene encoding α1,2-fucosyltransferase enzyme (HT, H transferase) under the human cytomegalovirus (CMV-IE) immediate early promoter was introduced by microinjection into a male pronucleus of the fertilized porcine oocyte. As a result of this experiment, the founder male pig was obtained with the transgene mapped to chromosome 14q28. Approximately 35% of the founder's progeny demonstrated presence of transgene. The RT-PCR analysis revealed expression of HT gene in different tissues of transgenic pigs. Flow cytometry analysis revealed reduction of level of epitope Gal on the cell surface of skin fibroblasts isolated from transgenic pigs.

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P258

Ataxia with oculomotor apraxia type 2: Novel mutations in the SETX gene

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The recessively inherited ataxia-oculomotor apraxia 2 (AOA2) is characterised by an age of onset between 10 – 25 years, gait ataxia, cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia, and elevated serum AFP level. AOA2 is associated with at least 15 different mutations within the senataxin gene (SETX) that is localised on chromosome 9q34. Mutation scanning by HPLC followed by sequencing of electrophoretic variants has identified mutations

in eight of 100 patients tested. Sequencing the 24 coding exons revealed eight novel DNA variations: one patient was homozygous for the missense mutation C1554G, one patient was heterozygous for the exchange P311L and a four-nucleotide deletion affecting the 5´splice site of exon 22. Heterozygosity was found for the missense mutations M386T, L584V, T760A, and D1077N as well as for the nonsense mutation R1606X. Each missense mutation was found in one patient whereas the nonsense mutation could be identified in two unrelated patients. Haplotype analyses excluded a common founder for the R1606X nonsense mutation. In six of eight patients heterozygous for SETX mutations, the diagnosis of AOA2 could not be confirmed definitely. This finding reveals the deficiency of pure sequencing strategies and emphases the need for introduction of functional tests or at least measurement of mRNA levels.

P259

Adjacent segregation causes an additional microdeletion 1p31.2-1p31.3 in an offspring of a female schizophrenic patient with a cryptic complex de novo chromosomal rearrangement characterized by FISH and array CGH

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The important role of genetic factors involved in the development of Schizophrenia, a frequent psychiatric disorder, is increasingly better understood. Beside linkage and association studies, cloning of chromosomal breakpoints in familial cases showing that disorder has proven useful in the identification of genes related to Schizophrenia. Here we present a male patient (2 years of age) who currently shows still an essentially normal development and behaviour. Just minor dysmorphic features can be recognized thus far. His mother who developed Schizophrenia at about 10 years of age is carrier of a de novo complex chromosome rearrangement (CCR) with an interstitial microdeletion of about 600-700kb at the proximal part of 1p31.3, an insertion of a more distal segment of 1p31.3 into 2p31 and an insertion of the adjacent proximal part of 1p31.2-1p31.3 into 4p15.2. Since he inherited the aberrant maternal chromosomes 1 and 2 but not 4, he consequently shows a larger microdeletion of 1p32.2-1p32.3 material of about 2 Mb which is nearly three times larger than the deletion present in the maternal genome. His chromosomal aberration has been characterized by molecular-cytogenetic techniques as: ish del(1)(p31.2p31.3)mat(RP4-759M20dim,RP11-442I03-),ins(2;1)(p31;p31.3p31.3)mat(RP4-537F10+;RP4-537F10-). An early onset of Schizophrenia has been reported in children; therefore one should be aware of that risk and keep the boy in regular medical and psychological supervision. However, since he did not inherit the aberrant maternal chromosome 4, with a breakpoint according to linkage studies at or close to another putative Schizophrenia locus, one can not rule out, that his risk related to that disorder might be lower than for his mother. Lymphoblastoid cell lines of the patient and his mother have been established for further analysis including expression studies of deleted and potentially rearranged genes.

P260

Hypothyroidism leads to downregulation of the corepressor alien: A negative feed-back regulation for resistance to thyroid hormone syndrome

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Thyroid hormone is essential for normal neuronal differentiation. Hypothyroidism leads to various degrees of mental retardation and to cretinism. In line with this the syndrome of Resistance to Thyroid Hormone (RTH) – a rare disease, mostly dominantly inherited - is associated with various degrees of mental retardation, attention deficit, deafness and goiter. The molecular basis of the RTH-Syndrome is mostly due to just point mutations that lead to amino acid exchanges of the thyroid hormone receptor ß located on chromosome 3 (3p24.3).
However, the exact role of the thyroid hormone receptors and the influence of a particular mutation in neuronal differentiation is not fully understood. Interestingly, neuroblastoma cancer cells reduce proliferation and undergo differentiation upon treatment with thyroid hormone. The thyroid hormone receptor associated with corepressors to repress its target genes. Here, we show the role of co-repressors for the expression of neuronal specific genes and their ligand-controlled recruitment to chromatin at thyroid hormone response elements. Interestingly mutant thyroid hormone receptors from patients with RTH-syndrome lack or have reduced ligand-controlled corepressor binding. Furthermore, we show that the expression of the corepressor Alien is regulated by thyroid hormone in brain and implies a negative feed-back mechanism in vivo to attenuate the potent repressive function mediated by thyroid hormone receptors.
These data indicate that the receptor controls the level of its own corepressor and suggesting that reduced thyroid hormone receptor mediated transactivation leads to reduced gene silencing. It is therefore conceivable that reduced gene silencing function correlates with milder symptoms of RTH-Syndrome patients.

P261

Is the gene causative for Zimmermann-Laband syndrome in 8q24.3?

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Zimmermann-Laband syndrome (ZLS) is a rare autosomal dominant inherited disorder characterized by coarse facial appearance, gingival fibromatosis, and absence or hypoplasia of the terminal phalanges and nails of hands and feet. Mapping of the translocation breakpoints of t(3;8) and t(3;17) found in patients with typical clinical features of ZLS defined a common breakpoint region of ~280 kb located in 3p14.3, which includes the genes CACNA2D3 and WNT5A. Mutation analysis of nine genes located in 3p14.3, including CACNA2D3, which is directly disrupted by one breakpoint of the t(3;17), identified no pathogenic mutation in eight sporadic patients with ZLS. Southern hybridization analysis and multiplex ligation-dependent probe amplification did not detect a submicroscopic deletion or duplication in either CACNA2D3 or WNT5A in ZLS-affected individuals. Taken together, the lack of a specific genetic lesion in the common region, defined by two translocation breakpoints, in sporadic patients with ZLS and apparently normal karyotype suggests that some other underlying genetic defect elsewhere in the genome could be responsible for ZLS.
Recently, a patient with features consistent with ZLS has been described to carry an insertion of the long-arm segment between 8q11.2-q24.3 of one homologue of chromosome 8 into the short arm of one chromosome 12 homologue at p11.2 [ins(12;8)(p11.2;q11.2q24.3)]. Thus, one breakpoint of the inserted fragment (i.e., 8q24.3) corresponds well to the breakpoint of the t(3;8) on chromosome 8 (q24.3), suggesting that the gene for ZLS might be located in this chromosomal area. The NIBP gene was found to be disrupted in 8q24.3 by the t(3;8). Subsequent mutation screening of NIBP as well as five other genes (KCNK9, LOC644167, C8orf17, CHRAC1, and EIF2C2) located in this region did not yet reveal any pathogenic alteration in nine ZLS-affected patients. Nonetheless, comprehensive analysis of additional genes in this area is ongoing.

P262

Functional characterization of a novel sequence variation in CACNA1H (Cav3.2) associated with childhood absence epilepsy

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T-type Ca2+ channels, largely expressed in thalamus, can induce neuronal burst firing which is critical for thalamo-cortical oscillations underlying seizures in absence epilepsy. Previous studies have shown that sequence variants of CACNA1H encoding the Cav3.2 channel can be associated with childhood absence epilepsy (CAE) in the Chinese population. Biophysical analysis

of these variants revealed a gain-of-function for some of them compared with the wild type (WT) channel. We now identified a novel variant in CACNA1H cosegregating in a German nuclear family with CAE. It predicts substitution of serine for glycine 1158 located in the cytoplasmic loop connecting domains 2 and 3 of the channel. We used standard molecular biology and the patch clamp techniques to characterize the mutation when expressed in tsA201 cells. Our results did show subtle differences between the biophysical properties of the mutant and WT channels which might induce a gain-of-function effect. Since the D2-D3 loop harbours important sites for channel modulation by other proteins, further respective investigations of potential mutational effects have been initiated.

P263

Molecular analysis in primary and secondary laminopathies

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Laminopathies represent a group of human hereditary diseases comprehending a wide variety of at least 12 phenotypes. Primary laminopathies arise through mutations in genes that code for A type lamins like LMNA while secondary laminopathies are caused by genes encoding lamin-associated proteins like ZMPSTE24(FACE1) or NARF. In the present study, we investigated 190 unrelated patients and 46 individuals in 9 families with disease phenotypes of primary and secondary laminopathies including Emery-Dreifuss muscular dystrophy (EMD), dilated cardiomyopathy (DCM), mandibuloacral dysplasia (MAD), familial partial lipodystrophy (FPLD), Hutchinson-Gilford progeria syndrome (HGPS) and restrictive dermopathy (RD). All patients were analysed for mutations in LMNA, FACE1 and NARF by heteroduplex analysis and direct sequencing. The pathogenic effects of the mutations were confirmed by segregation analysis, population study and comparison with locus specific databases. Under 85 unrelated EMD patients, we found 12 LMNA mutations. Two, p.M348I and p.R249W, represent novel mutations not registered in the locus specific LMNA mutation database (http://www.umd.be/). The recurrent mutations R249Q and R453W representing mutational hot spots were found in 7 unrelated patients. In 9 families, 14 individuals were confirmed and 32 were excluded to carry a LMNA mutation. Of 11 unrelated RD patients, four patients were homozygote for the common mutation c.1085_1086insT in ZMPSTE24. One patient was a compound heterozygote for the novel mutation c.50delA combined with the common mutation. A second novel mutation c.209_210delAT found in the affected child of a consanguineous couple was homozygous. All three mutations found lead to a functional lack of zinc metalloprotease ZMPSTE24. No mutations were found in 81 patients suffering DCM, MAD, FPLD and HGPS. Additionally, another progeroid disease, the Hallermann-Streiff syndrome, was excluded to be associated with LMNA, FACE1 or NARF.

P264

Characterisation of the kcnq genes in zebrafish

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The human KCNQ gene family encodes potassium channels linked to several genetic syndromes including neonatal epilepsy (KCNQ2/3), cardiac arrhythmia (KCNQ1), and progressive deafness (KCNQ4). KCNQ subunits form M-type potassium channels, which are critical regulators of neuronal excitability that mediate autonomic responses, pain, and higher brain function. Fundamental mechanisms of the normal and abnormal cellular roles for these channels may be gained from their study in model organisms. Zebrafish as an emerging model organism offers not only a mostly sequenced genome, well established cell biological techniques but also the possibilty to create transgenic and knock-down animals. Thus, to identify kcnq channels in zebrafish and carry out functional study of these channels in fish model, we searched in zebrafish genomic and EST database with Blast programs and RT-PCR experiments for homologues of the human KCNQ genes.
We identified eight fish kcnq genes in zebrafish. They are localised on fish chromosome 1, 2, 7, 8, 13(2x), 19 and 25. Significant sequence similarity was observed between orthologue pairs in fish and human. For each fish gene, at least two 400bp – 1kb fragments in UTR or coding regions were cloned into TOPO PCR II vector. Antisense probes were made from these clones and used for whole-mount in-situ hybridisation experiments to reveal the expression pattern of the kcnq channels in zebrafish embryogenesis.

P265

Bardet-Biedl syndrome: Interactions among BBS proteins

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Bardet-Biedl syndrome (BBS) is a rare, developmental disorder characterized by retinal dystrophy, obesity, postaxial polydactyly, renal failure, learning difficulties and hypogonadism in males. BBS is genetically heterogeneous with at least eleven genes (BBS1 – BBS11) involved in its molecular pathology.
Historically it has been ascribed an autosomal recessive mode of inheritance, but in several families the phenotype appears to require three mutated alleles. This model of “triallelic inheritance” suggests BBS as a link between Mendelian and complex phenotypes.
BBS proteins seem to constitute part of a multisubunit complex causing basal body or cilia dysfunction when mutated. However, important questions concerning the precise functions and binding partners of individual BBS proteins have yet to be elucidated.
Here, we report on our efforts to identify new interaction partners of selected BBS proteins (BBS1, BBS2, BBS4, BBS7) applying the yeast two-hybrid (Y2H) technology:
i) We present novel plausible binding partners including enzymes, transcription factors, centrosomal proteins, and components of the cytoskeleton.
ii) We confirm our Y2H results by co-immunoprecipitation analysis and show by immunohistochemistry, which of the putative interacting factors co-localize.
iii) We validate our approach by confirming the interaction of BBS4 with PCM1 and DCTN1 described recently by other groups.
iv) We describe direct interactions between selected BBS proteins and corroborate these findings by immunoprecipitation studies. The biological relevance of our findings for the pathophysiology of BBS will be presented and discussed.

P266

Rapid and reliable determination of transgene copy numbers in mice by SYBR green-based real-time PCR

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For many in vivo studies using transgenic mice it appears to be crucial to accurately determine the transgene copy number. Semi-quantitative Southern blot analysis is a very elaborate and time-consuming technique. Moreover, a densitometric quantification of an autoradiogram is error-prone and needs to be repeated in independent experiments, which might be hindered by restricted amounts of DNA achievable from tail-tip biopsies. Here, we show that it is possible to measure the transgene copy numbers by rapid and easy real-time PCR using the power-SYBR green method but without the necessity of using probes.
Proximal spinal muscular atrophy (SMA) mice were used to investigate the number of the transgenic human survival motor neuron 2 (SMN2) genes. The human SMN2 gene, a slightly different copy of the SMN1 gene is the main modifier gene of the SMA phenotype: the severity of the disease correlates inversely with the SMN2 copy number. However, mice carry only one murine Smn gene, the knockout of this gene is embryonic lethal. One existing SMA mouse model carries the following genotype: Smn-/-; humSMN2tg+. In these mice, which survive only for a few days, the number of human SMN2 transgenes is crucial for developing the gene dosage-dependent SMA phenotype. Regarding the last point the knowledge of transgene numbers is useful in order to produce reliable results attempting to rescue the SMA phenotype by pharmaceutical treatment of the mice.
We could avoid a main problem in using tail-tip DNA for very sensitive real-time PC, which is the low quality of the DNA, by employing a combination of a magnetic beads-based DNA isolation method and a newly designed real-time PCR protocol (Taqman). Using this method we were able to reliably determine the SMN2 copy numbers and could clearly distinguish between homo- and heterozygous animals. Two main issues of the protocol were the use of a murine single-copy gene (ApoB) and two distinct DNA-dilution steps.

P267

Functional characterisation of the human SCYL1 and its alternatively spliced variants

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SCYL1, which has also been described as NTKL, was firstly investigated as potentially mitosis associated protein because the gene physically maps into a region on human chromosome 11q13, where cancer associated breakpoints frequently occur. So far, four protein-isoforms generated by alternative splicing have been described: SCYL1, SV1, SV2 and TEIF. Expression of the SCYL1 gene has been shown to be controlled by serum-response factor during cardiac cell differentiation. The scarce literature on SCYL1 function reports a possible role of an alternatively spliced isoform (SV2) in mitosis, during which it translocates from the cytosol to the centrosomes. Another isoform, TEIF, is a known transcriptional regulator of the telomerase-subunit hTERT, making it a potential candidate for association with cancerogenesis. Furthermore, a down-regulated expression of SCYL1 in EBV-associated epithelial cancers was recently detected . To further elucidate the function of SCYL1 and its alternative splices isoforms we firstly verified the existence of the isoforms SV1, SV2 and TEIF by molecular cloning. Thereby we discovered the new transcipt-variant SV17 lacking the entire exon 17. We then analyzed the expression pattern of Scyl1 and its alternatively spliced variants by northern-blot and real-time PCR in different human tissues. We detected the highest expression rates in muscle, brain and testes. Furthermore, the full-length protein SCYL1, TEIF and SV17 were cloned into the pDsRed expression-vector and fused to a red-fluorescent GFP-variant. Subcellular localisation studies are ongoing.

P268

Regulation of the leukemia associated oncogene and developmental regulator EVI1 by all-trans retinoic acid

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The EVI1 gene codes for a zinc finger protein with important roles in embryonic development and in myeloid leukemogenesis. It is transcribed into several mRNA species with variable 5´-ends. One of these mRNA variants, MDS1/EVI1, gives rise to an EVI1 protein with an extended N-terminus and with functions partially different from those of the shorter EVI1 protein type. The other EVI1 5´-end variants are most likely translated into the same protein, i.e. the short EVI1 protein variant, but their variable 5´-UTRs can be expected to affect the regulation of protein expression.

Despite of its important functions, little is known about the regulation of the EVI1 gene. So far, all-trans retinoic acid (ATRA) is the only known physiological regulator of EVI1 in mammalian cells. Using the teratocarcinoma cell line NT-2 we have investigated the induction of EVI1 by ATRA.

Time course analyses showed that ATRA rapidly induces the EVI1 mRNA in NT-2 cells. A maximum is reached after approximately 18 hrs, except for the MDS1/EVI1 mRNA, which was noticably induced only after 48 hrs. This response was already seen with as little as 10nM ATRA and affected all EVI1 mRNA variants, albeit to variable degrees. Using reporter gene assays, an ATRA responsive region of ~250 bp was identified within exon 1a of EVI1. Under specific conditions NT-2 cell are able to differentiate into postmitotic neurons and thus offer the possibility to study EVI1 within this context. After undergoing a two-step neuronal differentiation protocol consisting of an initial ATRA-treatment phase and a subsequent terminal differentiation phase cells show characteristic neuronal morphology and upregulation of neuronal markers. Both EVI1 and MDS1/EVI1 are induced during the ATRA-treatment phase, but only EVI1 is induced during terminal neuronal differentiation. Future experiments will address the question whether EVI1 is necessary and sufficient to promote neuronal differentiation.

P269

The c.495G>A silent mutation in exon 3 of the SMN1 gene identified in patients with mild spinal muscular atrophy does not affect splicing and is therefore not pathogenic

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Proximal spinal muscular atrophy (SMA) is an autosomal recessive inherited disease. In 96% of patients, SMA is caused by the homozygous loss of the survival motor neuron gene 1 (SMN1), while the severity of the disease is influenced by the number of the almost identical copy gene, SMN2. The functional loss of SMN protein causes degeneration of the α-motoneurons in the spinal cord which leads to muscle weakness and atrophy of proximal voluntary muscles.

In a group of 4 patients with type III SMA, who carry only one SMN1 copy, we identified in exon 3 of SMN1 the A-allele of the silent mutation (c.495G>A) as the only aberration. This mutation has earlier been described as a polymorphism occurring on both SMN copies. We proposed that this nucleotide exchange my affect an exonic splicing enhancer, which than will cause alternative splicing of exon 3. Since exon 3 encodes the Tudor domain of the SMN protein that is essential for the interaction with Sm proteins and has an important role in snRNP biogenesis, a reduction of full-length SMN may cause mild SMA. Here, we show that these 4 patients neither show an increased amount of SMNΔEx3-transcripts as measured in EBV-transformed lymphocytes by quantitative RT-PCR nor an exon 3 deleted SMN protein in Western-Blots. Secondly, a group of 45 SMA parents, which were tested afore positive for the A-allele, were assayed for the localization of this allele on SMN1 or SMN2. We could prove by designing a gene specific assay for SMN1 that in 7/45 healthy SMA parents the A-allele was present in the only SMN1 copy. Since SMN2 is considered to be only disease-modifying, we conclude that the A-allele is not disease-determining. Any protective effects by an increased number of overall SMN copies could be excluded by LightCycler measurements, and will be further verified by MLPA.

These data suggest that the A-allele of the polymorphism c.495G>A does not cause an increased alternative splicing of the exon 3 and is therefore not pathogenic.

P270

Surprisingly complex rearrangements in the dystrophin gene oberved by MLPA analysis

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A reliable gene dosage analysis of single exons in the dystrophin gene can be performed by MLPA method (multiplex ligation-dependent probe amplification). Forty fragments each (79 coding exons and the muscle promotor) can be quantified using two reaction mixtures. Deletions and duplications of exons can be detected in male patients as well as in female carriers with high reliablity. More than 600 patient samples were analysed since July 2004 and a series of until now unrecognized deletions and duplications were observed.

Three highly complex rearrangements illustrate the dynamics in this big gene with extremely long introns. A DMD patient with mental retardation had a duplication of exons 42-43 directly followed by a deletion of exons 44-47. A transcript analysis from two years ago has already revealed this rearrangement. In another DMD patient a „perforated“ deletion was found, that is a deletion at two different positions in the gene (exon 13 and exon 17-18). In a Turkish family the index patient and his mother had a „perforated“ duplication of exons 2-12 and exons 51-60. The clinically manifesting sister of the index patient showed a de novo deletion of the exons 13-50. The mechanism for the generation of this mutation is discussed.

P271

Direct sequencing of all 79 exons of the DMD gene reveals point mutations in 24 patients without gross genomic rearrangements

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Approximately two-thirds of the mutations either in Duchenne muscular dystrophy (DMD) or the milder Becker muscular dystrophy (BMD) are deletions of one or more exons in the dystrophin gene which is located on chromosome Xp21. In our routine genetic testing, the MLPA (multiplex ligation-dependent probe amplification) method is used to detect deletions and duplications of this gene.

Twenty eight patient samples without a gross genomic rearrangement were subsequently analysed by direct sequencing of all 79 exons and adjacent intronic regions of the DMD gene. The majority of samples (16/28) presented nonsense mutations which result in premature translation termination. Since these mutations were

located in different exons there was no obvious hotspot for premature stop codons. We also detected 6 mutations at canonical splice sites of the gene which presumably lead to aberrant splicing according to splice prediction programs. In two patients we found frame-shifting deletions of 2 or 4 nucleotides in exons 18 and 29, respectively.
In another four patients we could not detect any point mutations in the DMD gene thus the diagnosis DMD or BMD remains questionable.
Despite the high cost and workload, technical progress in sequencing chemistry and laboratory automation have made the direct sequencing of large genes a feasible task with acceptable turn-around times. It is a useful complementation of the standard screening for deletions or duplications in the DMD gene.

P272

Novel ACVRL1 and ENG mutations in patients with hereditary hemorrhagic telangiectasia

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Hereditary hemorrhagic telangiectasia (HHT), or Morbus Osler, is a heterogeneous inherited disorder characterized by multi-systemic vascular dysplasia and wide variation in its phenotypic expression. In approximately 70% of HHT-patients, mutations can be identified by sequencing the two known HHT genes, ACVRL1 (ALK1) and ENG. Previously, we and others showed that hepatic manifestation in HHT patients is associated with mutations in the ACVRL1 gene, but rarely caused through ENG mutations.
In order to identify the molecular bases of HHT in a series of 15 adult patients with proven or likely HHT, we sequenced the coding exons and flanking intronic regions of ACVRL1 and ENG. In eleven of the patients (77.3%), we identified twelve different mutations, six in ACVRL1 and six in ENG. In each gene, four of the identified mutations were novel. Eight of the twelve mutations were missense mutations, all of which alter highly conserved amino acid residues. Two mutations were in-frame deletions or premature stop mutations, respectively. One patient carried two ENG mutations. No mutations could be identified in the remaining four HHT patients. In accordance to our previous studies, patients with severe liver involvement either carried a mutation in the ACVRL1, or no mutation was identified. One of the HHT patients with an ENG missense mutation was also affected by retinitis pigmentosa (RP).
We conclude that in most cases with HHT, ACVRL1 and ENG mutation analysis will confirm the clinical diagnosis. Molecular genetic diagnostics of patients with hepatic manifestations should primarily consider the ACVRL1 gene.

P273

Otoferlin, a constituent of acoustic signal transmission, and its interacting partners in the inner ear

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Deafness as a neurosensory disorder is inherited in a majority of prelingual hearing defects in an autosomal recessive mode. Presently, more than 60 genes have been implied in contributing to hereditary hearing impairment. DFNB9 affected individuals were shown to carry mutations in the OTOF gene with 18 mutations (mostly nonsense with a few missense) detected to date. On the basis of sequence homology to proteins like Fer-1 (C. elegans), Dysferlin and Myoferlin, possible functions like an involvement in membrane trafficking were discussed and otoferlin's interaction with syntaxin/SNAP25 was indicated. We generated an antibody for immuno-histochemistry and Western blots and strived to assess the possible binding partners of OTOF by (i) immuno-coprecipitation and by (ii) the yeast-2-hybrid system. The first approach generated 19 and the latter over 80 candidates that are now being screened for their actual presence in cells of the cochlea. To this end a cochlea-specific cDNA library and an inner hair cell (IHC)/outer hair cell (OHC) library are being used. Following confirmation of specific expression patterns, colocalization of the OTOF-partner will be assessed by confocal microscopy of immunostained tissue sections. In parallel, a construction of a knock-in mouse model for OTOF-exon14 mutations found in patients is under way.

P274

The Oculo-Auriculo-Vertebral Spectrum (OAVS): Genetic testing of candidate genes

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The Oculo-Auriculo-Vertebral Spectrum (OAVS, OMIM %164210) is a common birth defect involving first and second branchial arch derivatives. It is characterised by hemifacial microsomia, epibulbar tumours, ear malformations and vertebral anomalies. Although rare familial cases suggest that OAVS has a genetic basis, no causative mutation has been identified, so far. Some patients with OAVS show overlapping clinical features with other birth defects such as Branchio-Oto-Renal dysplasia (BOR, OMIM #113650), Treacher Collins syndrome (TCS, OMIM #154500), Townes-Brocks syndrome (TBS, OMIM #107480) or Okihiro syndrome (OMIM #607323). Therefore, we have analysed genes in which mutations are causative for the above mentioned disorders. From our cohort of 140 patients with OAVS we sequenced EYA1 (n=12), TCOF1 (n=10), SALL1 (n=56) and SALL4 (n=3) in phenotypically selected patients. In addition, we sequenced the positional candidate gene RAB28, that is disrupted by a translocation t(4;8)(p15.3;q24.1) in a patient with OAVS, in 75 patients. As Pact-/— mice show atresia of the external auditory canal and malformation of some cranial bones we also analysed the PACT gene in 28 patients with OAVS who present with unilateral (n=4) and bilateral (n=24) atresia of the external auditory canal. We found no bona fide mutation in any of the investigated candidate genes. Thus, we propose that OAVS is not an allelic variant of BOR, TCOF or TBS. In conclusion, epigenetic dysregulation of the developmental gene BAPX1, which maps close to the t(4;8) breakpoint, remains the only described molecular defect in OAVS (Fischer et al., Hum Mol Genet, 2006).

P275

Methylation sensitive regulation of TNF alpha expression: implications for Parkinson's disease

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Inflammatory processes have long been noticed in neurodegenerative disorders. Whether the inflammatory response accompanies the pathology or constitutes a mechanism of the disease process is unknown. Activated microglia are attracted to plaques in AD tissue and activated microglia has been found in the substantia nigra of PD patients early in the disease process. Among the numerous cytokines involved in the inflammation in neurodegenerative disorders tumor necrosis factor (TNF) alpha seems to be of particularly importance as TNF-alpha is known as a strong promoter of inflammation and is elevated in AD, PD and ALS. TNF alpha is a multifunctional cytokine that promotes inflammation but also functions in protective immune response, cell proliferation, differentiation and apoptosis. Expression of TNF alpha is tightly regulated on the transcriptional, post-transcriptional and translational level. The core promoter region of TNF alpha contains several putative transcription factor binding sites for the activating factors NF-Kappa B, AP1, AP2 and Sp1 To test the hypothesis if the methylation state of the TNF alpha promoter is altered in the brain of PD patients we compared DNA from different brain regions of PD patients and neurologically healthy, age and sex matched controls. Comparing DNA from the substantia nigra, the region of the most prominent pathology in the PD brain and from the putamen and cortex, we analyzed the methylation state of the TNF alpha core promoter region by bisulfite sequencing. Further, as the TNF alpha promoter sequence contains several CpG dinucleotides located within or next to transcription factor binding sites, we used Electrophoretic Mobility Shift Assays to analyze whether the methylation state of these cytosine residues in vitro influences the binding of tran-

scription factors to the promoter. Luciferase-reporter-assay were performed for functional analysis of the methylated and demethylated TNF alpha core promoter in vivo.

P276

Mutation analysis of patients with Marfan syndrome or Marfan-related disorders

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Marfan syndrome is an autosomal dominant hereditary disorder of connective tissue. Cardinal manifestations include proximal aortic aneurysm, ectopia lentis, and involvement of the skeletal system. The clinical diagnosis is based on a set of well-defined clinical criteria, the Ghent nosology. Heterozygous mutations in FBN1 (chromosome 15q21.1, 230-kb gene, 65 coding exons), which codes for the protein fibrillin 1, are the main cause of Marfan syndrome. Overlapping syndromes such as the Loeys-Dietz syndrome or Marfan syndrome type II (MFSII) are caused by mutations in TGFBR1 or TGFBR2, genes coding for the transforming growth receptor type I and II, respectively.
Molecular genetic analysis of FBN1 was carried out by direct sequencing of the coding exons and flanking intronic regions in 34 patients fulfilling the Ghent criteria or having tentative clinical signs of Marfan syndrome. A total number of 16 mutations were identified: 10 missense mutations, 2 frameshift mutations, 1 splice site mutation and 3 nonsense mutations. Out of these mutations, 6 missense mutations (Y63C, C781Y, R954C, C1333R, C2053T, C2339R), 2 frameshift mutations (8461insGA in exon 65 and 1376_1377delCT in exon 11) and 1 splice site mutation (2419+1 G>A in intron 19) were previously not described.
The amino acids affected by mutation are highly conserved within the vertebrate system; all of them involve a mutation to or from a cystein codon. The pathogenic nature of the newly identified mutations was verified by analysing other family members, and genotype-phenotype correlations for selected patients will be discussed. Patients with no identified mutation in FBN1 and fulfilling the diagnostic criteria for the overlapping Loeys-Dietz or MFSII syndrome were tested for mutations in TGFBR1 and TGFBR2. This was performed by direct sequencing of the coding exons and flanking intronic regions of TGFBR1 and TGFBR2. Out of a number of 4 patients, one previously described missense mutation was identified.

P277

Multi-exon out of frame deletion of the FBN1 gene leading to a severe juvenile onset of cardiovascular phenotype in Marfan syndrome

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Marfan syndrome is caused by mutations in fibrillin-1, a large gene spanning ~200 kb of genomic DNA on chromosome 15q21. So far, more than 600 different mutations have been identified, accounting for 60-90% of all Marfan syndrome cases, the vast majority being single nucleotide exchanges as well as small deletions and insertions. Only four major rearrangements have been described in the literature so far. We have screened 11 individuals fulfilling the diagnostic criteria of Marfan syndrome but negative for point mutations in the fibrillin-1 gene by SSCP and/or direct sequencing, for large rearrangements. We report here the largest known de novo and out of frame deletion in the fibrillin-1 gene in a patient fulfilling the diagnostic criteria of Marfan syndrome. We identified the deletion breakpoints at the genomic and transcript levels and studied the expression of the mutated allele at the transcript and protein level. We conclude that large rearrangements may account for a non-negligible proportion of all Marfan cases.

P278

Identification of transgene integration sites in a transgenic rat model for Huntington's disease

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Generation of transgenic animals by DNA microinjection has become one of the most important technologies in studying gene function in vivo. Random integration of transgenes often results in insertional mutation, which may complicate phenotype analysis of transgenic animals and/or create a good opportunity to study the function of endogenous genes.
We recently described a transgenic rat Model for Huntington's disease with a slowly progressive behavioral phenotype with emotional disturbance, motor deficits and cognitive decline. The tgHD rat model reflects to a remarkable extent cellular and subcellular neuropathological key features as observed in human HD brains. To exclude that some of the phenotypic features of the tgHD rats are caused by the transgene integration event, we cloned the transgene/genome junction sequences of two independend transgenic lines using a genome walking approach. We localized the transgene integration sites to the rat chromosomal band 3q21 in transgenic line 2762 and 7q13 in transgenic line 2771.
Preliminary FISH results indikate a single integration site in both transgenic lines. On chromosome 7q13 the integration does not affect any known gene. On chromosome 3q21 the transgene integrated in intron 23 (of 24) of XM_345366, a predicted gene similar to myosin IIIB. Class III myosins are actin-dependent motor proteins that contain amino-terminal kinase domains and are expressed in photoreceptors. However, all tgHD rats did not show any signs of disturbed vision. Taken together, it seems unlikely that the phenotypic hallmarks of the tgHD rat model are caused by an insertional mutation of a functional gene. However, the role of the integration into an intron of a putative gene in line 2762 has to be further investigated.

P279

Do polymorphisms in interaction partners of ataxin-3 influence the age-at-onset in SCA3?

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Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder which is also known as Machado-Joseph disease (MJD). SCA3 is caused by the expansion of a CAG stretch in the MJD1 gene encoding a polyglutamine repeat in the respective ataxin-3 protein. SCA3 therefore belongs to the group of the so called polyglutamine diseases.
Statistically a correlation between the number of CAG repeats and the age-at-onset in SCA3 patients is obvious: Patients with a low number of about 60 CAG repeats develop symptoms in their 60s and 70s whereas the age-at-onset in patients with a high number of CAG repeats (e.g. higher than 75 repeats) might be around 25 years or earlier. However, this correlation is not perfect since it was show that some patients with 71 CAG repeats have an age at onset of 29 years whereas other patients develop symptoms not until the age of 50 years. For this reason it has been proposed that the number of CAG repeats contribute only 55 % to the age at onset and that the remaining 45 % is influence by other factors, which we try to identify in this study. Up to now, the actual function of ataxin-3 is unknown. Recently, HHR23A and B, the human homologues of yeast RAD23 as well as p97/VCP were identified as interaction partners of ataxin-3. In this study we focused on single nucleotide polymorphisms (SNP) within these interaction partners. We first tested SNP published in relevant databases in our cohort of SCA3 patients, and later performed own screenings for novel SNP using dHPLC. We correlated the occurrence of the respective SNP with the clinical data and calculated whether these SNP influence the age-at-onset in SCA3.

P280

Functional analysis of the transcription factor-like nuclear regulator (TFNR) protein by generating conditional knock out mice

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The transcription factor-like nuclear regulator (TFNR), also known as BDP1 is part of the transcription IIIB complex (TFIIIB) and plays an important role in transcription initiation of genes transcribed by polymerase III. TFNR comprises 39 exons, encoding a 10.8 kb transcript which is ubiquitously expressed with notable abundance in cerebellum. The protein encoded by TFNR consists of 2624 amino acids and is subdivided

into three regions: an N-terminal part (B"), a middle part which contains 9 repeats of a 55 amino acid motif and a C-terminal part, the last two with yet unknown function. TFNR maps on 5q13, distal to the duplicated region that includes SMN1, the spinal muscular atrophy (SMA) determining gene. In rare cases SMA patients exhibit additional atypical features such as axonal neuropathies or cerebral atrophy. These phenotypes correlate with large deletions in the SMA region. We hypothesized that haploinsufficiency of TFNR may cause brain atrophy and neuronal dysfunction.
The human TFNR sequence reveals a homology of 98% to its murine counterpart. To investigate the role of TFNR protein in mammals in more details, we developed a conditional knock-out strategy by which exon 2 will be floxed and therefore will be removed by using the Cre/loxP system. We have been already successful in generating the targeting vector for ES cell transfection and established the Southern blot strategy for screening transfected clones after selection. Using the advantages of the Cre/loxP system various investigations will be possible under the consideration that TFNR has an important role in brain development and that therefore a homozygous ubiquitous knock-out of the TFNR would be lethal.

P281

A new POMT1 mutation in a case of Walker-Warburg Syndrome diagnosed prenatally
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Walker-Warburg Syndrome (WWS, MIM 236670) is one of the autosomal recessive type 2 (cobblestone) lissencephalies that are characterized by the triad of congenital muscular dystrophy, cobblestone lissencephaly, and eye abnormalities. Mutations in six genes are known to cause Type 2 lissencephaly, and the genes all encode putative or proven glycosyltransferases responsible for the glycosylation of α-dystroglycan. We describe a case of WWS that was diagnosed prenatally with an ultrasound finding in the 22nd week of pregnancy, of hydrocephalus with aqueduct stenosis and bilateral retinal aplasia. The parents are first cousins and had previously lost a child with hydrocephalus shortly after birth. Sequencing of the POMT1 gene in DNA extracted from cultured amnio cells revealed the homozygous mutation c.2164G>A in exon 20, which results in the missense mutation, Gly722Arg. A different mutation affecting the neighbouring codon in POMT1 has previously been reported in another patient with WWS. Twenty-seven mutations in the POMT1 gene have been reported and different allelic mutations in POMT1 result in extremely variable phenotypic severity. Due to the extensive heterogeneity of the disorders classified as type 2 lissencephaly, the description of cases of Walker-Warburg syndrome with mutation of the POMT1 gene is important for future diagnosis and prognosis.

P282

CHRNA2 is not mutated in a large sample of families with nocturnal frontal lobe epilepsy (ADNFLE)
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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a partial epilepsy characterized by clusters of seizures emerging nearly exclusively from sleep. The seizures are usually brief, lasting 30 to 60 seconds, and are characterized by motor phenomena with hyperkinetic or tonic manifestations. Two genes are known to cause ADNFLE, CHRNA4 and CHRNB2 encoding the alpha4- and beta2—subunits of the neuronal nicotinic acetylcholine receptor (nAChR), respectively. All known ADNFLE mutations are located in either the second or third transmembrane region, affecting the opening and closing mechanisms of the ion pore. Recently, a third nAChR subunit gene, CHRNA2 on chromosome 8p12.3-q12.3 was reported to be associated with nocturnal frontal lobe epilepsy. A missense mutation was found in an Italian epilepsy family, in which nocturnal seizures were accompanied by fear sensation, protruding tongue movements and nocturnal wandering (Aridon et al., 2006). We have now screened a sample of 82 index patients from unrelated ADNFLE families to assess the frequency of CHRNA2 mutations in this familial epilepsy syndrome. Mutation screening was performed by amplification and subsequent sequencing of part of CHRNA2 exon 6 containing transmembrane regions 1-3. Detected variants were tested in a case-control association study. Within our large sample of ADNFLE families no mutations could be identified in the parts of CHRNA2 that contribute to the ion pore. Sequencing identified a novel samesense nucleotide exchange (c.771C/T) that was also present in controls and is therefore likely to be non-pathogenic. The absence of mutations in our large sample of 82 families renders a major role of CHRNA2 in ADNFLE unlikely.

P283

Codon 101 in PRKCG: „Hot spot" for SCA14-causing mutations?
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Spinocerebellar ataxia type 14 (SCA14) is an autosomal dominant neurodegenerative disorder characterized by slowly progressive ataxia, dysarthria and nystagmus. Additional symptoms such as myoclonus, tremor, dystonia, depression, and cognitive impairment can occur in patients. Mutations in PRKCG, encoding protein kinase Cγ, cause the disease. To date, a minimum of 20 different PRKCγ mutations have been identified worldwide in single families. 12 mutations are clustered in exon 4, the remaining ones are located in exons 1, 2, 5, 10 and 18 of PRKCG. A patient of German origin with slowly progressive ataxia and a questionable family history for a movement disorder was diagnosed clinically as SCA6. However, SCA6 was excluded molecularly as were other SCAs caused by CAG expansions. We then considered SCA14 based on slow progression of symptoms and pure cerebellar ataxia. We identified an exchange of a histidine for a tyrosine at amino acid 101 (H101Y) in our patient. Interestingly, this mutation was described earlier in a large American family of English and Dutch extraction (Brkanac et al., 2002). Furthermore, a histidine to glutamine change at amino acid 101 was detected in a Portuguese family (Alonso et al., 2005). Given the different ethnic origins of the patient presented here and those described by Brkanac and Alonso, codon 101 in PRKCG may be a hot spot for mutations causing SCA14.

P284

A novel EP300 gene mutation in a patient with mild Rubinstein-Taybi syndrome
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Rubinstein-Taybi syndrome (RSTS, MIM 180849), a dominant Mendelian disorder with typical face, short stature, skeletal abnormalities, and mental retardation, is usually caused by heterozygous mutations of the CREBBP gene but recently, EP300 mutations were reported in three individuals (Roelfsema et al. Am J Hum Genet 2005). Using quantitative PCR (CREBBP and EP300 gene) and EP300 genomic sequencing, we studied here 13 patients who had shown no mutation after genomic sequencing of the CREBBP gene in a previous investigation (Bartsch et al. Hum Genet 2005). Two new disease-causing mutations were identified: a partial deletion of CREBBP and a 1-basepair deletion in EP300, c.7100delC (p.P2366fsX2401) representing the fourth EP300 gene mutation reported to date. The low rate of EP300 gene mutations in patients with RSTS and the unusual location of these mutations (outside the critical HAT domain) suggest that most EP300 gene mutations could be associated with other phenotypes, not RSTS. The comparatively high rate of single nucleotide polymorphisms (SNPs) in the EP300 gene (2.23 per individual) as compared to the CREBBP gene (.71 per individual) ($p<0.001$, Wilcoxon test) is another (indirect) evidence that EP300 gene mutations should be as frequent as CREBBP gene mutations.

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P285

Creation and characterization of a knockout mouse for the VKORC1L1-gene
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Introduction: Vitamin K is a cofactor for carboxylation of glutamate residues of proteins by vitamin-K-dependent gamma-glutamyl carboxylase. This reaction is important for activation of sev-

eral blood-clotting proteins and hence for blood coagulation. Carboxylation is essential for proteins of the bone metabolism and cell cycle regulation. The resultant vitamin-K 2,3-epoxide is recycled to the reduced active form by vitamin-K epoxide reductase (VKOR) in the vitamin K cycle. By sequence search algorithms, a paralog of VKORC1 has been identified in vertebrate genomes, called VKORC1-like1 (VKORC1L1). The gene is more conserved across the vertebrates than VKORC1. No function whatsoever is known for this L1-gene. We therefore plan to generate a KO-mouse line as a first approach to the analysis of this gene's function.
Methods: A plasmid-vector was generated with a short and a long homology arm to the VKORC1L1 gene in order to ensure homologous recombination. Exon 2 is flanked by loxP-sites to induce conditional inactivation of VKORC1L1 in selected tissues. After plasmid-transfection the VKORC1L1-targeted embryonic stem cells were confirmed by Southern blot and PCR. Positive ES cells are microinjected into pseudopregnant mice. The descendants, carrying the inactivated VKORC1L1-gene, will be analysed for viability and phenotype changes.
Results: 400 stem cell clones were analyzed by Southern blot. Therefore a probe which is homologous to the 5'-site of the gene was created. BamHI digested DNA of stem cells were blotted on a membrane and hybridized with the probe. Wildtype cells showed a 7,8 kb band on x-ray film, recombinant cells had a secondary 4,9 kb band. Two of the 400 stem cell clones were detected as recombinants.
Outlook: The next step, the microinjection of the positive selected clones, is in progress. The results will hopefully give new information about the function of the gene. The final goal is to understand the role of the VKORC1L1-gene in the entire organism.

P286

Cross-reactivity of cystic fibrosis mutation R117P with Inno-LiPA probe R117H

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Cystic fibrosis is one of the most common hereditary diseases worldwide. In Germany, 1 in 2,500 newborn children is affected by the disorder that is caused by mutations within the cystic fibrosis conductance regulator gene. To date, more than 1,000 mutations are known, many of them are rare mutations, occurring as spontaneous alterations affecting only one family or individual.
Here, we describe the cross-reactivity of the rare CF mutation R117P with a probe for the mutation R117H which accounts for 0.9% of CF disease cases in Germany.
DNA of the same patient was used for detection of CF mutations using the Inno-LiPA CFTR kit from Innogenetics as well as for sequence analysis for the R117H mutation. Interestingly, when analysing the DNA for R117H, sequencing did not reveal a nucleotide substitution leading to the relatively common replacement of argenine 117 with histidine, but with proline. Nevertheless, probing of the same DNA with the Inno-LiPA test, we found strong hybridisation with the R117H probe, indicating a substitution of arginine with histidine.
This finding shows that by using the Inno-LiPA test, R117P mutations can not be distinguished from R117H mutations and thus, it is not recommendable to calculate R117P frequencies using this test principle.

Genetic Analysis, Linkage and Association, Complex Genetics/Diseases

P287

First results of the first genome wide association study on genetic determinants of cardiac mass and structure: Analysis of 500 000 SNPs

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Introduction: There is increasing evidence that left ventricular (LV) mass and structure are in part determined by genetic factors. However, the causative genetic variants are incompletely understood.
Methods: A total of 592 probands from the population-based KORA/MONICA Augsburg survey were carefully phenotyped by physical examination, biochemical analyses, standadized questionnaires and repeated echocardiographies. All individuals were genotyped with the GeneChip® Human Mapping 500K Array from Affymetrix. In multivariate analyses SNPs were tested for association with different measures of cardiac mass and geometry (LV Mass indexed to body surface area or to fat-free mass, septal and posterior wall thickness, end-diastolic diameter) using non-parametric tests (Kruskal-Wallis test und Jonckheere-Terpstra test).
Results: In a first analysis, a total of 368 SNPs displayed association with both measures of LV mass ($p<0.001$). Of these, 25 were additionally associated with septal and posterior wall thickness. Twelve SNPs displayed association with both LV mass measures as well as with end-diastolic diameters and 60 SNPs were associated with septal and posterior wall thickness. Several significant SNPs clustered in genomic linkage disequilibrium blocks. The further strategy includes validation of most promising SNPs in a larger sample (n=712) and thereafter replication of the most interesting association findings in independent populations.
Conclusions: To our knowledge this is the first genome wide association study which identified interesting genomic regions with significant association to cardiac mass and structure. Currently, validation and replication of the most promising findings in independent populations is being performed.

P288

Fine-mapping of myocardial infarction susceptibility locus on chromosome 14

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Introduction: Myocardial infarction (MI) and coronary artery disease (CAD) are the most frequent causes of death in Western countries. Besides classical risk factors like smoking, hypertension, dyslipidemia, and diabetes, a familial background of disease is a strong predictor for MI and CAD. In the previous systematic genome-wide scan in 513 affected sibs using microsatellite markers a MI susceptibility locus on chromosome 14 was identified with a significant linkage (LOD score of 3.9, genome-wide $p<0.05$).
Methods: Study population used for case-control association analysis consisted of 826 MI male patients and 801 healthy control subjects. We performed a high-scale linkage disequilibrium (LD) mapping using SNP marker evenly covering –1 LOD drop interval of the maximal linkage signal (genomic region of 7 Mb containing 47 genes) using the ILLUMINA high-throughput genotyping platform.
Results: Altogether, 628 markers fitted the predefined criteria (call rate > 90%, Hardy-Weinberg equilibrium, and minor allele frequency >1%) and were used in statistical analyses. The most significant association signal (P=0.000009, OR 1.39) was identified in a 118 kb region with high LD containing two genes. This association remained significant after permutation adjustment for multiple testing (table-wide p=0.0056).
Conclusion: Using a high-scale LD mapping approach we identified a region contributing to a genetic susceptibility of myocardial infarction. Replication studies as well as detailed genetic and biological analyses of this genetic locus are currently under way.

P289

Genome-wide linkage interaction analysis of bipolar affective disorder

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We recently performed a genome-wide analysis in three European samples with bipolar affective disorder (BPAD) consisting of 52 families of Spanish, Bulgarian, and Roma descent (Schumacher et al. 2005). In the present study, we extended our analysis by performing a systematic genome-wide linkage interaction analysis using the full linkage data set. Non-parametric linkage analysis was performed after conditioning on linkage evidence to each of the 435 genotyped STR markers. Evidence for interaction was observed between chromosomes 2 and 6 as well as 2 and 15. In order to confirm our interaction results and to narrow down the linked regions we analyzed additional STR markers covering all three regions. We observed a ΔNPL-Score of 6.60 (increase of the initial NPL finding) on chromosome 2q when conditioning on linkage signals to chromosome 6q. Vice versa, we found a ΔNPL-Score of 4.85 on 6q when conditioned on the initial linkage data to chromosome 2q. Fine-mapping on chromosome 15 is still ongoing and would be presented at the congress. Our results point to an interaction between chromosomal loci on 2q and 6q as well as 2q and 15q. Susceptibility genes for BPAD located in these regions may have interfering regulative effects or interact together within the same biological pathway.

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Genetic analyses in multiplex families with myocardial infarction: Identification of novel chromosomal loci

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Background: In rare cases, coronary artery disease (CAD) and myocardial infarction (MI) cluster in families. In recent years we identified a total of 25 multiplex families with at least 4 and maximal 22 MI patients per family by systematic large scale screening of 200.000 patient records. We intend to demonstrate the inheritance pattern of MI and to localize the underlying genetic defects.
Methods: Power simulations using the SLINK software package applying several models of inheritance we performed segregation analysis with JPAP. To detect new candidate regions, we performed model-based and model-free multipoint linkage analyses in these families using 805 microsatellite markers. Methodologically, we employed the Lander-Green as well as a Markov chain Monte Carlo algorithm. Genome wide significance was assessed by simulations.
Results: In most families analyses revealed the highest theoretical LOD scores under the assumption of an autosomal-dominant inheritance. With the segregation analysis, we could verify an autosomal-dominant inheritance pattern. We detected a single locus in two independent families on chromosome 8q23.3-8q24.4 (D8S1179: LOD_dom=2.4 (p_emp=0.0004), LOD_npl=2.3 (p_emp=0.0007); 4/6 MI patients). Moreover, we identified a family with 7 MI patients which maps to region 4q34.1-4q35.1 (D4S2431: LOD_dom=2.1 (p_emp=0.0010), LOD_npl=2.0 (p_emp=0.0011)). The same region has previously been reported.
Conclusions: To our knowledge, we present the first systematic screen for Mendelian inheritance of MI. The results of segregation analysis and the positive linkage results strongly suggest that MI may be inherited in an autosomal-dominant pattern, at least in some families. The chromosomal localization of MI genes in selected families may further facililiate the identification of genetic defects leading to MI.

P291

Analysis of segregating SNP in a CNG in the schizophrenia locus SCZD10 on chromosome 15q15

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The human genome contains approximately 1-2% single copy, highly conserved sequences that are not functionally transcribed (CNG). Despite many studies the functional attributes of CNGs remain largely unknown. The evolutionary depth of their conservation, though, indicates that genomic variation in conserved non-genic sequences may be associated with phenotypic variability and human disorders.
Previously we identified a schizophrenia locus (SCZD10) on chr.15q15 with genome-wide linkage studies of 12 extended pedigrees. We refined the critical region to a 7.49Mb interval with further extensive haplotyping studies. In our efforts to reveal the underlying genetic defect, we performed mutation analysis up to now for 79 candidate genes by automated sequencing (see Ekici et al.). Due to absent functional mutations, we also investigated the CNGs in this 7Mb region on chromosome 15q15. We used the global alignment programme VISTA to identify all highly conserved elements in the region with >100 bases. In total, we could identify 3,099 CNGs with more than 70% identity, 65 of them with an identity of 100%. We sequenced 8 affected individuals for the 65 perfectly conserved CNGs and surprisingly detected 9 SNPs within these CNGs. Three were not contained in public databases and were observed only in patients. The three SNPs were genotyped in large families showing linkage to this locus and one of them segregated with the disease. Genotyping of the entire study group is ongoing. Our initial data warrant further exploration of the hypothesis, that variation in CNGs might be associated with this trait.

P292

Identification of a novel gene locus for autosomal dominant optic atrophy and sensorineural hearing loss

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Diseases of the optic nerve rank among the most common causes of vision loss in childhood and early adulthood. Autosomal Dominant Optic Atrophy (ADOA) has an estimated prevalence of up to 1:12.000 and therefore is the most frequent hereditary optic neuropathy besides LHON. Mutations in the *op*tic *a*trophy gene one (OPA1) are responsible for approximately 40-70 % of all ADOA cases. In addition a few families with mutations in the OPA3 gene have been described and two further gene loci (OPA4 18q12.2-q12.3 and OPA5 22q12.1-q13.1) have been mapped, demonstrating genetic heterogeneity in ADOA. Here we report on a novel locus causing ADOA and sensorineural hearing loss in a large family of Italian ancestry, in which optic neuropathy was undistinguishable from that associated with OPA1 gene mutations. Mutations in OPA1 were ruled out by DNA sequencing, cDNA analysis and finally marker segregation analysis. Furthermore the gene loci encoding Mitofusin 1 and 2 revealed no significant linkage. A subsequent Whole Genome Scan identified a region on chromosome 16q21-q22. Refined meiotic breakpoint mapping finally revealed a non-recombinant genetic interval of 6,96 cM with a maximum twopoint LOD score of 8.84. Genomic screening of four candidate genes in this region (CALB2, CYB5-M, DHODH and PLEKHG4) presented with no disease causing mutation. The identification of a new optic atrophy locus in this pedigree emphasises further genetic heterogeneity in ADOA.

P293

Mutations in the ribosomal protein gene RPL10 suggest a novel modulating disease mechanism for autism

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Autism is a distinct neurodevelopmental syndrome and is characterized by marked social

deficits, deviant language and a restricted range of stereotyped repetitive behaviors. These symptoms usually occur within the first three years of life with lifelong persistence. Autism has a strong genetic background with a higher frequency of affected males suggesting involvement of X-linked genes and possibly also other factors causing the unbalanced sex ratio in the etiology of the disorder. Syndrome pathogenesis is associated with abnormal brain development and manifest in several specific brain regions, especially cerebellum, amygdala and hippocampus. We have identified two missense mutations in the ribosomal protein gene RPL10 located in Xq28 in two independent sib-pair families with autism. We have obtained evidence that the amino acid substitutions L206M and H213Q at the C-terminal end of RPL10 confer hypomorphism with respect to the regulation of the translation process while keeping the basic translation functions intact. This suggests the contribution of a novel, possibly modulating aberrant cellular function operative in autism. Previously, we detected high expression of RPL10 by RNA in situ hybridization in mouse hippocampus, a constituent of the brain limbic system known to be afflicted in autism. Based on these findings, we present a model for autistic disorder where a change in translational function is suggested to impact on those cognitive functions that are mediated through the limbic system.

P294

A German genomewide linkage scan for type 2 diabetes supports a metabolic syndrome locus on chromosome 1p36.13 and a type 2 diabetes locus on chromosome 16p12

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Aims: In Germany, type 2 diabetes shows increasing prevalence with 5 to 8 million people having any form of diabetes (prevalence: 6-10%). In an effort to identify genetic factors contributing to this disorder, we performed a linkage study in 253 German families (affected-only analyses in affected sib pairs) using 439 STRPs at an average resolution of 7.76±3.80 cM (Marshfield).
Results: We found suggestive linkage on chromosomes 1p36.13 (D1S3669, LOD=1.49, nominal p=0.004) and 16p12 (D16S403, LOD=1.85, nominal p=0.002).
The region on chromosome 1 overlaps with linkage reports on symptomatic/clinical gall bladder disease with type 2 diabetes in the Mexican American population from the San Antonio Family Diabetes/Gallbladder Study, on the body size-adiposity factor in another Mexican American population, on lipid abnormalities, and on hypertension in Australian sib pairs. The region on chromosome 16 overlaps with a linkage signal identified in a meta-analysis of four European type 2 diabetes-related genome scans. In linkage analyses conditional on evidence for linkage at D16S403 we identified a LOD increase (ΔLOD) D17S1780 (p=0.0075). Conditioning on D17S1780 revealed evidence for interaction with D1S3669 (p=0.0055), D16S403 (p=0.0091), and another locus on chromosome 1 at ~200cM where several genome scans reported evidence for linkage as well (p=0.0066).
Conclusions: Summarising, the reported genome wide linkage scan in German families with type-2 diabetes linked to two previously published loci on chromosomes 1p36.13 and 16p12. Conditional linkage analyses suggest interaction between T2DM susceptibility regions on chromosomes 16 and 17, as well as between those at chromosomes 17 and 1. The region on chromosome 1p36 might be a candidate for complex metabolic syndrome susceptibility, either by one gene contributing to variable metabolic syndrome traits, or by harbouring several genes each causing a different metabolic syndrome subtype.

P295

The first association study between G72 and unipolar depression in a large sample of patients and controls of German descent

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G72 is considered a strong susceptibility gene for both schizophrenia (SZ) and bipolar disorder (BD). We recently reported association between identical G72-haplotypes and SZ, BD and panic disorder (PD). An association study on major depression (MD) has not yet been conducted. We studied a German sample of 500 MD patients and 1030 population-based controls. We were interested whether our previously identified risk haplotype of markers M23 and M24 was also associated with MD. To further explore any relationship between G72 and MD, we genotyped 10 additional SNPs highlighted in other studies. The haplotype distribution differed significantly between cases and controls (p=0.04). The previously identified individual risk haplotypes C-T and T-A also showed differential distribution between cases and controls. The exploratory analysis including 10 further G72 markers of interest and using a haplotype-sharing approach supported our findings, implicating the distal markers. This is the largest case-control study on G72 and MD to date. We found an association between MD and the same risk haplotype that we previously found associated with SZ, BD, or PD. We also found suggestive associations with other variants. Given that depressive symptoms are present across these diagnostic groups, G72/G30 might predispose to an endophenotype common to all four disorders. Our ongoing studies suggest that the personality dimension neuroticism could constitute this underlying endophenotype.

P296

Novel DFNB loci in consanguineous families with autosomal recessive nonsyndromic hearing loss from Morocco

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Hearing impairment is the most common inherited disorder of a person's senses and therefore understanding the various pathophysiological mechanisms causing hearing loss has an important medical impact. We used genome-wide homozygosity mapping approach in four large consanguineous families from Morocco with autosomal recessive nonsyndromic hearing loss (ARNSHL) and mapped two of the families to novel DFNB loci, one family to the DFNB33 locus on 9q35 with unknown causative gene, and one to the ESPN gene locus DFNB36 on 1p36.3. We identified the homozygous c.1757insG in ESPN in all affected family members of the SF18 family leading to a frame-shift and a premature truncation of the espin protein. Espin is a multifunctional actin cytoskeletal regulatory protein that is expressed in stereocilia of hair cells in the inner ear, and mutations have been described before in two families from Pakistan. The SF40 family was mapped to the DFNB33 locus on 9q34.3 to a 3 Mb region between marker D9S312 and 9qter. Since no causative gene is known for this locus, we tested the CACNA1B gene as a highly relevant candidate, but not mutations were identified. Furthermore, a novel locus, DFNB69, on 3p21.1-3p14.2 was identified in family SF33 located to a 9 Mb interval between D3S1588 and D3S3698, with a maximum multi-point LOD score of 3.01. SF27 family was linked to the novel DFNB70 locus on 2p21-2p16. The critical interval of 13 Mb is flanked by the markers of D2S119 and D2S378. Several cochlear expressed candidate genes are currently under investigation in order to find the

causative genes and to identify new pathophysiological mechanisms involved in hearing loss.

P297

Systematic association study of the dopamine ß-hydroxylase gene in migraine with aura

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Migraine with aura (MA) is an episodic neurological disorder with complex inheritance. Although the molecular pathophysiology of MA is largely unknown, there is functional and pharmacological evidence for an involvement of the dopaminergic system in migraine pathogenesis. One important component of this system is the dopamine ß-hydroxylase (DBH) that plays an essential role in dopaminergic and noradrenergic neurotransmission.
In order to determine whether genetic variation of the DBH locus is involved in the molecular pathogenesis of MA, we performed a systematic association approach by genotyping 11 SNPs capturing the main haplotype diversity of the DBH locus in a sample comprising 268 German MA patients and 272 gender-matched control individuals. In the single marker analysis we found two significantly associated SNPs (rs2097629: p = 9.3x10-4, OR = 1.51, CI [1.18-1.94]; rs1611131: p = 0.012, OR = 1.39, CI [1.07-1.81). The replication study for rs2097629 in an independent sample of 375 MA patients and 370 healthy controls was marginally non-significant. However, both samples taken together displayed a significant result (p = 5.3x10-4, OR = 1.33, CI = [1.31-1.56]). The genotype distributions of both samples did not deviate significantly from the Hardy-Weinberg equilibrium as analyzed by the exact test. To confirm our results, the replication study for SNP rs1611131 as well as a haplotype-based analysis in the extended sample are in process. Furthermore, a significant association between migraine and two length polymorphisms in the promoter region of DBH were reported recently. In order to replicate these findings and to confirm our results, the analysis of these polymorphisms is performed in our large sample comprising around 640 MA patients and 640 controls.

P298

Preferential reciprocal transfer of paternal/maternal DLK1 alleles to obese children - first evidence of polar overdominance in humans

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DLK1 is part of the Notch signaling pathway that controls many developmental processes. Several animal models suggest a function of DLK1 in adipogenesis. The gene is imprinted in eutherian mammals and located on chromosome 14 in human. To investigate a possible effect of DLK1 on human body weight we analyzed 32 polymorphisms in a 109 kb region encompassing the DLK1 gene. Upon study of 773 nuclear families based on extremely obese offspring and both parents we detected a single nucleotide polymorphism that is associated with child and adolescent obesity. Analysis of the allele transmission pattern within families indicated the existence of polar overdominance in humans, an unusual mode of non-mendelian inheritance known from the callipyge mutation in sheep.

P299

The DCDC2 compound deletion/STR polymorphism does not explain association with reading disability in a German dyslexia sample

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Dyslexia, or reading disability, is a complex disorder manifested by difficulties in learning to read and spell despite conventional instruction, adequate intelligence and sociocultural opportunity. Dyslexia is amongst the most common neurodevelopmental disorders with a prevalence of 5-12%. The DYX2 locus on chromosome 6p21-p22 is one of the best replicated linkage regions in dyslexia. Based on systematic linkage disequilibrium (LD) studies, we and others have reported the doublecortin domain containing protein 2 gene (DCDC2) as a strong candidate gene in this region (Meng et al. 2005, Schumacher et al. 2006). The group of Meng et al. (2005) has suggested a compound deletion/STR polymorphism in intron 2 of DCDC2 as the causative mutation. In the present study, we tested this compound deletion/STR polymorphism in 396 German dyslexia trios. We observed no significant deviation from random transmission, neither for the deletion nor for the alleles of the STR. We also did not found the deletion or any of the STR alleles to be in LD with the 2-marker haplotype which was associated with dyslexia in our sample (Schumacher et al., 2006). We thus conclude that the true causative mutation/s in DCDC2 remain/s to be identified.

P300

Lack of genetic association between components of the extracellular matrix (FBN1, LTBP2, MFAP2 and TGM2) and pseudoexfoliation syndrome in German patients

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Pseudoexfoliation (PEX) syndrome is a complex age-related disease which is characterized by deposits of a fibrillar extracellular material in the anterior segment of the eye, as well as in various extraocular tissues, e.g. in heart, lung and liver. The pathogenetic mechanism is still unknown, but overproduction and accumulation of abnormal fibrillar extracellular material have been suggested as a cause of PEX syndrome. A differential expression of genes mainly related to extracellular matrix metabolism has been reported in PEX patients. Since a genetic predisposition to this disease has been postulated, we investigated polymorphisms in four candidate genes coding for components of the extracellular matrix – FBN1, LTBP2, MFAP2 and TGM2 – for association with PEX syndrome. Therefore we recruited a large cohort of 333 unrelated German PEX patients and 342 healthy individuals who had repeated ophthalmologic examinations. Based on LD-data from HapMap we selected 42 haplotype tagging SNPs (29 in LTBP2, 5 in MFAP2, 5 in TGM2 and 3 in FBN1) covering more than 95% of genetic variation in Europeans at these loci and genotyped both groups using a combination of SNPlex and TaqMan assays. Five SNPs showed weak association (rs10146812, p=0.0414; rs862046, p=0.0049; rs2302114, p=0.0242; rs699370, p=0.0104; rs6023527, p=0.0289), but none remained significant after correction for multiple testing using permutation tests (n=10,000, Haploview 3.2). In conclusion, we found no significant differences in allele or haplotype distribution between PEX patients and control individuals in these four candidate genes. Hence, we suggest that genetic variation at FBN1, LTBP2, MFAP2 and TGM2 does not play a major role in the development of PEX syndrome.

P301

Periodic catatonia: mutation analysis of all coding genes in the schizophrenia linkage interval on chromosome 15q15

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Periodic catatonia (MIM 605419) is a familial subphenotype of schizophrenic psychoses with a strong genetic component. The genetically heterogeneous disorder is characterized by psychomotor disturbances with akinetic and qualitative hyperkinetic episodes, grimacing facial movements and apathy. Parametric as well as haplotype analysis were consistent with an autosomal dominant inheritance with reduced penetrance and a morbidity risk of ~27% for first degree relatives. Previously we have identified a major disease locus on chr.15q15 in a genome-wide linkage study of 12 extended pedigrees. We also replicated the chromosomal locus in an independent set of four families. Linkage and haplotype analyses in three exceptionally large pedigrees linked to chr.15q15 disclosed a critical region between markers D15S1042 and D15S659, which could be further refined with extensive haplotyping to a 7.49 Mb interval, containing 123 known genes (hg18).
In our efforts to reveal the underlying disease gene we prioritized all genes from the critical region with expression profiles using RT-PCR and determined their putative function based on extensive database searches. We first selected all 57 brain-expressed genes for mutation analysis by automated sequencing of DNA fragments in 8 individuals from linked families and 8 controls. No disease-causing mutation was identified in the coding region of any of these genes. These findings suggested, that variants in either
i) genes or parts thereof currently not known to be brain-expressed or
ii) in non-coding sequences such as regulatory elements may be the underlying genetic risk factor predisposing to periodic catatonia.
To address these questions we started to analyse all coding genes in this region and up to now sequenced 79 of them whithout identifiying any segregating variant. Independently, we are also sequencing a subgroup of conserved non-coding sequences from this locus (see Schanze et al.).

P302

The interferon-gamma receptor 1 gene modulates intrapair discordance in cystic fibrosis sib pairs

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Cystic fibrosis (CF) is the most common severe autosomal recessively inherited disease in the Caucasian population. CF is caused by a defect in the CFTR gene leading to disturbed ion transport of exocrine glands. The diversity of the clinical course among patients carrying the same CFTR mutation genotype implies that environmental influences and other genes modulate the course of CF disease. We have targeted the IFNGR1 gene as a modulator of CF disease severity because of its role as a transducer of the immunmodulatory and antiviral effects of interferon gamma. 37 families with F508del homozygous CF siblings, exhibiting concordant severely, concordant mildly or discordant clinical phenotypes, were selected from the European CF Twin and Sibling Study. Siblings and their parents were genotyped at microsatellite D6IFNGR1Sat1 within intron 5. Allele distributions were significantly different comparing concordant (CONC) and discordant (DIS) siblings (single marker p-value=0,00594). Hereupon, 19 SNPs within the IFNGR1 gene were tried on internal control-samples, 12 SNPs were excluded because of uninformativity and the remaining 7 SNPs (D6-134, D6-17, D6-113, D6-107, D6-133, D6-108, D6-101) within the promoter and intron 1 were chosen to genotype the same families. Two-marker haplotypes were constructed. For SNP D6-133, genotype and allele distribution was significantly different comparing CONC and DIS patient pairs (single marker p-value =0,00685). Haplotype distributions for D6-107 — - D6-133 and D6-133 —- D6-108 differed comparing CONC and DIS sib pairs (P = 0,01145). Based on the differences in genotype, allele and haplotype distributions, we conclude that IFNGR1 variants in intron 1 modulate CF disease severity. Sequencing of the fragment D6-107—- D6-108 from CONC and DIS patients will indicate possible modulation mechanisms.

P303

Molecular genetics of alopecia areata: characterization of rats and humans

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Alopecia areata (AA) is an inflammatory disease of the hair follicle that is characterized by circumscribed patches of hair that present with sudden onset and may multiply, expand and coalesce into extensive alopecia areata. It affects males, females and children at any age. Numerous studies indicate that AA is a multifactorial disease with a genetic basis, more frequently found in genetically related people as 10-20% of patients indicate at least one other affected family member. The identification of genetic factors involved is difficult because of the high heterogeneity displayed.
Rodent models have provided evidence in support of AA as an autoimmune disease. Dundee experimental bald (DEB) rat is a frequently used model with up to 70% of the experimental animals developing patchy hair loss spontaneously and presenting similar histologic features as humans. By crossing with PVG/Ola rats we have obtained 130 affected F2 animals and another 60 animals displaying the specific histopathology but no hair loss. Genome-wide and fine genetic mapping analysis was performed on the resulted population, using microsatellites as established or newly developed genetic markers. Employing different tests, suggestive non-parametric Z scores of 2.4 ($p<0.008$) and 2.5 ($p<0.006$) were obtained on chromosomes 5 and 8, respectively. Another chromosomal region of interest was selected after saturation mapping, leading to a detailed search through candidate genes, first constructing a transcript map in the area. In parallel, samples from 120 human families with at least two siblings with AA have been collected until now to perform genome-wide SNP-based linkage analysis. Candidate genes are being studied for association on chromosomes 2, 21, as well as from the HLA region.
Identification and characterization of new genes involved in AA etiology will lead to a better understanding of the developing mechanism and pathways and, therefore, novel approaches to a cure of the disease.

P304

A likely association between genetic variants at the GRIN1 gene and schizophrenic patients with a lifetime history of major depressive episodes in a German sample

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Disturbed glutamatergic neurotransmission has been implicated in the pathogenesis of schizophrenia (SCZ). The NR1 subunit of N-methy-D-aspartate receptors (NMDARs) is encoded by the gene GRIN1 on chromosome 9q34.3, a linkage region for SCZ. Controversy surrounds a potential association between SCZ and GRIN1. We present a case-control study on GRIN1 in a German sample. In 354 SCZ patients (44% female, 56% male) and 323 population-based controls (58% female, 42% male), we genotyped a microsatellite (position 137303343, build Nov 2002) and the SNPs rs4880213, rs11146020, rs6293 and rs10747050 (called Marker 1 to 5). Previous genetic studies in SCZ suggested that SCZ cases with a history of major depressive episodes (MDE) outside of psychotic episodes delineate a genetically distinct subgroup. Therefore, we also analysed the subgroup of 87 patients with MDE. There was no differential distribution of alleles or haplotypes between the cases and controls. The comparison between the subgroup of SCZ patients with MDE and controls showed several significant associations, e.g. a global p of 0.0113 for the four-marker-haplotype 1-2-3-4. When comparing the MDE subgroup with the remaining SCZ patients, this association still held. However, after Bonferroni-correction for multiple testing owed to the sliding-window approach, our results were shy of significance.

Although our exploratory study revealed no associations holding up to correction, our results suggest that GRIN1 be considered a candidate for future investigations. These should consider stratifying SZC cases on the presence of MDE.

P305

NOTCH2 as a modifier for JAG1 in causing Alagille syndrome

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Alagille syndrome (ALGS) is clinically heterogeneous with severe and mildly affected cases even within a family. Mutations within JAG1 are detected in only ~80% of patients. The clinical heterogeneity and the cases without JAG1 mutations remained unexplained so far. McCright et al. (2002) studying transgenic mice for JAG1 and NOTCH2 suggested that NOTCH2 might be involved in addition to its ligand JAG1 in causing and/or modifying the phenotypic expression of ALGS. To investigate whether NOTCH2 is involved either in causing the ALGS alone or even in cooperation with JAG1 we studied a sample of 103 ALGS patients by screening for JAG1 and NOTCH2 sequence variants. We identified disease causing JAG1 mutations in 64 patients (62%). Two further patients were heterozygous for c.2612C>G, p.Pro871Arg, considered as JAG1 polymorphism P2612 due to its presence in patients, clinically normal family members and in controls. Screening for NOTCH2 revealed several sequence variants present in only 1 or only few patients. But one specific NOTCH2 variant, IVS17-44C>T, 44 base pairs upstream of the acceptor splice site of exon 18, was recorded at an unexpected high frequency and only in those patients being heterozygous for a JAG1 mutation. This heterozygous NOTCH2 CT-genotype was detected in 25 (39,1%) of 64 patients with a JAG1 mutation but not in any of the 153 healthy controls (306 chromosomes) which is highly significant. It was also not present in any of the 37 patients without JAG1-mutation and without P2612. Even more surprisingly was the observation that the 2 ALGS patients being heterozygous for P2612 within exon 22 of JAG1 were, indeed, homozygous TT for NOTCH2 IVS17-44C>T. Our study provides the first evidence that sequence variants of 2 significant genes within the NOTCH signalling pathway, i.e. JAG1 and NOTCH2, appear to interact in causing ALGS. As JAG1 mutations are the predominant cause of ALGS it is reasonable to assume that NOTCH2 functions as a modifier of JAG1.

P306

Genetics of human worm infection: Variant SNPs of the IL-10 promoter responsible for low IL-10 secretion are associated with levels of circulating Brugia timori microfilariae but not with filarial lymphedema

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Lymphatic filariasis infections can present as clinically asymptomatic or have chronic pathology from mild to severe lymphedema. Studies have shown that pathology and whether a person has microfilaremia cluster in families. The immune response of patients with asymptomatic infections or chronic disease differs significantly. High levels of pro-inflammatory cytokines and no worms/microfilariae (MF, first stage larvae) characterize chronic pathology, while asymptomatic patients are characterized by immunosuppression, but presence of many worms/MF. IL-10, secreted by T-regulatory and other immune cells, plays a role in the immunosuppression seen in human and animal filarial infections. The IL-10 promoter single nucleotide polymorphisms (SNPs) at positions -1117, -854 and -627 are associated with different levels of IL-10 secretion. In this case-control study, an isolated population infected with Brugia timori from Alor Island, Indonesia, was genotyped to elucidate if there was an association of the IL-10 SNPs with pathology or microfilaremia. While no association with pathology was seen, the SNPs at -854 and -627 were associated with the number of MF in patient blood. These results suggest that genetic factors which determine IL-10 secretion by immune cells are involved in the wide range of MF levels seen in human populations.

P307

Sequence-based quantitative methylation analysis (SeQMA) of five imprinted loci

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Parent-of-origin-specific expression of imprinted genes is controlled by allele-specific DNA methylation. Proper control of DNA methylation is needed for normal development. Bisulfite DNA sequencing allows the determination of the methylation pattern of differentially methylated regions, but it does not allow the quantification of methylation. In fact, it is quite difficult to determine exactly the degree of methylation. Sequence-based Quantitative Methylation Analysis (SeQMA) is one solution to this problem. First, genomic DNA is treated with sodium bisulfite to convert unmethylated cytosine residues into uracil. Methylated cytosine residues remain unchanged. PCR products are subjected to sequencing with fluorescently labelled dideoxynucleotides and analyzed on an ABI Genetic analyzer with the help of the GeneScan software. By comparing thymine peak areas at CpG-sites with genomic thymine peak areas at control-sites (non-CpG-sites), the degree of methylation can be calculated. Several cytosine residues can be analyzed independently in a single assay. So far we have developed SeQMA assays for IGF2, H19, LIT1, GTL2 and PEG1. The analyzed regions span 6, 14, 6, 11 and 8 CpGs, respectively. Hyper- and hypomethylation of IGF2 and H19 in two patients with Beckwith-Wiedemann and two patients with Silver-Russel syndrome could clearly be detected. We conclude that SeQMA is suitable for the analysis of methylation patterns in a sizable number of genes and samples.

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P308

Loss of function variants of the filaggrin gene are not major susceptibility factors for psoriasis vulgaris or psoriatic arthritis in German patients

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Psoriasis vulgaris and atopic dermatitis share a number of features such as chronic cutaneous inflammation and disturbed epidermal barrier function. Genome wide scans have revealed a conspicuous overlap of susceptibility loci for both diseases involving chromosomal regions 1q21, 3q21, 17q25 and 20p12. Recently, two loss of function variants in the gene encoding filaggrin at 1q21 were shown to be strongly associated with atopic dermatitis. In view of a possible genetic overlap of the two skin diseases we investigated 375 patients suffering from psoriasis vulgaris, 375 patients with psoriatic arthritis, and 376 control probands. Moreover we directly studied expression of filaggrin in ten patients suffering from psoriasis vulgaris. Our immunohistochemical analysis revealed a checkered pattern with alternating positive broadened or almost absent filaggrin expression. However, no association was found for the two variants of FLG.
We conclude that despite a markedly altered filaggrin expression in psoriatic skin, loss of function variants of the FLG gene are neither associated with psoriasis vulgaris nor with psoriatic arthritis. The abnormal staining might reflect the altered epidermal differentiation. Our findings imply that the genetic background underlying the epidermal barrier defect in psoriasis is distinct from that found in atopic dermatitis.

P309

The cortisol and ACTH responses in the combined Dex/CRH test of acutely depressed patients vary significantly between 5HT2A receptor genotypes

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An excessive cortisol and ACTH response to the combined dexamethasone/corticotropin releasing hormone test (Dex/CRH test) is characteristic for acutely depressed patients. This hyperactive state is due to an impaired regulation of the hypothalamic-pituitary-adrenocortical (HPA) system. Serotonin is a known stimulator of the HPA axis and the 5HT2A receptor is the main receptor mediating the serotonergic stimulation of the HPA axis.
Patients were recruited from the Munich Antidepressant Response Signature (MARS) project. Psychopathology was assessed using the 21 items Hamilton Depression Scale. We examined 154 depressed patients with a Dex/CRH test within 7 days after admission and 92 remitted patients who underwent a second Dex/CRH test in the week prior to discharge. Genotyping was performed on a MALDI-TOF massspectrometer employing the Spectrodesigner software.
Two completely linked SNPs at the 5'end of the 5HTR2a gene, the –1438G/A SNP nearby the promoter region and the 102T/C synonymous SNP in exon 1 were examined. Genotype frequencies were in Hardy-Weinberg equilibrium. We performed ANOVA statistics on cortisol and ACTH responses with correction for age and gender. While depressed patients showed an statistically significant 5HTR2A-genotype dependent cortisol and ACTH response (cmax: p= 0.008; cauc: p= 0.004; amax: p= 0.019 and aauc: p= 0.013), this association was not observed in remitted patients. Heterozygous depressed patients showed the most pronounced hormone responses in the Dex/CRH test. In summary, our results point to an involvement of 5HTR2A genotypes in the degree of dysregulation of the HPA system in acute depression. In remitted patients, when the HPA system regulation has normalized, 5HTR2A effects were no longer observed. Our results suggest that 5HTR2A genotypes may contribute to HPA system dysregulation.

P310

Genetic association studies of the chromosome 15 GABA-A receptor cluster in migraine with aura

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Recently, a novel susceptibility locus for migraine with aura (MA) on chromosome 15q containing three GABA-A receptor subunits has been identified by linkage analysis in several large pedigrees. To further study the role of this locus in MA etiology we genotyped 56 SNPs capturing the known common haplotype variations of these three candidate genes in a sample comprising 270 MA patients and 273 matched controls. In a single marker analysis, four SNPs displayed nominally significant ($p<0.05$) association with MA. However, after permutation-based correction for the number of tests performed, the P-values of these SNPs were non-significant. Furthermore, a replication study of two of these SNPs in a second independent sample of 379 MA patients and 379 controls did not result in a significant finding. We also performed a haplotype-based analysis of case-control genotypes. Again we could not demonstrate a significant association with the phenotype after correction for multiple testing. In summary, we found no convincing evidence for an involvement of common sequence variants of the GABA-A receptor cluster on 15q11-q12 in the pathophysiology of MA.

P311

Refined localization and mutation screening in punctate palmoplantar keratoderma

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Palmoplantar keratodemas (PPK) are a family of diseases characterised by hyperkeratosis of the palms and soles. PPK are clinically and genetically heterogeneous. Autosomal dominant punctate PPK, also referred to as PPK type Buschke-Fischer-Brauer, is a rare hereditary skin disease clinically characterized by hyperkeratotic papules irregularly distributed on palms and soles. Lesions usually start to develop in late childhood to adolescence but may also start to appear up to the fifth decade in life. Walking may be painful because of larger hyperkeratoses over pressure points. Two loci for punctate PPK were described recently, located on chromosomes 8q24 and 15q22-q24. We have recruited two large families with punctate PPK and several core families. Now we have analysed the two large families, comprising 25 affected and 29 unaffected individuals, for linkage using microsatellite markers. Genetic linkage analysis clearly showed that both families mapped to the candidate region on chromosome 15 with a combined maximum lod score 5.2 at D15S983. Calculations were done with affecteds only because penetrance is reduced and the age at first manifestion may be rather high. Haplotype analysis then localized the punctate PPK locus within the 11.6-cM region defined by D15S1020 and D15S114. We have combined our results with data from the earlier report describing linkage to chromosome 15. The candidate region is then 2.7 Mb in length and harbours 34 positional candidate genes but no obvious functional candidate. In this work with two large families of punctate PPK analyzed for linkage, the locus on chromosome 15 was clearly confirmed. We are now analysing positional candidates in single patients and our families with mutation screening. The identification of a gene underlying punctate PPK will shed light on the etiopathogenesis of this elusive form of PPK and contribute to our understanding of pathomechanisms leading to the phenotype of palmoplantar hyperkeratosis.

P312

Homozygosity mapping and candidate gene screening in a consanguineous family with Seckel Syndrome

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Seckel syndrome is a rare, heterogeneous autosomal recessive disorder characterized by growth retardation, microcephaly with mental retardation, and a characteristic ´bird-headed´ facial appearance. So far, three loci at chromosome 3 (q22.1-q24), 14 (14q21-q22) and 18 (p11.31-q11.2) have been identified and one disease gene, ATR (Ataxia-telangiectasia and Rad3-related) isolated. This study is based on a consanguineous family in which four of five sibs were affected with SS, and in another part of this family one fetus. We first excluded the three known loci by microsatellites, confirming that the disease must be due to a new, fourth locus. We then used the 10K Affymetrix SNP chip for a whole genome scan and found significant linkage on chromosome 6 (Lod score 3.2). Based on microsatellites the candidate region could be condensed to 4 Mb. This interval contains 43 genes. Mutation screening of candidate genes is in progress.

P313

Concordance for the Bladder Exstrophy-Epispadias Complex (BEEC) in twins: Suggestive of genetic susceptibility

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The role of genetics in the etiology of the bladder exstrophy-epispadias complex (BEEC) is unknown. Several epidemiological studies and various reports of cytogenetic aberrations point towards a multifactorial mode of inheritance underlying the BEEC. Although twin studies provide an efficient method to differentiate the relative importance of genetic and environmental factors, to our knowledge, this approach has not been applied in the genetic dissection of the BEEC. An extensive review of the literature identified 73 reported BEEC twin pairs and we added a further 8 previously unreported. 56 out of the 81 BEEC twin pairs were informative for comparative genetic analysis. Based on these data we calculated pairwise/probandwise concordance rates of 45% (95% CI: 29% - 62%) / 62% (95% CI: 48% - 75%) for monozygotic (MZ) twins which drop to 6% (95% CI: 0.1% - 27%)/ 11% (95% CI: 1.3% - 33%) in dizygotic (DZ) twins. The concordance rates are significantly different between MZ and DZ twins (p = 0.009 and 0.0003 for the pairwise and the probandwise, respectively). The heritability (h^2) for the BEEC for MZ and DZ twins were estimated to be about 92% and 55%, respectively. Corresponding to the implications of the multifactorial threshold (MFT) model, we determined the MZ:DZ familial risk ratio (RMD) for all informative BEEC twin pairs with an RMD of 7.5. All these values provide strong support that, aside from environmental factors, genetic susceptibility contributes to the formation of BEEC.

P314

Association of the R620W polymorphism in PTPN22 with alopecia areata

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Alopecia areata (AA) is a common form of hair loss affecting approximately 1-2% of the general population. The progression of AA can be extremely variable as well for females as for males. A classification in three subtypes is established referring to the amount of hair loss. The etiopathogenesis of AA is not completely understood. However, AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle, and may be associated with other autoimmune diseases. To date, it has been postulated that various genes related to immune response are associated with AA, but only the involvement of the major histocompatibility complex (HLA) has been confirmed through independent replication.
Genetic association for a number of autoimmune diseases has been repeatedly reported for the R620W (c.1858C>T rs2476601) variant of the protein tyrosine phosphatase non-receptor type 22 (PTPN22, Lee et al. 2006). A recent study including 196 English patients with AA has suggested that this variant of the PTPN22 gene also contributes to susceptibility to AA (Kemp et al. 2006).
We attempted to replicate this finding by genotyping a case-control sample of Belgian-German origin that includes 436 patients with AA and 461 healthy controls. Significant results were obtained for the overall collective (P = 0.006). By stratification the sample we detected highest significant P-values for the severe group of AA (P = 0.005), the patients with positive family history (P = 0.006) and the patients with age at onset < 20 years (P = 0.007). Finally, we observed that the effect was mainly attributable to female patients. In conclusion, our results herewith confirm a significant association of the PTPN22 risk allele in our AA patient sample and the PTPN22 gene.

P315

The common non-synonymous variant G38S of the KCNE1- (minK)-gene is not associated to QT interval in Central European Caucasians: Results from the KORA study

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Aims: The QT interval in the general population is a complex trait with 30 to 50% heritability. QT prolongation is associated with an increased risk of sudden death. A recent family based study found association between QT interval and the common non-synonymous Glycin 38 Serine variant (G38S, rs1805127) of the KCNE1 gene coding for the minK-potassium channel subunit. We intended to replicate this finding in a large population sample of central European Caucasian ancestry as part of our ongoing search for genetic variants predisposing to arrhythmias.
Methods and results: We studied 3,966 unrelated individuals from the KORA S4 population based study without atrial fibrillation, pacemaker implant or pregnancy. Individuals were genotyped by MALDI-TOF mass spectrometry. We did not detect any significant association between the genotypes of the G38S variant and the QT interval in the entire population or in any gender.
Conclusion: Unlike the common Lysine 897 Threonine variant of KCNH2 (K897T, rs1805123) the G38S variant of KCNE1 does not appear to have a strong modifying effect on QT interval. However, we cannot rule out an effect of G38S on QT in other ethnic groups, under exercise or medications or on the risk for arrhythmias and sudden death.

P316

Evaluation of allele-specific methylation at the SNRPN u1B region and its duplicon u1D on chromosome 15q11-q13

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The chromosomal region 15q11-q13 is subject to genomic imprinting. The paternally expressed genes MKRN3, NDN, SNRPN and two alternative start sites of SNRPN (u1A and u1B) are differentially methylated. U1A and u1B share a high degree of sequence similarity and are methylated on the maternal chromosome and unmethylated on the paternal chromosome. Both loci contain at least one methylation sensitive HhaI site, which has been previously used for methylation analysis by Southern blot technique. In routine diagnosis for PWS and AS with the Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA), which detects CpG methylation throughout 15q11-13, we found hypomethylation in six of 164 individuals at the u1B locus. Genomic sequencing displayed no sequence changes at the three HhaI restriction sites used in the MS-MLPA. To analyze the methylation status of CpG dinucleotides of u1B in detail, we performed bisulfite treatment of DNA, cloning and sequencing and found predominant completely methylated or completely unmethylated clones. To quantify the methylation status with an independent quantitative method, we used Sequence-based Quantitative Methylation Analysis (SeQMA). Our results obtained by SeQMA showed no significant changes in the methylation status in the six individuals with hypomethylation in the MS-MLPA compared to normal controls. The hypomethylation obtained with MS-MLPA could therefore be due to a technical problem of the MS-MLPA. Another sequence closely related to u1A and u1B, termed u1D, has previously been shown to be not expressed and therefore does not represent an alternative start site for SNRPN upstream transcripts. U1D contains fewer CpGs than u1B and u1A. We analyzed four CpGs in PWS and AS patients with a deletion of 15q11-q13 by sequencing and found that methylation at these CpGs is not allele specific.

P317

Cystic fibrosis disease severity is determined by a paternally imprinted gene on 7q33-q34

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Analysing 7q31-7qtel for cystic fibrosis (CF) modulators in F508del-CFTR homozygous CF twins and siblings, we have previously described an excess of sharing of paternal chromosomes among concordant sibling pairs in comparison to discordant sib pairs who are composed of one mildly and one severely affected sibling. Here, we report on the fine-mapping of the paternally imprinted gene by genotyping 10 microsatellite markers on 29 concordant and 19 discordant CF sibs and their families. Apart from the established microsatellites D7S1521 and D7S550, 17 sequences containing repetitive motivs were selected from the 25 Mbp candidate gene region on NT_079596 and tested for informativity on a set of non-CF controls. Three dinucleotide-repeats (PIC values of 0.76, 0.80 and 0.85 estimated on 96 F508del-CFTR chromosomes), one trinucleotide-repeat (PIC = 0.53 estimated on 96 F508del-CFTR chromosomes), three tetranucleotide-repeats (PIC 0.42, 0.53 and 0.73 estimated on 96 F508del-CFTR chromosomes) and one pentanucleotide-repeat (PIC = 0.81 estimated on 96 F508del-CFTR chromosomes) were used for comparative recombination breakpoint mapping of the paternal chromosomes in 48 CF sib pair families. Between CFTR and 7qtel, the first recombination event on paternal chromosomes in concordant pairs was observed 10 Mb further towards 7qtel in comparison to the discordant pairs. The contrast between the observed recombination rates on paternal chromosomes comparing concordant and discordant pairs was strongest in a 6 Mb segment at 7q33-7q34 delineating the localisation of the paternally imprinted locus that determines CF disease severity.

P318

A familiar case of CHARGE syndrome

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CHARGE syndrome is an autosomal dominant multiple malformation syndrome. In the majority of cases it is due to mutations in the CHD7 gene. Characteristic features are coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, ear anomalies and deafness often in combination with hypoplasia of the semicircular canal. Most cases are sporadic, only few familiar cases are reported. Here we describe a familiar case of CHARGE syndrome. In both affected sibs we detected the same new mutation c.7302dupA in exon 34. This mutation leads to a frameshift and a premature stop codon. Sequence analysis of the CHD7 gene in both parents revealed no mutation whether in the father nor in the mother. We performed on childrens DNA an analysis of the identified mutation in linkeage to a detected polymorphism in intron 34. This diagnosis showed a paternal derivation of the mutated allele. In this case it is likely that a germline mosaicism in the father is the cause of the familiar CHARGE syndrome. In consequence of this case a prenatal diagnosis should be recommended to all parents of an affected child with a CHD7 mutation to exclude the possibility of germline mosaicsm.

P319

Mapping of a genetic syndrome with ichthyosis

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Autosomal recessive congenital ichthyosis encompasses a large, heterogeneous group of disorders of cornification. Isolated forms and ichthyosis associated with other signs of disease can be differentiated. The association of congenital diffuse ichthyosis with follicular atrophoderma and hypotrichosis is a very rare disorder that may represent a unique autosomal recessive syndrome. We have now recruited two families with a similar phenotype from the United Arab Emirates and from Turkey, respectively, to study the genetic basis of this phenotype. Five sibs of the Emirati family, three girls and two boys, showed normal stature, diffuse congenital ichthyosis, patchy follicular atrophoderma, generalized and diffuse non-scarring hypotrichosis, and marked hypohidrosis. The parents were first cousins and free of any skin and hair lesions as were three other unaffected sibs. The affected girl of the Turkish family showed also dispersed congenital ichthyosis, follicular atrophoderma, hypotrichosis, and additionally woolly hair, the parents and her sister were free of skin disease. In order to identify the gene for this disorder, we conducted a genome-wide scan in the larger family. 381 microsatellite markers were analyzed, and parametric LOD scores were calculated. However, two different chromosomal regions were identified with significant values of 3.4 and 3.5, respectively. Considering the marked genetic heterogeneity of congenital ichthyosis, it is not yet clear whether both families present the same genetic entity. Therefore, we are at present analyzing the second, smaller family for homozygosity. Results will point out whether the phenotypes map to the same locus in both families. Combined findings and analysis of a denser set of genetic markers will be instrumental for refined mapping. Identification of underlying mutations will give further insight into the range of proteins involved in the manifestation of ichthyosis and the process of epidermal differentiation.

P320

Identification and characterization of variants in FGF8 and the 5' region of TBX1 as possible modifiers of the cardiac phenotype in monosomy 22q11.2

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Deletion 22q11.2 syndrome is the most frequent known microdeletion syndrome. It is associated with a highly variable phenotype including DiGeorge- and Shprintzen (VCFS) syndromes and is causative for 5% of congenital heart defects. In mice tbx1 and fgf8, both expressed in the embryonic branchial arches and involved in the same pathway, were identified as possible candidate genes influencing the cardiac phenotype. We had previously shown that common single nucleotide polymorphisms (SNPs) on the remaining allele of TBX1 are not associated with the heart phenotype in patients with 22q11.2 deletion (Rauch et al. 2004, J Med Genet 41:e40). We now investigated the possible modifying role of SNPs in the TBX1 promoter region as well as in the coding and promoter region of FGF8. We therefore resequenced 191 patients with monosomy 22q11 (122 with cardiac defect, 69 without) to detect variants in the 5´ region of TBX1 and the coding region and RARα binding site of FGF8. Association studies for 58 identified variants showed significance for two variants in the 5`region of TBX1. In a luciferase assay different conserved regions of the 5`promoter area of TBX1 were investigated for their activating effects and several variants were investigated for a possible impact on that function. For two of the conserved regions tested in our assay activation was found, confirming their regulatory function. For the yet tested 6 variants no significant alteration of activation was observed. We therefore conclude that neither FGF8 nor TBX1 promoter variants play a significant role as modifiers of the heart phenotype in monosomy 22q11.2.

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Loss-of-function mutations in the filaggrin gene and alopecia areata

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Alopecia areata (AA) is a common dermatological disease which affects nearly 2% of the general population. Association of AA with atopic disease has been repeatedly reported. Loss-of-function mutations in the filaggrin gene (FLG) may be considered promising candidates in the development of AA since they have been observed to be a strong risk factor in atopic dermatitis. The FLG mutations R501X and 2282del4 were genotyped in a large sample of AA patients (n=449) and controls (n=473). Our results suggest that although no significant association was observed in the patient sample overall, the presence of FLG mutations has a strong impact on comorbidity with atopic disorders (atopic dermatitis, asthma, allergic rhinitis). This effect is dependent on the strength of the loading for atopic disease, the strongest effect being observed in patients who are comorbid for all three atopic diseases. Finally, we observed that the effect was mainly attributable to female patients, suggesting a previously unrecognized sex influence on the risk conferred by FLG mutations. In conclusion, our data provide the first molecular genetic support for the hypothesis that an ´atopic type´ of AA constitutes a distinct subtype of AA.

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Association between G72 and Neuroticism crosses diagnostic boundaries

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Introduction: The G72 gene has been previously implicated in schizophrenia, bipolar and panic disorder. The associated haplotype (M23-M24) occurs at a high frequency in the general population. Studying the impact of this haplotype with respect to disease-associated personality traits could lead to a better understanding of the basic mechanisms underlying the observed associations. We hypothesized that specifically Neuroticism might be an excellent candidate for conferring such an effect. Individuals who score high on Neuroticism readily destabilize emotionally and are at increased risk for several psychiatric diseases, e. g. panic disorder, generalised anxiety disorder, phobias, and alcohol dependence.
Methods: We assessed Neuroticism scores in a population-based sample of 166 German individuals using the Neo-Five-Factor Inventory and divided the sample in probands with high and low scores applying a median-split. Individuals were genotyped for the G72 markers M23 and M24, haplotype analysis was performed with the program COCAPHASE 2.40. Ten thousand permutations were performed.
Results: Single-marker analyses revealed significant associations between high Neuroticism scores and the C-allele of marker M23 (OR=1.63, p=0.028) and the T-allele of marker M24 (OR=1.62; p=0.029). The haplotype C-T was more frequent in individuals scoring high on Neuroticism (57% vs. 45%; p=0.028), the haplotype T-A was significantly less frequent (42 vs. 53%; p=0.037). Results remained significant after permutation.
Discussion: Association of a single specific haplotype across different psychiatric diagnostic categories raises the possibility that a phenotype dimension exists which is common to all diseases and that drives the observed associations. Our association finding suggests neuroticism as an excellent candidate for such a mechanism.

P324

Three circadian clock genes contribute to the seasonal pattern in mood disorder

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Seasonal affective disorder (SAD) is characterized by both, genetic and environmental, components. We hypothesized that three circadian clock genes, which form a functional unit, period homolog 2 (Per2), aryl hydrocarbon receptor nuclear translocator-like (Arntl) and neuronal PAS domain protein 2 (Npas2), could affect the phenotype of SAD. We analyzed each of the three genes in a sample of 189 patients and 189 matched controls.
The a priori hypothetical framework for selection of SNPs was guided by assessing potential functionality in silico. Gene-wise logistic regression analysis revealed that SAD is associated with variations in each of the three genes: in a combined genetic analysis, we found additive effects of the three genes. We hypothesize that the circadian clock genes play a role in the pathogenesis of SAD and present a hypothetical mechanism of action for the observed effect:
SNP10870 of Per2, which is located in an intronic region, showed association with SAD phenotype. Per2 is known to be a transcriptional repressor of the E/E'-box, which is an important node of the entire transcriptional network of endogenous clock genes. The stretch of DNA, in which SNP10870 is located, may constitute an intronic transcriptional enhancer element. The rare risk allele of the SNP may create a binding site for SP1 (GGGCGT), which is not present in most of the normal population, as indicated by the low abundance in the controls. Supportive evidence for the hypothesis of quantitative Per2 action is provided by fibroblast cell lines overexpressing mPer2 mRNA levels, which severely impaires the expression of circadian clock genes (Yamamoto et al. 2005).

P325

Functional promoter polymorphism in the VKORC1 gene is no major genetic determinant for coronary heart disease in northern Germans

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Background: Most recently, the C-allele of polymorphism rs2359612 (VKORC1: c.283+837C>T) in the VKORC1 gene has been reported to represent a major risk factor for coronary heart disease, stroke, and aortic dissection in Chinese patients. VKOR activity itself is the rate limiting step in gamma-carboxylation of vitamin K-dependent coagulation factors (factors II, VII, IX, X, protein C, S, and Z), as well as proteins of calcium metabolism (matrix Gla protein and osteocalcin). Gamma-carboxylation is essential for the biological activity of these proteins that have been previously hypothesised to play a role in the pathogenesis of atherosclerosis.
Aim: Analysis of VKORC1 genotype distribution in patients with CHD and controls from northern Germany, to verify the association of VKORC1 and CHD risk in patients with caucasian background.
Patients: CHD Patients (n = 901) and healthy controls (n = 521) are part of the PopGen biobank. Case and control samples were matched for ethnical and geographic origin, age and gender.
Results: Genotyping German CHD patients and control individuals, no evidence for a statistically significant association was detected between VKORC1 genotype and CHD phenotype. Similarly, case-control analysis with gender-stratified samples showed no association to CHD phenotype, nor did stratification for myocard infarction.
Conclusions: The discrepant association findings in Chinese and Germans may be due to population-specific differences in genetic and perhaps environmental predisposition, modifying the polygenic CHD phenotype by interacting with VKORC1 variants and thus conferring disease susceptibility in some populations, but not in others.

P326

Prenatal and molecular analysis of an unknown autosomal recessive syndrome with microcephaly and mental retardation

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The starting point of this analysis was a pregnant woman (12th week of gestation) who had three children probably affected with a hereditary disease. The parents were first cousins and had 5 children (three affected and two unaffected) as well as one affected cousin in a second family branch. The phenotype of this novel syndrome is characterized by severe mental retardation, muscular hypotonia, postnatal microcephaly, dwarfism and myoclonic seizures. Faced with this situation we decided to map the disease gene and to perform an indirect prenatal DNA diagnostics. Under the assumption of autosomal recessive inheritance we performed a 10K Affymetrix linkage SNP scan (including the parents and their two unaffected and three affected children) and found linkage to a 16 cM region on chromosome 1 with a LOD-Score of 2.7. The fetus was homozygous for a 5 cM interval. Furthermore analysis of the affected cousin, belonging to a second branch of the consanguineous family, indicated the same candidate locus. Based on these findings, the critical region of homozygosity could be narrowed down. Using microsatellite markers we reduced the risk region of the fetus to a 120.717 bp interval, containing 4 exons. We analysed these by sequencing but found no mutation in coding areas. Based on this information and assuming the current data base annotation is correct, the fetus was unlikely to be affected. The mother gave birth to a healthy baby. In order to identify the disease causing gene we are currently working on the analysis of 66 candidate genes in the critical region.

P327

No association between genetic variants at the ASCT1 gene and schizophrenia or bipolar disorder in a German sample

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Altered glutamatergic neurotransmission is considered a potential etiological factor of schizophrenia (SCZ) and affective disorders. The gene ASCT1 (SLC1A4), a member of the glutamate transporter superfamily, is located on 2p13-14, a region showing linkage to both SCZ and bipolar disorder (BD). ASCT1 can thus be considered a candidate gene for both disorders.
In a German sample, we tested for association between ASCT1 and both SCZ and BD (according to DSM-IV). Samples were as follows: for SCZ, 330; for BD, 306 patients; 319 population-based controls (CON). Genotypes for SNPs rs2075209, rs1064512, rs3732062, rs759458 and STR (STR-SLC1A4-11) were in HWE. Power to detect an effect at genotype relative risks of 1.3-1.5 was moderate to good (45-90 %).
Allele and haplotype frequencies did not differ between cases and controls. Recent findings from our group on the associations between BDNF and SCZ and between G72/G30 and BD suggest that SCZ patients with a history of major depressive episodes (MDE) outside psychotic episodes and BD cases with a history of persecutory delusions constitute genetically distinct subgroups of these disorders. Thus, we hypothesized that restricting case definition to those 95 SCZ individuals with MDE and to those 107 BD patients with a history of persecutory delusions might clarify the relationship between SCZ, BP and ASCT1. However, these stratification approaches did not yield any significant association either. Our results do not support an association of the ASCT1 gene with BD or SCZ in the German population.

P328

A novel intron alteration as a cause for familial Nail-Patella syndrome

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Nail-Patella Syndrome (NPS, OMIM: 161200) is an autosomal dominant disorder characterized by skeletal malformations (patellar hypo- or aplasia, elbow deformation, iliac horns) together with nail dysplasia. In addition, sensorineural hearing loss, ocular and renal abnormalities can be observed.
Mutations in the gene encoding transcription factor LMX1B, mapped to the long arm of chromosome 9 (9q34), are identified as the cause of NPS. More than 140 mutations are currently known to be associated with the phenotype. Most mutations are located in exon 2 to 6 affecting the functional domains LIM-A, LIM-B and the homeodomain.
Here we report the clinical findings of a family with several affected individuals. The physical examination of a mother and her 2 months old child revealed the presence of hypoplastic thumb nails and striking lunulae with triangular shape of the other finger nails, respectively. While the mother showed limited mobility of the elbow joints, the joint mobility was only mildly affected in the child.
For these two individuals, molecular genetic analysis of LMX1B- gene were performed. Further affected family members were reported to have similar nail features and restricted elbow mobility in some of them.
These revealed a G to C transition at position +1 of intron 2 (IVS2+1G>C) which was not described as a molecular cause for NPS so far. Characteristic clinical features seen in the patients are discussed in the context of a gentopype-phenotype correlation.

P329

Exclusion of genes from the EYA-SIX-DACH-PAX pathway as candidates for Branchio-Oculo-Facial Syndrome (BOFS)

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Recent reports have shown that mutations in EYA genes (eyes absent) or SIX genes (sine oculis) may contribute to disorders, which are characterized by defects in the development of eyes, ears, the derivatives of branchial sinuses, and the kidneys. The EYA-SIX-DACH-PAX gene pathway summarizes a few genes, which are crucial for appropriate embryonic development. It was the aim of our study to look for putative candidate genes for Branchio-Oculo-Facial syndrome (BOFS; OMIM 113620), a developmental disorder, which had been proven being distinct from the related syndromes Branchio-Otic (BOS1-3; OMIM 602588; 120502; 608389) and Branchio-Oto-Renal (BOR; OMIM 113650). Since mutations in SIX/EYA genes have been published to contribute to the BOS/BOR phenotype, we have analyzed the inheritance of markers of these genes and other members of the EYA-SIX-DACH-PAX interactive network in the largest family with BOFS.
All experiments have been performed by fragment analysis with fluorescence-labeled primers and/or sequencing of putative candidate genes. Here, we show for the first time the exclusion of many genes of the above-mentioned pathway. In a previous report, we already had rejected the members of the EYA gene family as BOFS candidates. We have now excluded all known members of the sine oculis gene family, namely SIX1, SIX2, SIX3, SIX4, SIX5, and SIX6. Further, FGF3, HOX11, IRF6, and FOXC1 were excluded. The members of the POU transcription factors gene family POU4F3 and POU3F4 were rejected by their segregation pattern or the X-chromosomal localization, respectively. DACH2 and PITX2 were excluded as well as the paired box genes PAX2 and PAX8. We draw the preliminary conclusion that the pathogenesis of BOF syndrome might rely on genes different from those genes,

which are members of the EYA-SIX-DACH-PAX network.

P330

Finding genetic modulators of a monogenic disease: The European Cystic Fibrosis Twin and Sibling Study

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Cystic fibrosis (CF) is caused by molecular lesions in the cystic fibrosis transmembrane conductance regulator gene (CFTR). To evaluate the cause of CF disease variability, the European CF Twin and Sibling Study collected data on two clinical parameters most sensitive for course and prognosis of CF for a cohort of more than 300 CF twin and sibling pairs. Application of genetic modeling algorithms enabled the dissection of the relative impact of inherited and environmental factors on CF disease severity. The European CF Twin and Sibling Study has chosen the following strategy to identify genes that modulate course and severity of CF: Firstly, we have included only F508del homozygotes in our study. The influence of the major disease-causing gene is normalised for all individuals of our study population, and consequently the role of factors other than the CFTR mutation genotype on the phenotype within our study population is emphasised. Secondly, the selection of concordant sib pairs provides an advantage compared with a set of unrelated patients as the phenotype of a concordant pair is determined by factors that are shared by both siblings. Individual factors that act on one out of two siblings only, such as random environmental effects, are unlikely to cause concordance within a pair, whereas the influence of shared environmental and genetic factors on the phenotype is accentuated in sib pairs. Finally, for the analysis of a quantitative trait extreme phenotypes are generally considered to be more informative. Dizygous patient pairs with extreme phenotypes were selected for the analysis of CF modulators in a candidate-gene based approach. Our results demonstrate that this design of an association study using affected patient pairs who exhibit extreme clinical phenotypes is effective and workable for the identification of genetic modulators.

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Pharmakogenetische Untersuchung von Dopamin-D2-Rezeptorpolymorphismen bei Neuroleptika-induzierter Gewichtszunahme

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Patienten mit schizophrenen Psychosen werden zum Teil über Jahrzehnte mit neuroleptischer Medikation behandelt. Hierbei kann es durch die Einnahme der Neuroleptika zu erheblichen Gewichtszunahmen kommen. Die Neuroleptika-induzierte Gewichtszunahme variiert zwischen den einzelnen Neuroleptika, jedoch hängt die individuelle Prädisposition stark von genetischen Faktoren ab. Genetische Assoziationsstudien haben bislang interessante und meist konsistente Befunde im 5-HT2C und ADRα2a Rezeptorgen sowie im Leptin-, GNB3- und SNAP-25-Gen erbracht. Überraschenderweise sind bislang eingehende Untersuchungen am Dopaminrezeptor unberücksichtigt geblieben gleichwohl das dopaminerge System durch Neuroleptika sehr stark beeinflußt wird. Außerdem ist das dopaminerge System auch in der Modulation von Belohnungskreisen involviert welche bei Nahrungsaufnahme aktviert werden. In dieser Studie wurden 12 SNPs, die das gesamte DRD2-Gen umspannen, untersucht. Es wurden 139 chronisch schizophrene Patienten eingeschlossen, die bis zu einer Dauer von 14 Wochen mit Clozapin behandelt wurden. Hierbei zeigten Träger des C/C Genotyps (n=52) des funktionell relevanten C957T Polymorphismus im Durchschnitt eine Gewichtzunahme von 5.76kg zu verglichen mit 3.42kg bei Trägern des C/T Genotyps (n= 50) und mit 2.51kg bei T/T Genotyp-Trägern (n=21; F[2,120]=4.29, p = .01).Haplotyp-Analysen erbrachten weitere signifikante Befunde mit den SNPS C939T and Taq1D die jeweils in der Nähe zum C957T-Polymorphismus liegen. Die Befunde legen den Schluß nahe, dass die Neuroleptika-induzierte Gewichtszunahme mit DRD2-Genpolymorphismen assoziiert ist.

P332

A familial periodic fever syndrome caused by a previously not described mutation in TNFRSF1A

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Hereditary periodic fever syndromes are characterized by periodic episodes of fever and signs of inflammation with or without involvement of inner organs leading to symptoms such as pericarditis, pleuritis, abdominal pain, or myalgia. The disease group includes syndromes such as familial mediterranean fever (FMF), tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and hyperimmunoglobulinemia D with periodic fever syndrome (HIDS). Recent advances in molecular genetics have lead to the identification of mutations in several genes that are the underlying causes of these syndromes. Here we report the results of molecular genetic analyses of individuals from a German family initially diagnosed as FMF for some clinical aspects. For the index case two nucleotide changes, c.263G>C and c.264C>A, were detected in the TNFRSF1A-gene. Analyses of the patient´s parents revealed that mutations were inherited in a monoallelic mode. Therefore it could be predicted that mutations result in the amino acid substitution cysteine to serine at position 88 of the protein (p.88Cys>Ser). The substitution that affects the cysteine-rich domain 2 (CRD2) of TNFRSF1A most probably interferes with the tertiary structure of the receptor by eliminating a disulfide bridge. Additional analysis of the mevalonate kinase-gene (MVK) revealed a G>A transition (c.155G>A) resulting in the amino acid substitution serine to asparagine (p.52Ser>Asn). This alteration is described to represent a common polymorphism. Clinical features presented by the patients of the family investigated are discussed within the context of molecular results.

P333

Identification of mutations in the human hairless gene in two new families with congenital atrichia

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Congenital atrichia (AUC) is a form of isolated alopecia with an autosomal recessive mode of inheritance. Patients are born with normal hair but this is shed almost completely during the first weeks or months of life and never regrows. In many families the development of papular lesions is noted as an additional phenotypic feature, which defines a related phenotype designated as atrichia with papular lesions (APL). Using positional cloning strategies and the molecular findings in hairless recessive (hr/hr) mice, an animal model for AUC, mutations in the human hairless gene (HR) have been identified as a cause of AUC and APL. To date, more than 20 different mutations of the HR gene have been reported in AUC and APL including different mutation types scattered over the entire HR gene length. Here, we describe two families of Saudi-Arabian and Jewish Iranian origin comprising a number of individuals with clinical features suggestive of APL/AUC. We therefore hypothesized that affected members may carry mutations in the HR gene. After sequencing the complete coding region of the HR gene in the Saudi-Arabian family, we identified a homozygous insertion of a G (c.2661dupG; p.Thr888DfsX38) in exon 12, resulting in a premature stop codon. In a Jewish Iranian patient, we identified a homozygous splice site mutation c.1557-1G>T in intron 4. The latter mutation has been previously reported in a compound heterozygous state. We describe the second exonic insertion mutation in the human HR gene and the first mutation in exon 12. Our study emphasizes the importance of sequencing the complete coding sequence and exon/intron junctions in the molecular diagnostics of AUC and APL.

P334

Analysis of sequence variants in DLG5 gene and haplotypes of CARD15/NOD2 gene involved in susceptibility to Crohn's disease (CD) in Polish patients

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Inflammatory bowel diseases (IBD) belong to chronic inflammatory diseases of the gastrointestinal tract with still unknown etiology. It is postulated that in etiology of the disease the genetic, environmental and immunological factors are involved. The morbidity in the European Union oscillates around 5 new cases per 100,000 people per 1 year and it is still growing. Some genes predisposing to CD were localized, including the most significant locus – pericentromeric region of chromosome 16p12-q13 – IBD1 and CARD15/NOD2 gene in this region. Polymorphisms in this gene are associated with susceptibility to CD. Recent studies implicate also regions 12p13, 6q13, 14q11, 5q31 and the region 10q23 with DLG5 gene. DLG5, encodes a scaffolding protein involved in the maintenance of epithelial integrity. Mutation in the DLG5 gene impedes scaffolding of DLG5 gene protein product.
Molecular studies involved polymorphisms in CARD15/NOD2 gene (P268S, R702W, G908R, 1007fsinsC) and in DLG5 gene (R30Q, A1490V). Screening analysis of DLG5 exon 10 and 23 were also performed. Group of 254 persons from Western Poland, including affected with CD with families and 100 individuals from population group were examined. Screening analysis (PCR-SSCP) and direct sequencing were performed followed by pyrosequencing. Statistical analysis performed for CARD15/NOD2 variant P268Ser indicates that allele T (Ser) occurs with higher frequency in affected with CD than in population group. We observed coexistence of polymorphic variants of CARD15/NOD2 and DLG5 genes. In 10 cases 1007fs was observed with homozygous presence of 268Ser. In two patients with homozygous 268Ser, homozygous 1007fs variant was found. Our results confirmed observations that approximately 50% CD patients carry at least one mutation and support hypothesis that a complete damage of the NOD2-signaling pathway and interaction between CARD15/NOD2 and DLG5 genes or other susceptibility genes may be necessary.

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Analysis of candidate genes modulating susceptibility to infection in cystic fibrosis

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While the major disease-causing gene CFTR determines susceptibility of the airways to the opportunistic pathogen P. aeruginosa in cystic fibrosis (CF) patients, progression and severity of the disease cannot be predicted by the CFTR mutation genotype. We hypothesized that genes involved in innate immunity modulate the susceptibility to infection in CF. 8 candidate genes such as toll-like receptors and other adaptors for bacterial ligands were investigated in a set 37 of F508del-CFTR homozygous European CF twins and siblings exhibiting extreme clinical phenotypes. Genes were targeted initially by one informative SNP. Further SNPs covering the candidate gene were typed if evidence for an association could be obtained. The genomic fragment containing the causative variant was identified by direct comparison of two-marker-haplotype-distributions between sib pair sets displaying a phenotypic contrast and validated by a permutation algorithm correcting for the dependence of the genotypes of the individuals within each sib pair. Individuals carrying contrasting haplotypes were subjected to confirmatory sequencing at the outlined genomic fragment. Candidate genes identified as modulators of overall CF disease severity were analysed for their role in CF infectious disease in 36 CF sib pairs for whom P. aeruginosa - related endophenotypes such as onset of initial and chronic colonization were known. In the context of CF, the continuously changing symptomatic treatment and its consequences have dramatically changed the manifestation of disease and improved survival. In order to ask whether this manifests in the CF population at the typed markers, we have retrospectively recruited CF patients born in 1959 – 1967 or 1970 – 1975 who were enrolled for CFTR mutation analysis at the CF clinic in Hannover in 1989-1994. Taken together, our data indicate an involvement of CF modulating genes that determine the interface of host and pathogen in CF.

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Der Methionin Synthase Polymorphismus c.2756A>G (D919G) beeinflusst den diastolischen Blutdruck

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Hintergrund: Für den funktionellen Polymorphismus des Methionin-Synthase-Gens (MTR) c.2756A>G (D919G) wurde in mehreren Studien ein Einfluss auf das Risiko von Gefäßerkrankungen nachgewiesen. Zusätzlich konnte gezeigt werden, dass er die krankheitsfreie Lebenszeit beeinflusst.
Methoden: Bei 187 Familien mit insgesamt 714 Personen wurden der systolische und diastolische Blutdruck gemessen, die Einnahme antihypertensiver Medikamente dokumentiert und der MTR Genotyp bestimmt.
Ergebnisse: Wir konnten eine signifikante Assoziation des MTR Polymorphismus mit den diastolischen Blutdruckwerten nachweisen: 79,2 ± 10,1 mmHg für den Genotyp AA, 77,7 ± 10,3 mmHg für den Genotyp AG und 73,7 ± 9,6 mmHg für den Genotyp GG (B=1,932; p=0,010 in der multilpen logistischen Regressionsanalyse mit Stratifikation für Alter und Geschlecht).
Schlussfolgerung: Der in dieser Untersuchung nachgewiesene Effekt auf den diastolischen Blutdruck könnte eine Ursache des günstigen Einflusses des G-Allels des MTR-Polymorphismus auf Gefäßerkrankungen sowie die erkrankungsfreie Lebenszeit sein. Weiterhin könnte er als Grundlage neuer antihypertensiver Behandlungsmethoden dienen.

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Role of genomic variants in the IGF-I gene and the IGF-IR gene in the aetiology of IUGR (intrauterine growth retardation) with ARED (absent or reversed enddiastolic) flow

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The growth factor IGF-I (insulin-like growth factor I) and its receptor IGF-IR are essential for the development of the fetoplacental system in human pregnancy. Reduced activity of the IGF-I-system leads to an impaired proliferation of cytotrophoblast cells and an inadequate placental angiogenesis. The resulting postplacental hypoxia is characteristic for pregnancies with IUGR (intrauterine growth retardation) and ARED (absent or reversed enddiastolic) flow in the umbilical artery. In contrast to this, the so-called uteroplacental hypoxia is associated with IUGR and PED (preserved enddiastolic) flow in the presence of a bilateral abnormal uterine artery Doppler waveform (uteroplacental hypoxia). To study a possible role of mutations in the IGF-I and the IGF-IR genes in the pathogenesis of placental dysfunction, we analysed the IGF-I and IGF-IR genes in an IUGR/ARED-flow group (20 mothers and 20 fetuses) and an IUGR/PED-flow group (13 mothers and 13 fetuses). DNA was extracted from venous blood samples (mothers) and umbilical cord blood samples (fetuses). The coding sequences of IGF-I (5 exons) and IGF-IR (21 exons), their intron/exon boundaries and the 5´- and 3´-UTRs of the IGF-IR gene were screened by single strand conformation polymorphism analysis (SSCP), restriction assays and direct sequencing. No variants could be identified in the IGF-I gene. In the IGF-IR gene, we identified 5 polymorphisms, 3 of them unknown so far: 2 polymorphisms in the 3´-UTR and 1 in the 5´-UTR of the IGF-IR gene, and 2 variations that had already been established (exon 16: silent mutation; 3´-UTR: deletion). Most of the variations are localized in the UTRs of the IGF-IR gene and were detected in similar frequencies in the patient and control groups. Therefore we conclude that the IGF-IR gene polymorphisms identified in our study do not play a relevant role in the aetiology of IUGR/ARED flow.

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No association of genetic variants of interleukin 6 and the susceptibility to periodontitis

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Periodontitis as a chronic inflammatory disorder is influenced by environmental and genetic factors. Several factors of the immune response and their genetic background have been proposed as potential markers for the susceptibility to this disease.
The aim of the present study was to evaluate the importance of genomic variants of the potent proinflammatory cytokine interleukin 6 (Il6) for the incidence of chronic and aggressive periodontitis. Patients and
Methods: In the present study 107 periodontitis patients (chronic: n=48, mean age: 48.1±10.1y, 33.3% males; aggressive: n=59, mean age: 41.6±9.8y, 35.6% males) and 40 control probands without periodontitis (mean age: 43.9±11.1y, 40 % males) were included. Clinical parameter including smoking status, plaque and bleeding indexes, pocket depth and attachment loss were assessed. Subgingival bacterial colonization was analyzed molecular biologically using the micro-Ident® test (Hain-Diagnostik, Nehren). We investigated genotype, allele and haplotype frequencies of the Il6-SNP –174G>C and –597G>A by use of PCR-SSP (CTS-Kit, Heidelberg).
Results: Hardy-Weinberg criteria were fulfilled for both SNPs. Investigating genotype, haplotype and allele frequencies no significant disease specific differences could be detected in comparison with healthy controls. Furthermore, the genetic background of Il6 was not associated with clinical and microbiological parameters investigated except attachment loss. In the group of patients suffering from aggressive periodontitis heterozygous genotypes were significantly associated with increased attachment loss (–174G>C: p=0.035; –597G>A: p=0.04)
Conclusions: Although, the genetic background of Il6 was associated with attachment loss representing a clinical parameter of periodontitis the genetic variants –174G>C and –597G>A could not be described as independent risk factors for chronic or aggressive periodontitis.

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Association analysis between bipolar disorder and variants at the G72/G30 locus in a Russian sample

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Since the initial report by Chumakov and colleagues association between G72(DAOA)/G30 locus on chromosome 13q and schizophrenia (SZ) have subsequently been replicated in many samples. Moreover, the G72/G30 locus has also consistently been found associated with bipolar affective disorder (BPAD) in independent studies. We recently reported association between identical G72/G30 haplotypes in SZ and BPAD in a German population. The strongest association was observed for M23 (p=0.013, BPAD sample; p=0.033, SZ sample) and M24 (p=0.036, SZ sample) markers. The present study examined involvement of the markers M22, M23 and M24 of the G72/G30 genes in etiology of BPAD in a case-control sample from the Russian population using single-marker and haplotype analysis.
We studied 253 BPAD patients (DSM-IV) and 246 controls of Russian ancestry. Three markers M22, M23 and M24 were selected for the MALDI-TOF Mass Spectrometry-based SNP genotyping. The single marker and haplotype case-control analysis with the program COCAPHASE 2.40 were performed. A stratified analysis limiting caseness to a history of persecutory delusions has also been done.
We did not detect an association between studied G72/G30 markers and BPAD. A stratification of the cases based on a life-time history of psychosis or persecutory delusions did not clarify the relation between G72/G30 locus and BPAD in the Russian population. Larger sample size allowing more powerful genotype-phenotype correlation analysis may be required to study the role of these genes in Russian population.

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Investigation of the functional variant c.-169T>C of the Fc receptor-like 3 gene (FCRL3) in alopecia areata

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Alopecia areata (AA) is a common skin disease presenting with patchy hair loss which affects approximately 1-2 % of the general population. The etiopathogenesis of AA is incompletely understood. However, AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle, and association with other autoimmune diseases including autoimmune thyroid disease (AITD) has been reported repeatedly. A functional variant in the FCRL3 gene has recently been implicated in susceptibility to autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and AITD. Given the autoimmune component of AA and the higher incidence of AITD in AA patients, we aimed to examine the role of the FCRL3 gene c.-169T>C polymorphism in the development of AA. We genotyped this polymorphism in a large Belgian-German case control sample including 266 unrelated patients and 281 healthy unrelated blood donors.
Our results do not support a significant association for the risk genotype in our AA patient sample (P = 0.595). Furthermore, the analysis of subgroups of individuals with either severe AA or an early age of onset (onset age < 20 years) did not reveal an association of this polymorphism (P = 0.731, P = 0.924). We also tested the subgroup of patients who had a positive family history, since a genetic effect might be stronger in familial cases where a genetic contribution to disease is more likely. No positive association emerged in this subgroup either (P = 0.884). Similarly negative results were obtained by testing for differences in global genotype distributions and allele frequencies. The findings from our case-control sample do not support an association between the FCRL3 variant c.-169T>C and AA.

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Angiotensin-converting enzyme (ACE) gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease

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Background: Abdominal aortic aneurysm (AAA) is a life-threatening condition affecting 4-9% of population with a risk increasing with age. Leriche syndrome characterized by aortoiliac obstruction (AIOD) is caused by advanced atherosclerosis and is a quite common vascular disease. Both AAA and Leriche syndrome are considered to have multifactorial etiology. Angiotensin I-converting enzyme (ACE) is one of the key factors affecting blood pressure regulation and electrolyte balance. It was shown that

half of the individual variability of the ACE plasma concentration is determined by an insertion (I)/deletion (D) polymorphism in intron 16 of the ACE gene. The relationship between insertion/deletion polymorphism in the ACE gene and number of cardiovascular diseases was observed. The aim of this study was to determine if there is an association between the ACE genotype and susceptibility to abdominal aneurysm or Leriche syndrome.
Methods: ACE genotypes (I/I; I/D and D/D) were determined in four selected groups: 133 patients with AAA and 152 patients with Leriche syndrome who underwent surgery, 152 individuals from control group and 392 samples of random Polish population group. Genotypes were compared with demographic and clinical data of subjects and analyzed in relation to risk factors.
Results: The genotype distribution and allele frequency of ACE I/D gene polymorphism were not significantly different between patients with AAA or AIOD and control or population group. Significant differences were found only between following groups: 1/ hypertensive patients with AAA and normotensive patients with AAA; 2/ hypertensive patients with AAA and population group.
Conclusion: ACE gene polymorphism is not a susceptibility factor to Leriche's syndrome and does not seem to be an important factor in development of AAA without considering coexistence of hypertension.

Normal Variation, Population Genetics, Genetic Epidemiology, Evolutionary Genetics

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Conservation of mammalian imprinted genes in marsupials and monotremes
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Imprinted genes are expressed from only one allele in a parent-of-origin-specific manner. Comparative analyses of the embryonal growth factor Igf2 and its receptor M6p/Igf2r are consistent with the idea that genomic imprinting evolved in a common ancestor of viviparous mammals after divergence from the the egg-laying monotremes. A characteristic hallmark of imprinted genes is the formation of larger clusters. To analyze the conservation and evolution of mammalian imprinted gene clusters, monotreme (Ornithorhynchus anatinus) and marsupial (Monodelphis domestica) orthologues of 76 known mouse and/or human imprinted genes were identified and in silico mapped by performing Ensembl TBLASTX searches with mouse cDNA sequences against the currently available genome sequences of O. anatinus (PreVersion OANA5) and M. domestica (MonDom4) or by using orthologue prediction in the Ensembl database. Up to now, 46 imprinted gene orthologues were identified and in silico mapped to M. domestica chromosomes. Because of lacking chromosomal assignments of 58 Blast hits only 12 orthologues could be mapped in O. anatinus. Interestingly, we did not detect any marsupial or monotreme orthologue of human and mouse imprinted H19, Gtl2, and Air, suggesting a very recent evolutionary origin of these non-protein-coding RNA genes. The Snrpn gene and its paralogue Snrpb which reside on different chromosomes in the human and mouse genomes are located directly adjacent to each other on Monodelphis chromosome 1. This argues in favour of an ancestral duplication event in a common ancestor of marsupials and eutherians, followed by chromosomal divergence of the two paralogues during eutherian evolution. Collectively, our results contribute to a better understanding of the molecular evolution, mechanisms and function of genomic imprinting and of the phylogenetic distribution of imprinted genes.

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Signature of a severe bottleneck in European NF1 haplotypes
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Despite of intense analyses, the genetic structure of the European population is still under debate. As contribution to the intense controversy, we analyzed the haplotype structure of the NF1 gene. This autosomal locus is ideal for population genetic studies because this highly conserved genomic unit spans 350 kb, is structurally and functionally well-defined and shows an extremely low recombination rate. A combined resequencing and SNP typing project in a German population revealed the presence of only two well separated subgroups of NF1 sequences, with a time to the most recent common ancestor of about 700,000 years and little variability between sequences belonging to the same haplogroup. This pattern of variability is incompatible with the assumption of a neutrally evolving locus in a stable population. Possible explanations for the deep split in the phylogenetic tree are balancing selection, strong genetic drift or a population admixture. To distinguish between these alternatives, we analyzed the haplotype structure of NF1 in a worldwide DNA panel. This SNP typing project revealed an African origin for both European haplogroups as their most basal haplotypes are found exclusively in Africa. Therefore, the two European haplogroups are not the result of a population admixture between one African and one non-African population. The action of balancing selection can be excluded because of the absence of a functional variant selection could act upon. Hence, during the emigration of the anatomically modern human out of Africa, genetic drift caused an extreme shift of the haplotype frequencies, resulting in the observed haplotype pattern in Europe.

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Lack of association of Pro279Leu variant and (CAG)n repeat in MEF2A gene with myocardial infarction
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Introduction: Coronary artery disease (CAD) and myocardial infarction (MI) are caused in part by genetic factors. Recently, a seven amino-acid-deletion in MEF2A gene was linked to MI/CAD in a large pedigree with autosomal dominant pattern of inheritance. This study intends to investigate the relevance of MEF2A gene mutations in both familial and sporadic forms of MI.
Methods: MEF2A gene was sequenced in one affected member from 23 extended MI families (> 5 affected members per family). The Pro279Leu variant in exon 7 was genotyped using the 5´ nuclease TaqMan assay in 1381 unrelated MI patients with positive family history for MI/CAD and in 533 patients with sporadic MI as well as in two control populations (n=1021 and n=1055). The (CAG)n repeat in exon 11 was genotyped in 543 sporadic MI patients and in 1190 controls.
Results: No MI-causing MEF2A mutation was identified in any of the extended MI families. Furthermore, the genotype distributions for Pro279Leu were neither different between familial MI cases (CC: 99.8%, CT: 0.2%, TT: 0%) and controls (CC: 99.7%, CT: 0.3%, TT: 0%; p=0.71) nor between sporadic MI patients (CC: 99.8%, CT: 0.2%, TT: 0%) and controls (CC: 99.7%, CT: 0.3%; TT: 0%; p=0.73). Furthermore, the frequencies of the different (CAG)n repeats were similar in MI patients and controls.
Conclusions: Mutations in MEF2A were not detected in this large series of MI families. Thus, the genetic variation linked to MI in a single family appears to be a private mutation. Furthermore, the two independent samples of more than 1,800 MI patients and two large control populations displayed no evidence for association of the Pro279Leu variant or the (CAG)n repeat in the MEF2A gene with sporadic or familial MI.

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The human androgen receptor locus: evidence for genetic variability distinct from the GGN repeat modulating the risk for androgenetic alopecia and test for positive selection

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Androgenetic alopecia (AGA, male pattern baldness) is the most common form of hair loss. It has been demonstrated that genetic variability in the androgen receptor gene (AR) is the cardinal prerequisite for the development of early-onset AGA. The genetic data suggest that a polyglycine encoding GGN repeat in exon one of AR is a good candidate for conferring the functional effect. In the present study we tested the hypothesis that further variability at the AR locus contributes to the development of AGA. This was accomplished by association analysis after stratification for the GGN repeat. We found evidence for genetic variability modulating the risk to develop AGA which is distinct from the GGN repeat. The comparison of bifurcation and extend of haplotype homozygosity of the AR haplotypes indicates that risk and neutral haplotypes have higher allelic-specific linkage disequilibrium than the non risk haplotypes although they are more frequent in the German population. This could be explained by positive selection of the risk and neutral haplotypes. To further test for positive selection at this locus we compared allele frequencies between different ethnic groups by means of FST. In a large region around AR we found a shift towards higher FST values across the three major HapMap populations compared to the FST distribution over the whole X-chromosome. This supports the hypothesis of positive selection acting at this genomic region. However, based on the FST analysis it is difficult to decide whether AR itself is the target of positive selection. Further studies are needed to clarify this issue.

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Determination of the lineage specificity of the large pericentric inversions distinguishing human and chimpanzee chromosomes using the macaque genome as outgroup

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The human and chimpanzee karyotypes are distinguised by 9 large pericentric inversions. It has been suggested that these inversions contributed to the separation of early humans and chimpanzees by suppression of recombination within inverted regions if the inversions are present in the heterozygous state. For eight of these inversions, the respective breakpoints have been characterized at the DNA sequence level. The availability of the macaque genome provides the means to determine the 'ancestral state' at the breakpoint sites and to determine the lineage specificity at the sequence level. Alignment of the human, chimpanzee and macaque sequences at the breakpoint sites indicated that 5 of these inversions must have occurred in the chimpanzee lineage on the basis that neither the human nor the macaque exhibit any syntenic disruption within the breakpoint regions, in contrast to chimpanzee. The inversions of human chromosomes 1 and 18 are however human-specific and hence 'derived'. In the corresponding breakpoint regions, the chimpanzee and macaque sequences are directly alignable without disruption and hence represent the ancestral state. By contrast, in the human lineage, the inversion breakpoints map to the respective regions. Our recent analyses suggest however that there may have been an assembly error in the breakpoint region of 16q in the macaque genome. The inversion that distinguishes human and chimpanzee chromosomes 16 has hitherto been considered to be chimpanzee-specific (Goidts et al. 2005, Genome Res. 15:1232-1242). Sequence alignments involving the macaque genome nevertheless suggest that macaque and chimpanzee share ancestral sequences in the breakpoint regions. To confirm or refute the correct assembly of the macaque sequence in the breakpoint region of 16q, we are currently performing discriminant PCR experiments. Our findings indicate that inclusion of the macaque genome contributes importantly to the comparative analysis of hominoid genomes.

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The degeneration of a mammalian X chromosome by accumulation of mutations (in non-essential genes)

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The mole vole Ellobius lutescens is an exceptional mammal that is known for its odd number of diploid chromosomes (2n = 17,X) with a single X chromosome in males and females. The absence of a second gonosome implies the lack of a partner for recombination between the homologous X chromosomes (in females) respectively X and Y chromosome (in males). Therefore, we suggest that the single X of E. lutescens will degenerate in consequence of an accumulation of deleterious mutations in non-essential genes. In analogy to the fate of the Y chromosome of male mammals we suggest mechanisms as genetic hitchhiking and Muller´s ratchet that are responsible for the accumulation and fixation of deleterious mutations.
To test this hypothesis, we analysed conserved regions of essential (Mecp2, Zfx, Flna, Xist) and non-essential (Atrx, Opn1mw) X-chromosomal genes. As reference sequences we used regions of the autosomal genes Sfrs3 (essential) and App (non-essential). In our phylogenetic studies, we compared the sequences of E. lutescens with the orthologous regions of other species (mouse, rat, dog, cow, rhesus macaque, chimpanzee, human) by alignment. These sequence data could be obtained from different databases (Ensembl, NCBI, Genome Browser). Altogether we compared about 5 kbp of sequences.
The results of DNA alignments confirmed our hypothesis of a single degenerating X chromosome. The sequences of the essential and non-essential autosomal and essential X-chromosomal genes of E. lutescens did not significantly differ from the sequences of the other tested species. By contrast, we could confirm the suspected accumulation of mutations in non-essential X-chromosomal mole vole genes. So we expect a progressive decay of the single X chromosome and as consequence the extinction of the species E. lutescens.

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Analysis of new cases with Pierson reveals novel LAMB2 mutations and founder alleles in European subpopulations

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Pierson syndrome (OMIM 609049) is characterized by severe congenital nephrotic syndrome (CNS) with diffuse mesangial sclerosis and ocular abnormalities including microcoria, abnormal lens, and retinal changes. As the underlying cause of this disorder we have recently identified loss-of-function mutations in the LAMB2 gene encoding the laminin beta 2 chain. Laminin beta 2 is an essential basement membrane component, which is particularly abundant in the glomerulus, lens capsule, intraocular muscles, and neuromuscular synapse. Due to increased awareness of Pierson syndrome several new cases have been diagnosed in the last two years.
Herein we report on 10 newborns from different ethnic backgrounds, who were prospectively ascertained to be affected by Pierson syndrome. Only two patients originated from consanguineous families. LAMB2 sequencing analysis was performed by bidirectional sequencing of all coding exons and revealed 9 novel and 6 previously described mutations in either homozygous or compound heterozygous state. In cases of recurrent mutations, haplotypes were determined by studying SNPs and microsatellite markers at the LAMB2 locus in the index patients and their parents. Thereby, we found evidence that the repeatedly observed mutations R246W and c.4504delA (1502fsX1519) each originated on a common ancestral haplotype. The increasing number of patients diagnosed with Pierson syndrome – also from non-consanguineous families – and the finding of European founder alleles suggest that this disorder is more common than previously assumed and has been overlooked in the past.

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The influence of the genetic background on the detoxification of heavy metals

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Detoxification processes are essential for all living organisms. Heavy metals are ubiquitous environmental pollutants with high toxic potential. Humans accumulate heavy metals primarily as a function of the environmental contamination degree. However, not all humans reflect their individual exposure as expected, suggesting genetically influenced regulatory mechanisms. In order to identify genetic factors underlying the inter-individual variance in detoxification capacity for the heavy metals mercury, lead, cadmium, nickel, and platinum, a group of 381 individuals was investigated for the relationship between polymorphisms in genes encoding metal metabolizing proteins (metallothioneines (MTs), glutathion-S-transferases (GSTs)), and metals content in hair, urine and blood. The correlation between metal concentrations and expression of genes of the MT1- and MT3-family was evaluated in a subgroup of thirty individuals. In brief, our results revealed, that
1) mercury concentrations correlate with polymorphisms in genes MT1a and MT1k,
2) mercury concentrations are significantly increased in persons with the GSTT1 -/- and GSTM1 -/- genotypes, as compared to persons with GSTT1/GSTM1 +/+
3) distinct genotypes of MT2 and MT4 genes were found to be associated with increased concentrations of several heavy metals
4) mercury apparently suppresses MT1X expression in dependence of the GSTT1/GSTM1-genotype.

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RYR1 Mutations are regionally diverse – RYR1 Mutations are globally limited

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Malignant Hyperthermia (MH) is caused by an dominantly inherited susceptibility to drugs used for general anaesthesia. Muscular rigidity causes rhabdomyolysis, hyperthermia, hyperkalaemia, and renal failure and, eventually death. Individuals with malignant hyperthermia susceptibility (MHS) can be identified by either a positive muscle contraction test or analysis of a RYR1 mutation previously detected in the family's index case.
The size of the gene (102 exons) hampers complete sequencing for routine diagnostic, and the diversity of mutations makes genotype phenotype correlation difficult. The European Malignant Hyperthermia Group (EMHG) compiled a list of 28 mutations that are considered pathogenous and common. They are scattered among 17 exons and should be included in any mutation analysis. We sequenced DNA of individuals from 35 different families according to a graduated scheme (1st step exons 17, 39, 44-46, 2nd step exons 11, 14, 15, 40, 3rd step exons 2, 6,9) and identified a mutation causative for MHS in 13 families. Two families had 742G>A, three 1840 C>T, three 6617 C>T, and three 7300G>A. Two families had mutations that had just recently been amended to the EMHG list (6481C>T and 7361G>A). No novel mutations had been found, polymorphisms were frequently observed. The mutation frequency in Germany is regionally diverse (hitherto 1840C>T and 7300G>A had been found most often) but is covered by the list of known mutations.Interestingly, a recent study in Italy identified 31 different mutations in 43 out of 50 MHS patients tested, 16 of which were novel, eight of them would have been covered by our analysis.
Conclusion: Analysis of a limited number of exons or mutations will only be successful in a minority (e.g. 30-50%) of MHS-individuals. Expanding the number of exons for analysis is an ambiguous approach. Our results support previous studies that had achieved little extra success after undertaking extensive additional sequencing workload.

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RNAi sequence-dependent off-target gene silencing can not be explained by overall identity or 3´UTR seed matches

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The gene SLC12A8 on 3q21 (PSORS5) was recently confirmed by our group as susceptibility locus for psoriasis vulgaris and psoriatic arthritis. In an effort to clarify its function, we performed a knockdown of the gene in SW48 human cells using three different chemically synthesized siRNAs at three time intervals (24, 48 and 72h after chemical transfection), followed by microarray expression analysis. To obtain a specific response and minimize off-target effects (OTEs), experimental controls were performed, e.g. use of the minimal effective siRNA concentration (10nM), appropriate positive and negative control, monitor transfection efficiency and cell toxicity and confirmation of knockdown at mRNA and protein levels. Gene cluster analysis determined siRNA1 and siRNA3 (24 and 48h) as the most effectives, and genes with fold-change >1.5 were identified. Real-Time RT-PCR analysis of OAS-1 and IFIT-1 demonstrated the absence of an interferon response in the transfected cells, and there was no effect of the delivery agent alone in the expression of SLC12A8. Nevertheless, expression profiling identified up to 57 (siRNA1) and 216 (siRNA3) off-target transcripts (modulated only by one of the siRNAs). We used web-based search tools for the prediction of potential unintended targets for a given siRNA based on overall sequence identity (Specificity Search) and on 3´UTR seed matches (siRNA Seed Locator). However, the first method recognised none of the off-targets, and the second explained just some of the induced OTEs for siRNA3 (p=0.0004 for multiple seed matches after 24h, compared with random untargeted transcripts). Our data indicate that unspecific gene targeting might be common in siRNA-treated cells even in the absence of interferon response, and that current protocols used to predict potential sequence dependent OTEs fail to identify these. In order to make siRNA a clinically viable therapeutic approach, additional factors with a role in off-targeting should be identified

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Extensive in silico analysis of NF1 splicing defects uncovers determinants for splicing outcome of 5' splice site disruption

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Since splicing outcome of sequence alterations can severely influence the activity of the protein it is important in diagnostic settings to determine the splicing effect of the alteration. In a cohort of 97 Austrian neurofibromatosis type 1 patients we identified 94 mutations that were fully characterized at the genomic and mRNA levels. 36 of these (38%) altered pre-mRNA splicing and fall into five groups: exon skipping resulting from mutations at authentic splice sites (type I), cryptic exon inclusion caused by deep intronic mutations (type II), creation of de novo splice sites causing loss of exonic sequences (type III), activation of cryptic splice sites upon authentic splice-site disruption (type IV), and exonic sequence alterations causing exon skipping (type V). In an effort to gain insight into the determinants of the splicing pathway activated upon mutation of the natural 5´ splice sites (5´ss) i.e. skipping of the affected exon (type I) or activation of a cryptic splice site (type IV) we performed extensive in silico analysis of 37 NF1 exons carrying 84 novel and published type-I and type-IV 5'ss mutations. Our results indicate that the availability of a good cryptic splice site is an important determinant of cryptic splice-site activation upon 5'ss disruption. Furthermore, the exonic sequences downstream of exonic cryptic 5'ss resemble intronic more than exonic sequences with respect to exonic splicing enhancer and silencer density, helping to distinguish between exonic cryptic and pseudo 5'ss. This study provides valuable predictors for the splicing pathway used upon 5´ss mutation, as well as parameters that help distinguish cryptic from pseudo 5'ss. Since these measures have predictive value they are not only relevant to a better understanding of the mechanisms of splice-site selection, but may also constitute a step towards developing tools to predict the outcome of splice-site mutations associated also with other disease gene

P353

Epigenetic modifications change at isochore boundaries

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The mammalian genome is not a random sequence but consists of long genomic stretches called isochores, that are characterized by a distinct GC content and are often separated by sharp transitions. Throughout mammalian evolution, this isochore pattern of the genome was conserved. GC-rich human sequences tend also to be GC-rich in other species and vice versa. Many different biological processes are related to isochores, and transitions between neighbouring isochores function as genomic landmarks, separating genomic regions with different structural and functional characteristics from each other. The NF1/RAB11FIP4 locus on chromosome 17q11.2 contains a clear-cut isochore transition that exhibits a close interrelation of genome structure and function; features like recombination rate, replication timing and mutation rate experience drastic synchronous changes at exactly this border. To determine whether epigenetic modifications and the GC content of the underlying DNA sequence show a similar interdependency, we now analyzed the DNA-methylation pattern of Alu elements on behalf of the global methylation pattern of both isochores. The use of lymphoblastoid cells and fibroblasts, which are characterized by the differential expression of the GC-rich RAB11FIP4 gene whereas the GC-poor NF1 gene is expressed in both cell lines, allowed us to determine the interrelation of the expression level of the two genes and the degree of DNA methylation. Independent of the gene activity, the GC-poor isochore is significantly less methylated than the GC-rich isochore in both cell lines. Thus, the genomic environment has a stronger impact on the overall DNA-methylation pattern of a gene than its transcriptional activity. Consequently, even for epigenetic modifications, isochore borders serve as genomic landmarks.

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Association DB - exploring genome-wide association

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The advance of genotyping assays capable of generating 500,000 or more genotypes per sample makes genome-wide association studies feasible. However, the sheer amount of data renders a quick interpretation of the results almost impossible for humans as, depending on the sorting, either the positional information or the significance will go under in the flood of data.
Here we present Association DB, a web-based database application that analyses and visualises genome-wide association. Genomic data is integrated into the results and both are presented in an intuitive graphical interface allowing an interactive exploration of the data. Up to three different comparisons of cases and controls can be overlaid to reduce spurious hits, e.g. by population effects. The use of colours gives a direct hint on the relevance of the results. This and the possibility to include OMIM reports or gene expression greatly facilitates the search for candidate genes in the haystack of association results. Clicking on a gene will display detailed information about this gene and hyperlinks to external resources.
Association DB is an open source application and no commercial software is required to run or use it.

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Sensitivity and specificity of high throughput melting curve analysis for the detection of private mutations and other rare genome variants in PCR amplicons

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High throughput genotyping of common gene variants (SNPs) has recently seen remarkable advances. High throughput screening for unknown and rare genome variants (point mutations) is similarly a major need and challenge in human genetic research and molecular diagnostics. The need is reflected by ongoing initiatives to achieve genomewide resequencing at $100K by 2009 and $1K by 2014 (The Personal Genome Project, Shendure et al. Nat. Rev. Genet. 2004; lazej et al. PNAS, 2006). Classical screening methods have not shown convincing potential beyond medium throughput (chemical or enzymatic cleavage, SSCP, TGGE, DHPLC) or are too expensive and labour intensive for high throughput implementations (Sanger resequencing)
Recently melting curve analysis (MCA) has been proposed as an innovative screening method for unknown genome variants in PCR amplicons. Current commercial implementations are capable for high throughput in 384 well plates with costs about an order of magnitude less compared to Sanger resequencing. We have evaluated MCA in a series of 362 patients with autosomal dominant dilative cardiomyopathy. A total of 26 Exons from three genes have been investigated by this method in all patients so far. Specificity of the method was higher (78% average) than with previously used methods, avoiding unnecessary resequencing expenses. In 96 patients who had undergone complete Sanger resequencing of 18 exons we could evaluate the sensitivity of the method. We found it to be 96,5% (range: 74-100%) if amplicons were no longer than 350 bp even if several common (SNPs) and rare variants (point mutations) were present in one amplicon. As expected it was decreased in longer amplicons and in amplicons containing several variants.
Current implementations of melting curve analysis offer a good detection of private mutations and other rare variants in PCR-amplifiable short genomic regions, which is applicable in high throughput with a very reasonable cost-efficiency ratio.

P356

Tetrapod specific conserved non-coding elements (CNEs) from intronic and upstream region of GLI3 are transcriptional enhancers

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During early vertebrate development the GLI3 transcription factor is required for multiple activities including specification of mesodermal, neural and heart tissue, as well as gastrulation movements and regulation of differentiation and pattern onset in the extending body axis. GLI3 is also a key player in pattern formation during brain and limb development. Expression studies of GLI3 witness a high-level and complex regulation. A current challenge is to comprehend how GLI3 dynamics generates such diverse outcomes. Towards this end we intend to uncover cis-regulatory elements for limb-specific developmental role of GLI3. We have employed comparative genomics and phylogenetic footprinting to identify conserved non-coding elements (CNEs) as candidates for limb development from intronic and upstream GLI3 genomic intervals. All the selected CNEs are conserved up to the tetrapod lineage which radiated ~400 million years ago but do not reveal deep phylogenetic footprints (no conservation up to fish). As a initial step to define the putative regulatory function we have checked in vitro luciferase activity of eight intronic and four long-range upstream CNEs by transient transfection in three different cell lines with or without endogenous GLI3 expression. We show that all the intronic CNEs behave as transcriptional enhancers, mostly activators, both with a GLI3-minimal promoter construct and with a heterologous promoter, and are specific to GLI3 expressing cell lines. We further show that all the selected upstream CNEs are generally transcriptional repressors, and their activity is not confined to the GLI3 expressing cell lines. As a next step we intend to evaluate the transcriptional potential of these CNEs in limb buds by electroporation into chick embryos. These analyses could help appreciate the complex regulatory landscape of GLI3.

P357

The ontologizer: Improved detection of overrepresentation of gene-ontology annotations with parent-child analysis

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Gene Ontology (GO) is a controlled vocabulary describing different features of gene and gene products for any organism. GO terms are arranged as a directed acyclic graph in which child terms represent subclasses or parts of parent terms. Exploring Gene Ontology annotations for lists of genes sharing certain characteristics (such as differential expression in a microarray hybridization) has become a widespread practice. In particular, one is often interested in terms which are overrepresented in such lists as this gives first insights into the potential biological meaning of the experiment.

The standard approach to measuring the overrepresentation of a term involves the one-sided Fisher exact test. Importantly, as each GO term is analyzed in isolation, the structure of GO is neglected. We have shown that this approach can lead to false-positive results with potentially misleading biological interpretation. We have developed a novel method for the analysis of GO term overrepresentation that determines overrepresentation of terms in the context of annotations to the term´s parents. This approach reduces the dependencies between the individual term´s measurements, and thereby avoids producing false-positive results owing to the inheritance problem (Grossmann et al., 2006).
Here, we present the Ontologizer, a Java WebStart or desktop application that can be used to perform statistical analysis for overrepresentation of Gene-Ontology terms in sets of genes or proteins derived from an experiment. The Ontologizer provides both the standard approach to statistical analysis based on the one-sided Fisher exact test as well as the novel parent-child method together with an easy to use user interface. We will demonstrate the analysis of microarray datasets using both approaches and discuss important differences in interpretation. We show how the Ontologizer can be used to explore the graph of GO terms and annotated genes.

P358

Intragenic conserved non-coding elements from human GLI3 are cell- and tissue-specific enhancers

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GLI3 is a zinc-finger transcription factor acting during development as primary transducer of Sonic Hedgehog (SHH) signaling in a context dependent combinatorial fashion.
Mutations in the human transcription factor GLI3 cause a variety of dominant developmental defect syndromes subsumed under the term “GLI3 morphopathies”, such as Greig cephalopolysyndactyly syndrome (GCPS), Pallister-Hall syndrome (PHS) , postaxial polydactyly type A (PAPA), and preaxial polydactyly type IV (PPD-IV). Mutations affecting murine Gli3, such as extra toes (Xt), anterior digit deformity (add), and polydactyly Nagoya (Pdn) serve as models for GLI3 morphopathies.
GLI3 has essential functions controlling multiple patterning steps in different tissues/organs which render a tight temporal and spatial control of gene expression indispensable. As a first approach to determine the landscape of GLI3 cis-regulatory elements, we show that 11 ancient genomic DNA signatures conserved in human-pufferfish Takifugu (Fugu) rubripes are distributed throughout the introns of human GLI3. They constitute ancient nucleation sites within non-coding sequence elements (CNEs) highly conserved among tetrapods. Both these human-fugu conserved modules and the surrounding sequences act in combination as cell and tissue specific enhancers of gene transcription when transiently transfected into human cell cultures or zebrafish embryos. The human-fugu conserved sequences within the CNEs from GLI3 introns are essential but not sufficient for transcriptional regulation. The CNEs tagged by DNA sequences highly conserved in a teleost represent an ancient part of a larger repertoire of cis-acting elements controlling time, location, and quantity of GLI3 expression.

P359

Functional high-throughput screening of candidate genes involved in cholesterol metabolism

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High blood cholesterol levels are considered responsible for excess cardiovascular morbidity and mortality. Current pathophysiological models of hypercholesterolemia assume a tight association of environmental as well as genetic factors, many of which are yet unknown. For the identification of genes predisposing for hypercholesterolemia we examined the effects of RNAi-mediated candidate gene knockdown on cholesterol metabolism using a cell-based cholesterol uptake assay.
HeLa cells were grown and transfected on prefabricated siRNA-microarray slides. Cholesterol uptake was quantified using an automated microscope and analyzed at the level of single cells by customized cell-recognition software.
Results show significant and robust changes in cholesterol uptake for control candidates such as the LDL receptor as well as for candidates previously unknown to be linked to cholesterol metabolism. By adapting transfection, uptake and imaging to high-throughput conditions, we can now functionally examine up to hundreds of candidate genes on a single chamber slide with greatly reduced time and effort. This will allow us to systematically identify new candidate genes involved in regulating cellular lipid metabolism on a large scale.

P360

New diagnostic resequencing microarray for hereditary spastic paraplegia

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Hereditary Spastic Paraplegia (HSP) is a heterogenous group of inherited neurological disorders. Insidiously progressive spastic weakness of the lower extremities is the common criteria in all 33 forms described so far. Autosomal dominant, autosomal recessive and X-linked mode of inheritance have been described. Clinically HSP is differentiated into pure (uncomplicated) and complex (complicated) forms, depending on isolated impairment of corticospinal tracts or more wide spread affection of neuronal circuits or systemic involvement.
For proper diagnosis molecular genetic analysis is fundamental since clinical parameters alone are not reliable in distinguishing HSP forms. In order to establish high throughput genotyping, we designed a HSP resequencing microarray (Affymetrix platform) covering the coding exons and flanking intronic sequences of the HSP genes L1CAM (SPG1), PLP1 (SPG2), Atlastin (SPG3A),Spastin (SPG4), NIPA1 (SPG6), Paraplegin (SPG7), KIF5A (SPG10), HSP60 (SPG13), BSCL2 (SPG17), Spartin (SPG20), Maspardin (SPG21), (13 - 94kb, 5 - 28 coding exons), as well as the 59 most frequent small deletions, insertions and insertion/deletions in these genes. We present data on microarray analysis of 20 autosomal dominant HSP index patients.

P361

Assessment of SNP genotyping consistency between whole-genome amplified and genomic DNA using 768 SNPs from different genomic regions

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High-throughput genotyping of SNPs has become an important research strategy in biomedical research. Although current genotyping protocols require minimal amounts of DNA per assay, repeated use often leads to depletion of these in many cases non-replaceable samples. Whole-genome amplification technologies have recently been developed to overcome this problem, some are based on the highly processive φ29 DNA polymerase. In most cases, the amplification is successful. However, it has been controversially discussed whether the whole-genome amplified (wga) DNA exactly mirrors the information content of the genomic DNA (gDNA) template.
In the present study, we aimed to assess the genotyping consistency between 25 wga DNAs (generated using GenomiPhi V2 DNA Amplification Kit, GE Healthcare) and their corresponding gDNA samples. gDNAs had been prepared by conventional salting-out methods of EDTA whole blood samples or by using the chemagic Magnetic Separation Module I (Chemagen). 768 SNPs from different genomic regions were genotyped by Illumina’s GoldenGate Assays and produced a total of 19,200 possible “genotype pairs” between wga and gDNA. 22 of the total 25 pairs performed well, with comparable average genotype call frequencies of 97.3% (gDNA) and 97.1% (wgaDNA). The average genotype consistency between gDNA and wgaDNA samples was 99.6%. Performance of the remaining 3 DNA pairs was noticeably worse (average genotype call frequency of 97.4% (gDNA) versus 60.1% (wgaDNA), average genotype consistency was only 34%). Possible explanations for the observed discrepancies include the age of

gDNA, the extraction method as well as the presence of unknown inhibitors interfering with the ö29 DNA polymerase. For the general use of wgaDNA samples as templates in high-throughput genotyping studies, it will be important to recognize and exclude such 'outlier' DNAs. They may possibly be identified by their relatively low genotype call frequency.

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Proteomic analysis of cathepsin B and L-deficient mouse brain lysosomes

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Combined deficiency of both cathepsin B and cathepsin L results in early-onset neurodegeneration in mice reminiscent of neuronal ceroid lipofuscinoses in humans. Only a few protein substrates of cathepsins B and L have thus far been described and the mechanisms by which these cathepsins regulate cell proliferation, invasion, and apoptosis remain poorly understood. Therefore, we intended to quantify protein changes in brain lysosomes of double deficient mice.

A combination of subcellular fractionation and LC-MS/MS using isobaric tagging for relative and absolute quantitation (iTRAQ™) allowed us to simultaneously assess wildtype and cathepsin B-/-L-/- cerebral lysosomes. Altogether, 19 different proteins were significantly increased in cathepsin B-/-L-/- lysosomes. Most elevated proteins had previously been localized to neuronal biosynthetic, recycling/endocytic or lysosomal compartments. A more than 10-fold increase was observed for Rab14, the Delta/Notch-like epidermal growth factor-related receptor (DNER), calcyon, and carboxypeptidase E. Intriguingly, immunohistochemistry demonstrated that Rab14 and DNER specifically stain swollen axons in double deficient brains. Since dense accumulations of expanded axons are the earliest phenotypic and pathognomonic feature of cathepsin B-/-L-/- brains, our data suggest a potential role for cathepsins B and L in recycling processes during axon outgrowth and synapse formation in the developing postnatal central nervous system.

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Effect of SNP selection on haplotype block patterns in two genomic regions

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Single Nucleotide Polymorphisms (SNPs) in close physical vicinity tend to be in linkage disequilibrium (LD) and "travel together" in a given population. This principle is of major importance for many LD-based gene identification projects in the field of biomedical research. The International HapMap Project provides detailed information on patterns of LD, defines haplotype blocks and reduces the amount of redundant SNP information by identifying a minimum set of SNPs (haplotype tagging SNPs, htSNPs) to capture all common variants of these blocks. Several publications have shown that, apart from differences between populations, SNP density and sample size have a strong impact on LD and block patterns. Current block structures in HapMap must therefore be regarded as preliminary and dependent on the sample. It is usually suspected, however, that a higher SNP density will resolve this problem and give a more stable and uniform picture of the "true" underlying block structure, regardless of the particular choice of SNPs. This view has been challenged by a recent paper by Nothnagel and Rohde (AJHG 77:988-998, 2005). In the present study, we aimed to test the effect of comparable but complementary SNP sets on the resulting block structure in two genomic regions comprising the genes DISC1/DISC2/TSNAX on 1q42.1 and NRG1 on 8p21. A total of 484 HapMap-derived htSNPs, covering about 1.5 Mb of genomic region, were genotyped in a sample of 738 German population-based control individuals. LD patterns and haplotype blocks were defined according to the standard definitions used in HapMap. Our results provide confirmatory evidence that, while global LD patterns are comparable, complementary SNP sets result in profound differences in haplotype block patterns. As a consequence, the use of block-free tagging SNP approaches should be considered as an alternative to common block-based approaches when planning systematic LD studies.

P364

Application of the E-painting technique for karyotype reconstructions and breakpoint characterisation

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The whole genome DNA sequence and annotation of genes is available for several vertebrates. We have concentrated on a comparison of synteny conservation in human, mouse, rat, dog, and cattle from the mammals and chicken from the birds. The chicken was used as an outgroup. For all genes for which the orthology to human genes is proven the chromosomal position of these genes was listed. By this segments of common synteny are visualized. At the same time the borders of these synteny segments and with these borders the sites of evolutionary breakpoints are delineated. At all 351 chromosomal breakpoints were assigned with a median precision of the breakpoint interval of 130 kb. In general there is no overlap of these evolutionary breakpoints with cancer breakpoints and with fragile sites. A considerable variation in the local rate of rearrangements became apparent. To our surprise this rate is highest in chromosome regions which in human are known to be gene rich. Further, the analysis provides insight in chromosome evolution. In most cases the already known associations and fusions of the ancestral eutherian founder karyotype were confirmed, but some chromosomes of this founder karyotype are described in more detail and others have to be reconsidered as e.g. the human specific fusion of chromosome HSA 2.

P365

Analysis of genetic variability by resequencing

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The analysis of genetic variability in candidate genes is the central step in the discovery of disease genes. An assumption for disease gene identification is the sequence analysis of candidate genes in patients and controls to identify all specific sequence variations (SNPs or indels) associated with the disease. We have installed an automated high-throughput re-sequencing service platform within the NGFN open to all NGFN members, but also to scientific cooperations outside the NGFN. In addition, we offer bioinformatic techniques for the analysis of the data sets. During NGFN I we have sequenced over 20 candidate genes for different diseases. Currently, we are sequencing different candidate genes for complex diseases (cardiovascular diseases) or pharmacologically relevant genes in cooperation with our clinical partners. The coordination of the various sequencing requests from the network partners will be performed through our WEB portal (http://www.resequencing.mpg.de/). The Coordinator of the subproject is responsible for quality control of samples and communication with partners. The cost for this service pipeline were calculated to 0.045 Euro per sequenced base/sample, to be financed by the user requesting the service. This price included PCR amplification, sequencing reactions and data analysis. If exon-intron amplicons are already available, it is possible to send in the PCR products and primer for sequencing and data analysis (4.4 Euro per sequencing read). In this case we will work with the PCR products of our cooperation partners.

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Automated cycle sequencing of DNA samples using an AVISO ´Theonyx Liquid Performer´ robotic platform

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The increasing number of sequence reactions to be set up is a growing problem in current molecular diagnostics. Since automated capillary electrophoresis has helped to reduce hands-on time and sequence analysis software assists in finding causative mutations in hereditary diseases, manual setup of sequencing reactions has become the bottleneck within the process. We have successfully adapted a ´Theonyx 440H Liquid Performer´ robotic platform to the demands of automated DNA sequencing in a sequencing core of a routine molecular laboratory. In the fully automated process PCR products of genomic DNA are provided in 0.2 ml reaction tubes and are vacuum-purified on board using filtration plates. Subsequently, purified DNAs are spectrophotometrically measured, thus enabling a definite template input into the cycle sequencing process which is set up and performed in a thermal cycler on the platform. After thermal cycling further purification of the sequencing reactions is performed using ultrafiltration plates on board. The RoboManager software controlling the process enables import of up to 6 sample plates and corresponding Excel input sample files and up to 95 primers with a total of 96 samples per run using bidirectional sequencing or 192 samples using single sequencing primers. The 96-well output plates containing up to 192 sequences are generated together with Robomanager plate output files. Subsequently, after conversion to the ABI (.plt) input format using the ´ABI File Converter´ software input files can be uploaded and samples can be electrophoresed on an ABI 3100 genetic analyzer, generating up to 192 sequence files in 18 hours total time. Sequences show very low background, perfect dye blob removal, and readlengths up to 500 base pairs with 99% accuracy, thus meeting the demands of post analysis in a molecular diagnostics laboratory. Additional speed-up of total turn around times to 12 hours will be possible using the ABI 3130XL upgrade and POP-7 polymer.

Prenatal Diagnosis, Reproductive Medicine

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Differentiation of pluripotent spermatogonial stem cells to haploid male germ cells

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Spermatogonial stem cells (SSCs) provide the basis for spermatogenesis throughout adult life by undergoing self-renewal and differentiation into sperm. Fascinating breakthroughs have been achieved in recent years in regard to our knowledge and to novel applications on SSCs. They were recently shown to be pluripotent and to have the same potential as embryonic stem cells (ES cells).
In a differentiation protocol using retinoic acid (RA) based on a double selection strategy we have shown that ES-cells are able to undergo in vitro meiosis and produce haploid male germ cells. These cells – by using ICSI- are able to fertilize oocyes. Retransfer of the early embryos into pseudopregnant mice resulted in viable offspring.
We have now succeeded in establishing a protocol for differentiation of haploid male germ cells from spermatogonial stem cells in vitro. The functionality of the cells is now proven by ICSI. If functional sperm can be differentiated from SSCs, our results could have an impact for couples suffering from male infertility.

P368

Polar body testing on different array platforms

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Chromosomal copy number changes are among the most important factors that negatively influence embryo survival. Currant estimates are that more than half of all human preimplantation embryos contain aneuploid cells. For an efficient screening of aneuploidies in polar bodies, we developed several strategies. First, we take advantage of our sequential approach of interphase FISH first, followed by single cell CGH of the same cell (Langer et al. [2005] Lab Invest 85:582-592). We analysed more than 20 polar bodies without prior FISH analysis and 5 polar bodies with sequential analysis by interphase FISH and CGH. In previous experiments we used our single cell linker adaptor approach to amplify the entire DNA content of a single polar body for a subsequent CGH evaluation, but the long duration of this protocol (32 h) represents a disadvantage for polar body testing. Hence we tested the commercially available WGA4 (Sigma-Aldrich) kit, which allows DNA amplification within 5 hours. First, we tested the quality of polar body DNA amplification product, by chromosome CGH, then we hybridized the DNA on different array platforms. To this end we used the 1 Mb array (Fiegler et al. [2003] Genes Chromosomes Cancer 36: 361-374, in collaboration with H. Fiegler and N. Carter, Welcome Trust Sanger Institute, UK), the 8 k array (in collaboration with P. Lichter, Heidelberg, Germany) and a mini- array consisting of pooled BACs covering all human chromosome arms (Knijnenburg et al. [2005] Nucleic Acid Res 33:e159; in collaboration with J. Knijnenburg, K. Szuhai and H. Tanke, Leiden University Medical Center, The Netherlands). Our data suggest that a high resolution analysis of polar bodies on an array platform is feasible. This will greatly extend existing approaches in prenatal diagnosis. We will present detailed data about the performance of polar body amplification products on these array platforms.

P369

Multiplex RT-PCR of DNA modification and pluripotency genes in single blastomeres of mouse preimplantation embryos

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Over the last years, RT-PCR has become the preferred method for validating results obtained from microarray analyses and other techniques that evaluate gene expression changes on a global scale. RT-PCR is the most sensitive technique for mRNA detection and quantification currently available. On the other hand, there is an increasing requirement of techniques for expression analysis at the single cell level. We are specifically interested to study the expression of known and candidate genes for nuclear reprogramming in individual cells of preimplantation embryos. In a pilot study, we are using the AmpliGrid technology for comparing expression profiles in single cells of mouse morulae. The AmpliGrid is a glass chip with a surface structure that allows for the specific positioning of one single microliter on each of the 48 discrete reaction sites (enabeling to run 48 1 µl reactions in parallel). Single cells are placed under optical control onto these reaction sites followed by direct RT-PCR amplification. In a first set of experiments, we identified an appropriate control gene, histone H3 variant H3.3, which is expressed at rather constant levels in early embryonic cells. Similarly, expression of the transcription factor Oct4 (Pou5f1) was detected in essentially all cells of morula stage embryo, indicating a uniform reactivation of pluripotency gene expression. In contrast, expression of different DNA methyltransferases, methylcytosine-binding domain proteins and other candidate genes for DNA modification varied considerably between cells. Our preliminary results suggest that the transcriptome encoding the reprogramming machinery and, by extrapolation, genome reprogramming differs between individual cells of an early embryo. Future experiments will reveal whether expression profiling of 1 or 2 blastomeres can be used for embryo quality assessment.

P370

Integrated and sequential screening for fetal aneuploidies – consequences of the SURUSS- and FASTER study

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For the first time all currently applied methods of prenatal screening for fetal aneuploidies, especially trisomy 21, have directly been compared in two large observational population studies, the European SURUSS and the American FASTER study. Integrated and sequential screening turned out to be the most significant techniques yielding highest detection rates of about 95% at a fixed false-positive rate of 5% and lowest false-positive rates of about 1% at a fixed detection rate of 85%. In spite of high detection the new methods reduce the amount of invasive procedures and hence procedure related fetal loss rates to a minimum. In consequence health care professionals in other countries break new grounds by implementing these screening methods while in Germany they are almost unknown. With our presentation we want to give an overview of the new methodology. The results of SURUSS and FASTER are summarized and the pros and cons of integrated screening discussed.

P371

Five years polar body dignosis for monogenic disorders in Regensburg

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Due to the regulations of the embryo protection law in Germany polar body diagnosis (PBD) is still the only option of a preimplantation genetic diagnosis for couples at risk of transmitting a monogenic disorder. Here we report about the PBD results of our centre since 2001 for selected disorders.
Following the removal from the oocyte, the polar bodies are lysed and then subjected to a one step multiplex fluorescence PCR, using at least 2 closely linked informative VNTR Markers – if possible - together with a system for direct mutation detection. PBD is an indirect diagnosis, sequential analysis of the first and second polar body allows to deduct the genetic information of the respective oocyte.
As of today 22 PBD cycles have been performed at our center. From 140 Oocytes (mean 6.36 per cycle) 23 could be diagnosed as not containing the respective mutation. 23 embryos could be transferred in 15 cycles (mean 1.53 per transfer cycle) resulting in 5 clinical pregnancies (including two twin pregnancies). Five healthy children have been born; one twin pregnancy is still ongoing. Our preliminary pregnancy rate after PKD (33,3%) is not lower when compared to ICSI alone or PID with blastomere biopsy, indicating that there seem to be no adverse effects of the polar body biopsy.
However there are limitations of the PBD when compared to PID: (I) only maternally transmitted disorders can be analyzed and (II) in case of X-chromosomal or autosomal recessive disorders the number of embryos to be discarded is higher. However, polar body diagnosis for monogenic disorders has been proven to be a useful tool for couples at risk of transmitting a genetic disease to avoid recurrent pregnancies with subsequent prenatal diagnosis and termination of the pregnancies with an affected fetus. It requires a close interdisciplinary cooperation at the center and an intensive counseling of the couple regarding both genetics as well as reproductive medicine.

P372

Reproduction abnormalities and twin pregnancies in parents of sporadic patients with Oculo-Auriculo-Vertebral Spectrum (OAVS)/Goldenhar syndrome

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Background: A great number of case reports on concordant and discordant twins with Oculo-Auriculo-Vertebral Spectrum (OAVS) suggest that there might be an association between reproductive abnormalities, twinning and OAVS. The etiology of OAVS is unknown, but may involve epigenetic dysregulation of the oocyte or early embryo.
Methods: We collected data on fertility and pregnancy outcome of 72 parents of patients with sporadic OAVS. We also evaluated prospective follow-up data on 3.372 fetuses and children conceived by intracytoplasmatic sperm injection (ICSI).
Results: Parental age, duration of menstrual cycle and the incidence of spontaneous abortion was not different when compared with the German population. Time to pregnancy appears to be increased in the parents of patients with OAVS. Furthermore, there is an excess of parents who have used assisted reproductive techniques (ART; retrospective p=0.038, prospective p=0.023) and an excess of twins among naturally conceived patients with OAVS (p=0.015).
Conclusions: ART conceptions, longer time to pregnancy and monozygotic twinning in OAVS are compatible with the concept of overripeness ovopathy as proposed by Jongbloet (1968).

P373

High aneuploidy rates of human oocytes found with a broad indication for preimplantation genetic screening

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Introduction: The aim of PGS is to improve success rates in ART. A high aneuploidy rate also in young women justifies a broad indication. As we got comparable success rates with PGS as without, we analysed our success cascade.
Methods: After approval by the local ethics committee, in 2004 and 2005 we performed 60 cycles. PGS offered to all patients with 6 or more oocytes, independent of age or other indications, performed by laser biopsy of the first polar body on day 0, fixation for FISH and hybridization with chromosome 13, 16, 18, 21, 22. Zygote selection was done on day 1 in accordance with the German embryo protection law, embryo transfer usually on day 2, with an intended elective double embryo transfer from proven “euploid” (for 5 chromosomes) oocytes. If no two euploid oocytes were available, one or two not detected oocytes were transferred.
Results: The average age of the women was 36 years (range 25 – 44 years). ET was performed in 57 cases (95%), resulting in 14 (25%) pregnancies, 8 deliveries and 6 abortions. 584 mature oocytes were retrieved, of these 498 (85%) were biopsied, 454 (91%) survived, 365 (73%) fertilized. 449 (90%) could be fixed, 334 of these (74%) got genetic results, 129 of these (39%) were euploid, 205 (61%) aneuploid. 106 embryos were transferred, 66 of these (62%) were generated from euploid oocytes. The implantation rate was 14%. We saw in 47 cases (77%), with an average of 35 years, a 100% - 50% aneuploidy of all biopsied oocytes.
Conclusions: PGS is safe in the sense that the pregnancy rate per embryo transfer (25%) is comparable to the German national averages (28%).The aneuploidy rate per patient is high even with a broad, not age related indication. There is also a high rate of patients where the majority of the oocytes are aneuploid. To find the postulated benefits of PGS indications should be made embryologically (morphology of oocytes), not only clinically (age of patient).

P374

Mice deficient for RNA-binding protein Brunol1 show reduction of spermatogenesis but not fertility

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RNA-binding proteins are involved in post-transcriptional processes like mRNA stabilization, alternative splicing and transport. We report here the expression and functional analysis of murine Brunol1, a novel mouse gene related to elav/Bruno family of genes encoding for RNA-binding proteins. Expression analysis of Brunol1 during embryogenesis by RT-PCR showed that Brunol1 expression starts at 9.5 dpc and continue to the later stages of embryonic development. In adult mice, the Brunol1 expression is restricted to brain and testis. Expression analysis with testicular RNA isolated from mutant mice with arrested spermatogenesis at different stages and from mice at different developmental stages revealed a haploid germ cell specific expression of Brunol1. Furthermore, we showed a cytoplasmic and nuclear localization of Brunol1 using a GFP fusion protein. In order to elucidate the function of the Brunol1 protein, we disrupted the Brunol1 locus in mouse by homologous recombination. Male and female Brunol1+/- and Brunol1-/- mice from hybrid C57BL/6J x 129/Sv and inbred 129X1/SvJ genetic backgrounds exhibit normal phenotype and were fertile, although number and motility of sperm were significantly reduced. An intensive phenotypic analysis showed no gross abnormal-

ities in testis morphology. Collectively our results demonstrate that Brunol1 is a nonessential protein for spermatogenesis.

P375

Sequence variation in the common primer binding site for the sex determination amelogenin 6bp deletion can lead to misdiagnosis in rapid prenatal diagnostic QF-PCR

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Rapid prenatal diagnosis using QF-PCR is based on amplifiying 6 or 7 microsatellite markers from each chromosome under evaluation (usually 13, 18, 21 and X/Y). Analysis is done by counting alleles from each informative marker and comparing the signal intensity between these alleles. Three alleles or signal intensities in a 2:1 ratio (compared to the regular 1:1 ratio) are indicative of three chromosome copies. The so-called amelogenin marker makes use of a 6bp-deletion in the first intron of the amelogenin gene that occures only on the X-chromosome and not on the Y-chromosome. Hence, PCR-products spanning this 6bp-deletion will differ in size, and they are used to distuingish between male and female in samples of unknown sex. The signal intensities of the products are expected to be in a 1:1 ratio in males. The most common amelogenin primers are the so-called british primers. They generate PCR-products of 106 bp and 112bp from the X- and the Y-chromosome, respectively. These primers are used in several forensic and paternity test kits from various companies and are also frequently used for rapid prenatal diagnosis using QF-PCR.
We have identified a sequence variation on the X-chromosome in the primer binding site of these british primers. The variation is 4 nucleotides from the 3'-end and reduces the signal intensity of the X-chromosom peak by a factor of 2-3. Without information from other informative markers this could be interpreted as a XYY-genotyp in prenatal diagnosis. The sequence variation was originally found from a patient from frankonia and subsequently found in four patients from Southern Germany and one with turkish orign out of 1500 QF-PCR diagnosis, suggesting that the mutation is rare. However, we suggest the use of a second, different primer pair for amplification of the 6bp amelogenin deletion, use of markers amplifying other differences between the X- and the Y-chromsome and microsatellite markers from the pseudoautosomal region (e.g. X-22).

P376

Characterisation and growth potential amniotic fluid cells

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Despite the widespread and well established use of amniotic fluid cells in routine prenatal diagnosis is the current knowledge about origin and properties of theses cells still limited. For prenatal diagnosis the adherent and colony forming cells are selected and early classifications of theses cells based mainly on morphological criteria. Three major groups have been described: epithel-like (E-) cells, amniotic fluid (AF-) specific cells and fibroblast-like (F-) cells.
We characterised adherent and non-adherent cell populations of 56 amniotic fluid cell samples obtained from amniocenteses that were performed between 15th and 33th g.w. for routine prenatal diagnosis. For 41 samples the indications were advanced maternal age and the cytogenetic analyses revealed normal karyotypes. 16 amniotic fluid samples derived from abnormal pregnancies. Growth of amniotic fluid cells was studied with respect to cell concentration in the inoculum, blood contamination of the fluid, fluid colour, and gestational age of the pregnancy. The adherent amniotic fluid cells morphology was decribed as published data have shown.
Especially, it could be demonstrated how the AF-cells alter their morphology when they get older. To characerise this cell polulation better, we performed immunofluorescence on growth factor receptores (FGFR-1 and EGFR-1). We could demonstrate, that AF-cells were positive for both receptor types. Furthermore, adherent AF-cells produce human chorionic gonadotropin (hCG) as shown by immunofluorescence. The effect of hCG in AF-cells ist not understood in detail. It is discussed, whether hCG might play a role in cell attachment. In summary, under the well established in vitro conditiones is a strong selection towards AF-cells, thus being the predominant cell type used for prenatal diagnosis.

This project has been accepted by the ethics commission of the University of Marburg (project number: 56/06).

P377

Interphase FISH to detect fetal aneuploidy due to cryptic unbalanced chromosome rearrangement undetected by karyotyping

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We report on a prenatal diagnosis of an unbalanced translocation involving chromosomes 7 and 21 in a fetus with an abnormal facial profile, retrognatia, hypoplastic nose, cleft lip and palate, ventriculomegaly, hypoplastic left heart, pyelektasis and shortened humeri. Amniocentesis was performed at the 18th week of gestation because of increased nuchal translucency and fetal malformations. Interphase FISH on uncultured amniocytes with probes for chromosome 13, 18, 21, X and Y revealed three signals for chromosome 21. The chromosome analysis resulted in a normal numeric karyotype without any obvious structural abnormality. FISH analysis with a chromosome 21 painting probe and a locus specific probe for the critical Down syndrome region detected an additional signal on the long arm of chromosome 7. The unbalanced karyotype was confirmed by hybridization with a chromosome 7 specific subtelomeric probe [(46,XY,der(7)t(7;21)(q34;q21)pat]. Parental karyotypes showed a paternal balanced translocation 46,XY,t(7;21)(q34;q21). The mother opted for termination of the pregnancy because of the poor prognosis of the fetus. In genetic counselling, information was obtained that an uncle of the father clinically shows Down syndrome which had hitherto not been confirmed by cytogenetic investigation. Cytogenetic and molecular cytogenetic analysis in this patient revealed a numerical and structural aberrant karyotype with the balanced reciprocal translocation and an additional chromosome 21. This chromosomal aberration can be either the result of an interchange trisomy or an independent nondisjunction event. This case illustrates that a "conventional" prenatal diagnosis regimen (i.e. "excluding" Down syndrome due to malsegregation of a familiar translocation) could have lead to a false diagnosis.

Genetic Counselling, Education, Genetic Services, Public Policy

P378

Molecular neonatal screening for homocystinuria in the Qatari population

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Homocystinuria is an autosomal recessive disorder of methionine and homocysteine metabolism caused by a deficiency of cystathionine β-synthase (CBS). We previously reported a high incidence of approx. 1:3000 in Qatar, caused primarily by homozygosity for the mutation R336C in the CBS gene. In order to facilitate early recognition of the treatable disease we developed a novel metabolic and molecular testing strategy for neonatal screening. For mutation analysis, DNA was extracted from dried blood spots using the ABI Prism6100 PrepStation. Mutations R336C (c.1006C>T) and D234N (c.700G>A, previously found in a single Qatari family) were tested using PCR-based fluorogenic 5´nuclease probe (TaqMan) as a simple DNA diagnostic method suitable for processing a large number of samples. So far we have analyzed 2,396 newborns of Qatari origin. A total of 4 neonates with homocystinuria were identified. All showed highly elevated homocysteine concentrations in dried blood spots whilst methionine was elevated in two neonates only. Three children were homozygous for the common mutation R336C; follow-up sequence analysis in the fourth patient revealed homozygosity for muta-

tion G347S (c.1039G>A) in exon 9 of the CBS gene, not previously observed in the Qatari population. In addition we identified 36 heterozygote carriers for R336C equivalent to an allele frequency of 0.009 (95%-CI 0.006-0.012), and 2 carriers for D234N. Carriers showed no metabolic abnormalities. The G347S allele displays an extreme deviation from Hardy Weinberg equilibrium p ~ 0.0006 exact test method. The expected genotype counts are: 0.2; 41.6; 2354.2. Our results support a very high incidence of homocystinuria in Qatar, possibly reaching up to 1:600 and caused largely by a high degree of consanguinity. Molecular neonatal screening is feasible but metabolic screening appears to have a higher sensitivity for the detection of homocystinuria in Qatar.

P379

Assessing risk-predicting models in German high risk breast cancer families

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Germline mutations in BRCA1/2 account for approximately 30% of high risk hereditary breast and/ or ovarian cancer. A number of risk prediction models have been developed to assist in pre-test counselling and the selection of individuals likely to harbor a mutation in BRCA1/2. In particular, the German Centers Deutsche Krebshilfe for familial breast- und ovarian cancer are using the prediction model of Cyrillic 2.1.3 to restrict DNA diagnostics of BRCA1/2 to individuals who are predicted to have a 10% or more likelihood to carry a pathogenic mutation. Thus far, the predictive value of this and other modules have not been determined for the German population.

In our study various prediction models (e.g. Cyrillic 2.1.3, Manchester, Claus Tables) were retrospectively applied to 298 index cases which had undergone BRCA1/2 mutation testing. Several statistic properties such as sensitivity, specificity, positive and negative predictive values, likelihood ratios and area under the receiver operator characteristic (ROC) curve were evaluated. Of the 298 tested, pathogenic mutations were identified in BRCA1 (n = 56) and BRCA2 (n = 33) while 209 were negative after analysing with a combination of methods including dHPLC, direct sequencing and MLPA.

Compared to data collected in related studies for other populations (e.g. American, Australian, UK) our results show similar accuracies of the models. In particular ROC analysis suggests a reasonable but not ideal discriminatory value of 0,75 for the Cyrillic model. This leaves the option to apply additional criteria to program requirements such as user-friendliness or reduced errors and ambiguities in data input. In conclusion our data indicate that the statistical models have little impact on pre-test probability to harbor BRCA1/2 mutations. This implies a note of caution to preliminary exclude individuals which have a predicted risk of carrying a BRCA mutation of less than 10% purely based on statistical model calculations.

P380

Recurrence risk in children of patients with sporadic uniateral retinoblastoma

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Retinoblastoma (Rb) is the most frequent intraocular tumor in childhood. The hereditary form of Rb is caused by germline mutations in the RB1 gene. Molecular analysis in patients with unilateral Rb is based on identifying oncogenic mutations in DNA from tumor and, subsequently, testing for these mutations in DNA from blood. Previously we have found that 9% of patients with unilateral Rb are heterozygous for an oncogenic mutation and 3.7% show mutational mosaicism. In most patients oncogenic mutations were not detected in peripheral blood (87.3 %). The goal of the present study was (i) to specify the recurrence risk in children of these patients taking into account the results of mutation analysis and (ii) to determine the spectrum of phenotypic expression in children who have inherited a mutant RB1 allele. Thirty-six of 126 families with Rb recorded in our database have founders with unilateral Rb. DNA from blood was available from 23 of these patients. Molecular testing showed that 18 (78%) of these patients were heterozygous for the mutant allele and 5 (22%) patients showed allele imbalance indicative of mutational mosaicism. Applying Bayes theorem, recurrence risk estimates in children of patients with unilateral Rb can be obtained. Children of heterozygous patients have a recurrence risk of about 50%. Risk is lower (about 35%) in children of patients with mutational mosaicism. All children who inherited a mutation from a mosaic parent developed bilateral Rb. Recurrence risk in offspring of patients with no detectable mutation in peripheral blood is calculated to be below 1.1%. As one of five mosaic founders showed low levels of the mutant allele in peripheral blood, highly sensitive methods are needed for mutation detection.

P381

How many families suspected of having Lynch syndrome show colorectal cancer clustering by coincidence?

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The Amsterdam (A) and the less stringent Bethesda (B) criteria describe the familial clustering and early onset of colorectal and other cancers in patients suspected of having Lynch syndrome (LS), which is defined by carriership of a deleterious mismatch repair gene mutation. In the molecular diagnosis of LS these criteria are used to preselect patients for subsequent genetic testing. However, families may fulfill these criteria even if no hereditary background is present at all, just based on coincident clustering or early onset of sporadic tumors. We here present a model to quantify the probability of such coincidence based on information about pedigrees and cancer histories in over 3100 families enrolled in a large registry of the German HNPCC Consortium.

Applying Bayes rule in a general Mendelian modeling approach assuming monogenic autosomal dominant inheritance, the model calculates the posterior odds of having a hereditary disease background given a specific familial cancer history according to the unconditional (prior) odds and the likelihood ratio of observing the specific cancer history. The population prevalence of LS was taken from a literature review, serving as a lower estimate for the prior odds (de la Chapelle, 2005). The model utilizes age dependent gender specific penetrance functions for carriers and non-carriers derived from own data and the literature.

Model calculations with a prior odds of 1:1000 show that the median probability of coincidence is 0.10 for A and 0.94 for B families (0.01 and 0.61, respectively, with a prior odds of 1:100). For the subgroup of microsatellite stable patients, these probabilities are 0.61 for A and 0.95 for B families (0.13 and 0.68, respectively, with a prior odds of 1:100).

In conclusion, Bethesda families are more likely to be sampled by coincidence than by a hereditary background. This should have consequences for diagnostic strategies.

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A simple PCR based methylation test for GNAS1 / NESP55 imprinted loci improves PHP1b diagnostic testing

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Pseudohypoparathyroidism type Ib (PHPIb, OMIM #603233) is a rare disorder characterized clinically by parathyroid hormone (PTH)-resistant hypocalcemia and hyperphosphatemia, increased serum PTH, and a decreased response of urinary cAMP to exogenous administration of PTH. PHPIb is caused by mutations in regulatory regions of the GNAS1 (chr 20q13.2) gene in-

herited from the mother thus causing an imprinting defect of the Gs-alpha gene resulting in both alleles having a paternal epigenotype. PHPIb comprises at least 2 distinct conditions sharing the same clinical phenotype:
A) Loss of methylation only at exon A/B of the GNAS1 gene including no other epigenetic abnormalities. In most cases this is caused by a heterozygous microdeletion in the STX16 region, resulting in autosomal dominant PHP Ib (AD-PHP1b).
B) Methylation abnormalities at all GNAS DMRs (differentially methylated regions), including the DMR at exon A/B, caused by a deletion removing the NESP55-DMR and exons 3 and 4 of the antisense transcript. This has been found causative for most sporadic PHP1b and a few familial cases of AD-PHP1b. When inherited maternally, the deletion abolishes all maternal GNAS1 imprints and de-represses maternally silenced transcripts.
To elucidate aberrant methalytion patterns derived from these conditions, we have designed PCR testing of GNAS1- and NESP55-DMRs. Upon methylation sensitive restriction digestion either the methylated or unmethylated alleles of both DMRs are amplified. This allows proper diagnosis of PHP1b cases, by elucidation of abnormal metyhlation patterns within the amplified imprinted regions. Analyses of patients using this PCR test confirmed clinical PHP1b diagnosis. Thus this test enables proper diagnostic testing for both disease causing conditions, circumventing cumbersome testing via southern blotting.

P383

Public Health Genetics zwischen Wunsch und Wirklichkeit: Theoretische und ethische Anmerkungen aus dem NGFN

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Forschung im Bereich der Humangenetik und –genomik hat neue Erkenntnisse über die biologischen Grundlagen pathogenetischer Prozesse einschließlich der Wechselwirkungen des menschlichen Genoms mit Umwelt und Verhalten hervorgebracht. Obwohl mit dem Fortschreiten der Forschung die Einsicht in die Komplexität des Gegenstandes zunimmt und somit die genetischen Grundlagen von Gesundheit und Krankheit in vielerlei Hinsicht klärungsbedürftiger erscheinen, wird der genombasierten Identifikationen gesundheitsrelevanter Merkmale bereits jetzt Innovationspotential für den Bereich der Prävention durch so genannte Public Health Genetics zugeschrieben. Als relativ neues Problemfeld strebt Public Health Genetics die Integration molekulargenetischen Wissens in Strategien der populationsbasierten und individuellen Prävention an. Auf dem gegenwärtigen Stand molekulargenetischer Forschung drängen sich angesichts dieses Anspruches insbesondere wegen der relativen Reichweite genetischen Wissens und wegen der beschränkten Möglichkeiten der Identifikation von sicheren Prädiktoren für komplexe (Volks-)Krankheiten Fragen nach der medizinischen, sozialen, ökonomischen und ethischen Rechtfertigbarkeit von Public Health Genetics auf.
Der Beitrag analysiert wissenschaftliche Rahmenbedingungen und Grenzen von Public Health Genetics-Strategien. Dabei werden Anwendungsmöglichkeiten von prädiktiven und präventiven Interventionen zur Erhaltung und Wiederherstellung der individuellen und öffentlichen Gesundheit kritisch gewürdigt. Der Analyse liegt das Kriterium der (gesteigerten) sozialen Erreichbarkeit von Gesundheit als Maß für erfolgreiche Innovation im Gesundheitssektor einersesits und das Verfahren der „social accountability" biomedizinischer Innovation andererseits zugrunde.

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Phenotypes of complex combinations of globin gene mutations in hemoglobinopathy patients: implications for genetic counselling

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Introduction: Recent migrations of large populations result in an increased incidence of persons carrying several different α- and β-globin gene mutations. These genotypes are often difficult to predict from the haematological phenotype. In turn, it is not always possible to predict the phenotype to be expected from an unknown complex genotype. We describe four families with combinations of α- and β-globin gene mutations not described in the literature until now.
Methods: Hematologic analysis was performed with automated systems. The α-globin gene locus and the β-globin gene locus were analysed using polymerase chain reaction (PCR), oligonucleotide-strip hybridization, GAP-PCR, denaturing HPLC (DHPLC), and DNA sequencing. 7 amplicons covering the β-globin gene are amplified and analyzed by DHPLC and sequencing. 4 amplicons spanning the α2-globin gene were sequenced directly after amplification.
Results: Family 1: The woman of Italian origin is carrier of β-thalassemia minor, the husband is carrier of an α0-mutation. In addition, a point mutation in the α-globin locus of the woman was found, resulting in a 25% risk of this couple for children with HbH disease.
Family 2: A man of Turkish origin was upon haemoglobin HbA1c analysis found to be carrier of an anomalous Hb as well as of a β-thalassemia minor trait. Molecular analysis identified homozygosity for Hb G-Coushatta and heterozygosity for a β-thalassemia mutation.
Family 3: A family of Jamaican origin was identified to carry the sickle cell allele, a β-thalassemia mutation, and an α-globin allele. These mutations were combined in one of the daughters.
Family 4: The family in which Hb Altdorf originally was described in Aarau, was re-examined. The mutation was also identified in a woman of east-asian origin, indicating a multicentric origin of Hb Altdorf. In conclusion, molecular diagnosis must be used for risk ascertainment in populations with a high prevalence of both types of thalassemia.

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Two different gene mutations may cause inherited breast cancer in a German-Polish-family

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We report on three female cousins of wich two have inherited breast cancer. Two of the three cousins, a breast cancer affected and an unaffected woman, are sisters. All three women have a breast cancer affected mother and additionally a breast cancer affected maternal grand mother in common. The three young women are members of a large Polish-originating family. For the breast cancer affected woman living with her family in Germany we could identify a pathogenic BRCA1 mutation as well as a NBS1 mutation (657del5). For the other two women (sisters) living with her relatives in Poland the mentioned NBS1 mutation was identified in the still healthy sister whereas this mutation was already excluded in the breast cancer affected sister. Gene analyses to identify the familial known BRCA1 mutation in these two women are at the moment in Poland in progress. As we learned from literature the germline mutation 657del5 of the NBS1 gene contributes significantly to the incidence of breast cancer in Central Poland.
Conclusion: As a lot of Polish-originating families are living in Germany we recommend to perform also molecular genetic analyses in the NBS1 gene for at least an adequate index patient, especially if the BRCA1/BRCA2 mutation screening identifies no pathogenic mutation in the family.

Therapy for Genetic Diseases

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Impact of molecular cytogenetic analyses for the response to TMZ-chemotherapy of glioblastoma patients

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Glioblastomas are the most common malignant brain tumors. Surgical cure of these tumors is virtually impossible and their clinical course is mainly determined by the biological behavior of the tumor cells and their response to radiation and chemotherapy. Additionally, glioblastomas are tumors with substantial genetic changes and a remarkable large intratumoral and intertumoral genetic heterogeneity, which is histological only conditionally expressed, but obviously substan-

tial influence the therapy success. We investigated whether the different treatment success of glioblastoma patients with the commercial cytostatic drug Temozolomid (TMZ) differs in subsets of malignant glioblastomas defined by their genetic lesions. Eighty patients with newly diagnosed glioblastoma were analyzed with comparative genomic hybridization (CGH) and loss of heterozygosity (LOH). All patients underwent radical resection. Fifty patients received TMZ after radiotherapy (TMZ group), thirty patients radiotherapy (RT) alone.
The most common aberrations we detected were gains of parts of chromosome 7 and losses of 10q, 9p or 13q. The spectrum of genetic aberrations did not differ between TMZ and RT group. Patients treated with TMZ showed significantly better survival than patients with RT alone (19.5 vs. 9.3 months). Genomic deletions on chromosomes 9 and 10 are typical for glioblastoma and associated with poor prognosis. However, patients with these aberrations benefited significantly from TMZ in univariate analysis. In multivariate analysis, this effect was pronounced for 9p deletion and for elderly patients with 10q deletions, respectively.
This study demonstrates that molecular genetic and cytogenetic analyses potentially predict response to chemotherapy in patients with glioblastoma.

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Targeted correction of an Hprt point mutation in vitro by single stranded oligonucleotides

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Transfection of cells with specific single stranded oligonucleotides can induce the targeted exchange of one nucleotide in eukaryotic genes. We demonstrated the correction of a point mutation in the hypoxanthine-guanine-phosphoribosyl-transferase (Hprt) gene in V79-151 hamster cells. The correction rates are currently very low which may be due to inadequate oligonucleotide structures. Therefore we investigated the influence of different 3´- and 5´-modifications on the correction rate. Oligonucleotides modified with a TA-clamp on one side showed a higher correction rate than those with a GC-clamp. 5´-clamps induced higher correction rates than clamps on the 3´-end. These data indicate 5´-TA-clamps as the modification of choice.
It is not yet understood which cellular repair pathways play a role in this targeted nucleotide exchange. To investigate the involvement of DNA repair genes we established Hprt deficient mouse cell lines. Four different mouse embryo stem cell lines were differentiated into stable cell lines and one of them, MSK407, was used for further experiments. These cells have a T-G transition at Hprt cDNA position 407. The cells are tested for HAT sensitivity and transfection protocols were optimized. In a next step we will silence the repair gene Ercc1 by RNAi and investigate if this has an influence on the correction rate of the Hprt mutation. The Ercc1-Xpf complex is a structure specific nuclease that plays a central role in the nucleotide excision repair (NER). This may lead to new insights about the mechanism of the targeted nucleotide exchange.

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Pitfalls in the molecular genetic diagnosis of a TSC2-PKD1 contiguous gene syndrome in a patient with tuberous sclerosis under rapamycin therapy

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Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem disorder with characteristic tumour-like lesions called hamartomas. It results from mutations in one of two genes, TSC1 or TSC2. A subgroup of large deletions and rearrangements in TSC2 also affect the adjacent PKD1 gene causing the TSC2-PKD1 contiguous gene syndrome usually characterized by early-onset polycystic kidney disease.
We report a case with the definite clinical diagnosis of TSC based on major features including dermal, cerebral, renal and retinal lesions. Mutation screening of peripheral lymphocyte DNA by direct sequencing and subsequent MLPA analysis of both TSC genes identified a genomic deletion including TSC2 exons 36-41 and PKD1 exon 46. In order to define the deletion in an independent blood sample we performed long range PCR spanning the 3' parts of both genes and characterized a deletion including TSC2 exons 36-41 and PKD1 exons 30-46. It appeared as a somatic mosaic and indicated a TSC2-PKD1 contiguous gene syndrome not supposed clinically. The suspected mosaicism could be verified with a deletion specific TSC2-PKD1 FISH probe only in 10% of metaphases from a fresh blood sample.
TSC1 and TSC2 proteins hamartin and tuberin form heterodimers and act as tumour suppressors that inhibit the mammalian target of rapamycin (mTOR) cascade. Inactivation of either TSC1 or TSC2 in the complex activates the mTOR pathway. Rapamycin (sirolimus) is a immunosuppressant that inhibits mTOR and that is used in clinical trials for TSC associated renal angiomyolipomas and astrocytomas. During the term of the molecular genetic analyses, the patient has been treated with rapamycin. As rapamycin is known to inhibit cytokine stimulated T cell proliferation, we hypothesize that cells even harbouring the heterozygous TSC2-PKD1 deletion have been selectively repressed and therefore escaped detection. Attempts to verify the deletion in a therapy dependent manner in different tissues are currently ongoing.

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The natural compound atraric acid inhibits both the transactivation of human androgen receptor and prostate cancer cell growth

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Extracts from Pygeum africanum are used in the treatment of prostatitis, benign prostatic hyperplasia (BPH) and prostate cancer (PCa), a major health problem of men in Western countries. The ligand-activated human androgen receptor (AR) supports the growth of the prostate gland. Inhibition of human AR by androgen ablation therapy and by applying synthetic antiandrogens is therefore the primary goal in treatment of patients.
Bioactivity-directed fractionation of a selective methylene chloride extract from the stem bark of Pygeum africanum led to the isolation of the antiandrogenic compound atraric acid (AA). AA inhibits the ligand-activated human AR responsive reporter gene assays and its antihormonal activity is receptor specific. Importantly, AA is able to efficiently repress the growth of LNCaP and C4-2 prostate cancer cells but not of PC3, lacking endogenous expressed AR, or of CV1 cells. Mechanistically, AA competes with androgens for the binding to human AR and inhibits nuclear transport of AR suggesting a novel molecular mechanism for androgen antagonism.
Thus, AA as a natural substance and its derivatives may serve as novel therapeutic compound for prophylaxis and treatment of prostatic diseases.

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The simultaneous targeted exchange of two nucleotides by single stranded oligonucleotides is restricted to a distance of about 10 nucleotides

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Transfection of cells with single stranded oligonucleotides can promote targeted nucleotide exchange of one or two nucleotides in a gene. To investigate the DNA repair mechanisms involved we examined the maximal distance for the simultaneous exchange of two nucleotides by single stranded oligonucleotides. The experimental system was the correction of a hprt point mutation in a hamster cell line and the generation of an additional nucleotide exchange in variable distance to the first exchange position. The detected simultaneous nucleotide exchanges were clustered in a region of about 10 nucleotides upstream and downstream to the first exchange position. The maximal distance was independent of the position of the first exchange, and the modifications of the oligonucleotides. We suggest that the first repair step of targeted nucleotide exchange is related to a sequence of at least 10 nucleotides of the single stranded oligonucleotide used for the physical incorporation or as a matrix for a repair process.

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Targeting single base pair alterations of cancer cell mtDNA in vitro using peptide nucleic acids (PNA)

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Single base pair alterations in the mitochondrial genome (mtDNA) are frequently found in mitochondria of cancer cells and cells of aged tissue in human. We are interested in developing a method for targeting a specific mtDNA sequence in vitro by oligomers consisting of peptide nucleic acids (PNA). PNAs are synthetic nucleic acid mimics based on repeating N-(2-aminoethyl-glycin)-units in the backbone linked with purine or pyrimidine bases by a methyl-carbonyl-group. PNAs are uncharged, show higher binding affinity to a DNA target sequence than DNA oligonucleotides do and are able to distinguish a target differing by a single nucleotide. In addition they are able to invade a DNA-double strand. We investigated the binding of PNAs to mtDNA, using two mtDNA PCR products with a length of 420 bp who differ by a single base pair. In addition, we studied the specific binding of the PNAs to mtDNA in isolated, highly purified mitochondria as well as in live cancer and control cells. We aim at influencing the behavior of cancer cells by targeting a mtDNA sequence found exclusively in the cancer cells but not in healthy somatic cells.

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