VORTRÄGE

KEYNOTE LECTURE

The Genome of the Black Death

Johannes Krause[*]

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Genome wide data from ancient microbes may help to understand mechanisms of pathogen evolution and adaptation for emerging and re-emerging disease. Using high throughput DNA sequencing in combination with targeted DNA enrichment we have reconstructed the ancient genome of *Yersinia pestis* from skeletons securely dated to the Black Death pandemic from the East Smithfield cemetery in London, England, 1348 – 1350. Phylogenetic analysis indicate that the ancient organism is ancestral to most extant *Y. pestis* strains and falls very close to the ancestral node of human infectious *Y. pestis* that had their genome sequenced. Temporal estimates suggest that the Black Death of 1346 – 1351 was the main historical event responsible for the introduction and worldwide dissemination of currently circulating *Y. pestis* strains pathogenic to humans, and further indicates that contemporary *Y. pestis* epidemics have their origins in the medieval era. Comparisons against modern genomes reveal no unique derived positions in the medieval organism, suggesting that the perceived increased virulence of the disease during the Black Death may not have been due to bacterial physiology. These findings support the notion that factors other than microbial genetics, such as environment, vector dynamics, and host susceptibility should be at the forefront of discussions regarding emerging *Y. pestis* infections.

SYMPOSIA

S1-02

Somatic Mosaicism in Normal and Diseased Human Tissues Via L1 Retrotransposition

Geoffrey J. Faulkner
Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh

This talk will present data tracing somatic retrotransposition in the human body. In particular I will describe an updated retrotransposon capture sequencing (RC-seq) protocol used to map L1, Alu and SVA insertions in the brain (Baillie et al., Nature, 2011) and in non-neural tissues. I will also present RC-seq results from hepatocellular carcinoma, where L1 and Alu insertions are found in tumour suppressor genes expressed in liver. Finally, I will show that de novo L1 insertions occur in human induced pluripotent stem cells (hiPSCs) and contribute to genomic instability. These experiments elucidate new spatiotemporal contexts for somatic retrotransposition, and likely point to both positive and negative phenotypic outcomes for the phenomenon.

S1-03

Revertant Somatic Mosaicism in Dyskeratosis Congenita

Roland P. Kuiper

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One of the hallmarks of cancer is loss of tumor suppressor activity, which requires the inactivation of both copies of a tumor suppressor gene. Individuals that carry a germline mutation in such a tumor suppressor gene have increased risk to develop cancer, since only a single somatic hit in the remaining allele is required to induce tumor formation. When the germline mutation creates a growth disadvantage in proliferative tissues, like for example blood or skin, the opposite can occur: cells in which the pathogenic allele is replaced by wild-type will favor the survival of a cell. This phenomenon is referred to as revertant mosacism. I will present an example of this phenomenon in Dyskeratosis Congenita (DC), a multisystem disorder that is characterized by many clinical features including mucocutaneous abnormalities, dystrophic nails, bone marrow failure, leucoplakia, and increased risk for malignancies. Mosaic reversion of pathogenic *TERC* mutations to wild-type are recurrently encountered in blood of patients that lack a typical bone marrow

phenotype. This finding has important implications for improving diagnostic testing and understanding the variable phenotype of DC.

S2-01

BRAF-MEK signalling in Development, Melanoma and Behaviour in Zebrafish

E. Elizabeth Patton

Institute of Genetics and Molecular Medicine, MRC Human Genetics Unit & Edinburgh Cancer Research Centre, Edinburgh, UK

BRAF is the most frequently mutated gene in melanoma, and germline mutations cause cardio-facio-cutaneous (CFC) syndrome. We are using the zebrafish system to study BRAF-MEK mutations in the context of CFC syndrome in development, and in melanoma development in the adult. We have previously shown that human CFC mutations in BRAF and MEK cause cell movement defects during development. In addition, some CFC syndrome mutations expressed in zebrafish melanocytes lead to nevi, a feature similar to some CFC syndrome patients. Critically, we find that treatment with MEK inhibitors within an early time window can prevent these phenotypes and permit normal development, or that continual low treatment doses of MEK inhibitor can permit normal development in CFC expressing embryos, without additional toxicities later in development. Increased anxiety has also been reported in some patients with CFC syndrome. We have developed a model for anxiety in zebrafish larvae, and find that increased levels of MAPK signalling caused by the PDE4 inhibitor Rolipram lead to specific swimming behaviour. Importantly, we find that MEK inhibition reduces anxiety-like behaviour in this model. We suggest that low-levels of MEK inhibition in development, rather than a more potent MEK inhibition in the context of cancer, may help to manage or even prevent some of the clinical features of CFC syndrome.

S2-03

From High-Throughput Screens to Biomedical Knowledge

Frank Buchholz

UCC, University Hospital and Medical Faculty Carl Gustav Carus, University of Technology Dresden

Cancer research has, so far, primarily taken the form of intensive investigation of individual genes and a small number of interactions between genes. However, the identification of a large number of oncogenes and tumor suppressor genes in recent years has illustrated that cancer is much more a problem of the cell system than a problem with a single gene. Hence, it is becoming clear now that cancer can only be fully understood, and therefore combated, when the process of cellular transformation is understood at the systems level. To become cancerous, a cell has to change in many ways, overcoming numerous safeguards that normally keep renegade cells in check. For this reason, a comprehensive understanding of cellular transformation is required to find the best ways to treat this disease.

We are using endoribonuclease prepared (esi)RNA-mediated RNAi screens and large-scale protein-tagging (TransgeneOmics) to investigate stem cell and cancer relevant processes to obtain a more comprehensive picture of cellular transformation. We have recently extended our genome-wide esiRNA libraries to also include silencing triggers against long non-coding RNAs, a class of molecules that have recently been implicated in diverse biological pathways**.** Examples of our work to interrogate tumor-relevant processes in stem cells and cancer cells will be presented.

S3-01

Cornelia de Lange Syndrome and Other Disorders of Cohesin

Deardorff, M.

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Cornelia de Lange syndrome (CdLS; or Brachmann-de Lange Syndrome) is a dominantly inherited multisystem developmental disorder with distinctive facial features, growth retardation, a range of limb defects and variable cognitive delay. Over the past 8 years, causative mutations have been identified for many individuals with CdLS.

Mutations in the NIPBL gene have been identified in approximately 75% of patients with severe classical CdLS, but have been found in a far lower percentage of patients with mild or atypical features. NIPBL is a key regulator for loading Cohesin, a multisubunit protein comprised of SMC1, SMC3, RAD21 and additional regulatory proteins originally identified to maintain sister chromatid cohesion following DNA replication.

Some of the patients with mild or atypical CdLS have had mutations identified in the SMC1A or SMC3 genes. These individuals typically present with milder structural anomalies, more fullness of the eyebrows, a more prominent nose and significant cognitive impairment when compared with classical CdLS.

More recently, three de novo mutations in the RAD21 gene were indentified in individuals with a less striking CdLS clinical phenotype, most with very mild cognitive impairment. Even within this small group, there is heterogeneity of clinical features, which may be due to the nature of the RAD21 mutations. The identification of additional individuals will be essential to further delineate this subgroup.

Work in both yeast and vertebrate cell models has shown that SMC3 is acetylated to facilitate its cohesiveness to binding sister chromatids. Mutations in ESCO2, one of the vertebrate SMC3 acetyltransferases, cause a sister chromatid cohesin defect and result in Roberts Syndrome and SC Phocomelia, which present with clinical features distinct from CdLS, but affect similar organ systems.

HDAC8 serves to deacetylate Cohesin and allow it to be recycled for subsequent cell cycles. Mutations in HDAC8 can cause clinical features quite similar to classical CdLS, caused by NIPBL mutations. However, there is some degree of difference in severity within families as well as between males and females. The majority of this variability is likely due to the degree to which the mutant allele is expressed for this X-linked gene.

I will review the underlying causes and clinical features of these molecular subgroups and propose reasons why mutations in these different genes may result in varying clinical features.

S3-03

Cohesin and Germ Cell Aneuploidy

R. Jessberger, C. Blei, U. Biswas, C. Adelfalk, E. Revenkova, F. McNicoll*

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Cohesin complexes are essential for sister chromatid cohesion and play pivotal roles in regulation of gene expression, and in DNA repair. The canonical cohesin complex consists of SMC1, SMC3, and a kleisin, which form a tripartite ring. Additional proteins such as SA, WAPL, PDS5B, Sororin or an acetyl transferase (ECO1/2) interact with the complex.

Mammalian meiocytes form several different, spatiotemporally distinct cohesin complexes. Spermatocytes and oocytes cells express four meiosis-specific cohesin subunits, SMC1β, REC8, RAD21L and SA3, in addition to SMC1α, SMC3, RAD21, SA1 or SA2. The individual roles of the different meiotic cohesin complexes formed by these components are largely unknown and subject of current studies.

The only meiosis-specific SMC protein known is SMC1β. Mouse meiocytes deficient in SMC1β are impaired in sister chromatid cohesion (SCC), feature shortened synaptonemal complexes, extended chromatin loops, and defective telomeres. These mice are sterile, and in spermatocytes a specific checkpoint linked to a failure in synapsis of meiotic chromosomes in prophase I is triggered. SMC1β deficiency in oocytes causes loss of chiasmata and sister chromatid cohesion with increasing age and thus leads to chromosome mis-segregation. The Smc1β-/- mouse is now considered a key mouse model for age-dependent aneuploidy. On these grounds, the "cohesin deterioration hypothesis", suggesting cohesin decay as a leading cause for aneuploidies, was proposed, and is now supported by several, independent lines of evidence from different laboratories. These data and the deterioration hypothesis will be discussed.

S4-02

Modeling Neurodevelopmental Disorders using induced Pluripotent Stem Cells

Cassiano Carromeu

Sanford Consortium of Regenerative Medicine (SCRM)/University of California – San Diego (UCSD)

The human brain is an intricate circuit of specialized neural cells. A disturbance in the homeostasis of this circuit can lead to various neuronal pathologies, including neurodevelopmental disorders. In such cases, the unavailability of affected human neurons for research has hampered the elucidation of disorder etiologies. Induced Pluripotent Stem Cells (iPSC) represent a rapidly evolving technology with great potential in areas ranging from basic research to drug discovery. When applied to neurodevelopmental disorders, the iPSC technology allows for the derivation of patient-specific neurons. Here, we use iPSCs to model Rett Syndrome (RTT), a neurodevelopmental condition under the umbrella of the Autism Spectrum Disorders. The comparison of human RTT and non-affected neurons has generated insights into the molecular and cellular mechanism of the disease. Moreover, we used new techniques to measure neuronal network activity created by iPSC-derived neurons in a dish. Preliminary data points to a connectivity deficit and excitatory/inhibitory neurons imbalance in RTT neural networks, compared to controls. The study of RTT and

other neurodevelopmental disorders using iPSC-derived neurons represents a major step toward the understanding of human brain function.

S4-03

Intellectual Disability: Identification of gene mutations and commonly disrupted molecular networks

Hans van Bokhoven

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Intellectual disabilities (ID) comprise a highly diverse group of cognitive disorders. Gene defects account for about half of all patients and mutations causative for impaired cognition have been identified in more than 400 genes. While there are numerous genetic defects underlying ID, a more limited number of pathways is emerging whose disruption appears to be shared by groups of ID genes. One of these common pathways is composed of ID genes that encode regulators of chromatin structure and of chromatin-mediated transcription regulation. Already more than 20 “epigenetic ID genes” have been identified and this number is likely to increase in the coming years. A prominent example is the *EHMT1* gene, encoding euchromatin histone methyltransferase 1, which carries heterozygous mutations in 25% of patients with a recognizeable ID disorder, denoted Kleefstra syndrome (KS). We hypothesized that the remaining ‘‘EHMT1-negative’’ KS individuals have mutations in genes that share a biological function with EHMT1. Indeed, next generation sequencing in a cohort of such patients identified de novo mutations in four genes, MBD5, MLL3, SMARCB1, and NR1I3, all of which encode epigenetic regulators. Using *Drosophila*, we demonstrate that MBD5, MLL3, and NR1I3 cooperate with EHMT1, whereas SMARCB1 is known to directly interact with MLL3. Our results reveal a chromatin-modification module that underlies a recognizable form of ID. We propose a highly conserved epigenetic network that underlies cognition. Our current research aims to gain more insight into patterns of altered chromatin modifications that are caused by epigenetic gene mutations, how these will disrupt the brain-specific expression of target genes, and their effects on synaptic plasticity. In-depth knowledge about this network should allow the design of rational strategies to treat the growing group of ID pathologies that are caused by epigenetic defects.

S5-01

The genetics of intelligence

Lars Penke

University of Edinburgh, UK

Quantitative behavioural genetic studies show unanimously that general intelligence is one of the most heritable human traits, especially in middle and old adulthood. However, despite intensive research efforts the molecular genetic foundations of intelligence differences remain unclear. I will provide an overview of current molecular genetic results on human intelligence and its changes across the lifespan. In particular I will present our own studies from the “Disconnected Mind Project”, based on the Lothian Birth Cohorts of 1921 and 1936, population samples of Scots in their seventies and eighties for whom IQ scores from age 11 years are known and who are thus exceptionally well suited for the study of intelligence differences across the life span. Genome-wide analyse indicate no indicate common variants with substantial effects associated with intelligence, but a clear association between APOE and lifetime cognitive change, in both cases confirming candidate gene studies. Genome-wide complex trait analyses, on the other hand, are able to recover much of the heritability of intelligence and lifetime change. I will discuss this pattern from an evolutionary genetic perspective. Furthermore, I will present novel results on the neurostructural foundations of general intelligence. In particular, three independent biomarkers of the integrity of neuronal tracts in the brain explain 10% of the variance in intelligence, more than the best currently known neurostructural correlates in meta-analyses. These associations are completely mediated by cognitive information processing speed and provide evidence for a procedurally plausible neurostructural model of intelligence. First results on the genome-wide molecular genetic foundations of this model will be presented.

S5-02

Genetic Architecture of Multifactorial Disorders

Naomi R Wray

The University of Queensland, Queensland Brain Institute, Brisbane, Australia

By definition multifactorial disorders are caused by a combination of multiple genes and environmental effects. Traditionally the genetics of these disorders has been studied using concepts that refer to the combined effect of all genes (e.g., heritability), using the resemblance (or recurrence risk) between relatives. Genome-wide association studies (GWAS) facilitate the dissection of heritability into individual locus effects. They have been successful in finding many associated SNPs and have greatly increased our estimates of the number of genes involved in complex trait variation. To date, for many disorders, tens to hundreds of loci have been identified that explain in total up to 20% of narrow sense heritability. However, the proportion of additive genetic variation explained by all common SNPs together (not just the significant ones) is one-third to one-half for a range of quantitative traits and diseases. The variation is spread over all chromosomes in proportion to their length, implying that there are many more variants with effects sizes too small to be detected with sample sizes employed to date. Empirical observations are all converging to a highly polygenic model of multifactorial disorders, with a surprisingly large proportion of additive genetic variation due to variants that are in linkage disequilibrium with common SNPs. Results from empirical data across a range of disorders will be used to illustrate the emerging joint distribution of effect sizes and allele frequency in the population. Understanding the genetic architecture of multifactorial disorders is a first step in understanding their biological causes.

S6-01

Genome variation affecting cancer predisposition and response to therapy

Thomas J. Hudson

Ontario Institute for Cancer Research

Genomic variation, through its effect on gene structure and expression, plays an important role in disease predisposition, biology, and clinical response to therapy. Cancer mutations can be classified as germline (inherited) and somatic (tumoral). I will provide examples of ongoing projects that emerged from large-scale genome studies of cancer patients that are pertinent to both classes of cancer mutations.

Genome-wide association studies (GWAS) have led to the identification of many genetic loci that contribute to an increased predisposition to cancer. For example, to date more than 22 loci have been associated with colon cancer. Risk prediction models using combinations of genetic markers, age and family history are being developed as public health tools to stratify the population into colorectal cancer risk categories, thereby informing targeted prevention and surveillance.

In a study of a chromosome 11q23 locus that is genetically associated with colorectal cancer risk, we investigated two adjacent transcripts named COLCA1 and COLCA2 and show that inheritance of one or two protection-associated alleles correlates with increased expression of both proteins as well as lymphocyte infiltration at the periphery of neoplastic tissue, a histological feature that is associated with increased survival. Our findings provide evidence for a genetically regulated host immune and tumor response involving multiple cells that reside in the microenvironment of colon cancer.

Personalized cancer medicine is based on a rapidly emerging knowledge of the cancer mutation repertoire through comprehensive studies such as the International Cancer Genome Consortium and the increased availability of anti-cancer agents that target altered genes or pathways. In the second part of my presentation, I will present concepts and experiences gained from a pilot study involving patients with advanced metastatic cancers from five cancer centers in Ontario who are potential candidates for early phase clinical trials of targeted agents. The study includes rapid mutation detection using next-generation sequencing technologies in a set of genes deemed to be actionable, validation in a clinical molecular diagnostics laboratory, and reporting of actionable mutations to clinicians and patients.

S6-02

Brain tumor genomics – how do our patients benefit from the new knowledge?

Stefan M. Pfister[1,2], David TW Jones[2], Marcel Kool[2], Paul A Northcott[2], Natalie Jäger[2], Roland Eils[2], Peter Lichter[2]

[1]German Cancer Research Center & University Hospital Heidelberg

[2]German Cancer Research Center (DKFZ)

Novel developments in the 'omics'-technologies such as microarray-based profiling and next-generation sequencing approaches have enabled a thorough biological characterization of several clinically challenging childhood brain tumor entities including medulloblastoma, ependymoma, glioblastoma, and pilocytic astrocytoma. A wealth of novel predisposing conditions, somatic driver mutations, differentially regulated genes, and mechanisms of regulation has been identified in these tumors, but, most importantly, many of these characteristic aberrations seem to be restricted to biologically meaningful subgroups of each of these diseases that cannot be distinguished under the microscope. Integration of this molecular data with clinical variables now enables biologists, bioinformaticists, physician scientists, and clinicians together to implement this new knowledge into clinical trial settings, and aids to recruit patients for personalized therapy concepts.

Some examples will be presented in which molecular findings have already been or will shortly be incorporated in clinical decision making, e.g., de-escalation of therapy intensity for WNT-driven medulloblastoma, recruitment of SHH-driven medulloblastoma patients to SHH-inhibitor studies, assessment of MAPK hits in pilocytic astrocytoma to qualify for MAPK inhibitor studies, or a separate study design for medulloblastoma patients with underlying Li-Fraumeni Syndrome.

This is certainly just the beginning of a new era of personalized pediatric neurooncology, but these examples may demonstrate the usefulness of genomics analyses to improve therapeutic concepts and ultimately patient outcomes for these clinically challenging diagnoses.

S6-03

Epigenetic Tumor Characterisation and its Translation into Clinical Use

Henk Stunnenberg

Institute NCMLS/FNWI, Medical Centre, Radboud University Nijmegen, NL

Deciphering the human genome sequence has provided critical insight in genome function in relation to biological processes in health and diseases. Recent technological improvements have opened up the analysis of the epigenetic regulation of the information embedded in the genome. Epigenetic regulation takes place at many levels including histone modifications, positioning of histone variants, nucleosome remodeling and DNA accessibility. Together with DNA modifications, transcription factors and other DNA-binding proteins this information provides an epigenetic blueprint. The epigenetic features of each cell type in the body (>250) are distinct and once established during development and differentiation need to be maintained. Hence, the study of epigenetic processes go beyond DNA-stored information and provide essential insight in the manual of the genome, in deciphering derailed processes in disease.

Talk nach 12

Current status of the clinical/medical genetics in Europe

Milan Macek Jr.

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The European Society of Human Genetics (www.eshg.org; ESHG) is a non-profit, non-governmental organization which has two main aims: a/ to promote research in basic and applied human and medical genetics and to b/ to ensure high professional standards in diagnostic and clinical practice. ESHG also facilitates contacts between scientists and professionals who share these aims, in particular those working and/or residing in Europe. In this respect ESHG works closely with European National Human Genetics Societies (www.eshg.org/nhgs.0.html?&L=0). One the recent examples of successful collaboration have been joint initiatives leading to the official recognition of clinical-/medical genetics by the European Union (EEA) via inclusion of this specialty into Directive 2005/36 (www.eshg.org/index.php?id=111) or national endorsements of the ESHG official response to European Commission Public Consultation on the amendment of the "IVD" Directive 98/79/EC (ec.europa.eu/health/medical-devices/documents/revision/

index_en.htm), including the development of the consensus curriculum for the laboratory specialty in genetics (www.eshg.org/224.0.html?&L=0). Current status of the clinical specialty will be presented, including a pilot project on staffing at selected national levels, including cross-country comparisons.

Talk nach 12

The Clinical Laboratory Geneticists Section of the European Board of Medical Genetics (EBMG)

Thomas Liehr

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There is an increasing need for recognition of also a laboratory specialty in clinical genetics, in line with last year's recognition of clinical/medical genetics as an EU recognized specialty under the UEMS umbrella. As some of you may recall, the process of achieving such a clinical specialty was long and cumbersome, mainly because approval had to be given by an EU recognition committee that required almost unanimous agreement between all 27 EU countries. To ease the bureaucracy in such certification processes, the EU has proposed to establish a professional qualifications directive scheme. Hopefully this proposal, where also ESHG has given substantial input to the process, will be passed within the next 2 years. In order to have a laboratory genetics curriculum ready if such a proposal is passed, an ESHG ad hoc committee has been quite active the last year.

A proposal for core curriculum has now been finalized, called Clinical Laboratory Genetics as an EU-recognized specialist profession, and it can be found on our web-page under *About ESHG/Committees*. The curriculum has been approved by the ESHG Executive Committee and Board. The curriculum has received support from 14 country representatives, representing 204/345 (59%) of the EU weighted (qualified majority) votes.

At present we align the requirements of the Clinical Laboratory Genetics as an EU-recognized specialist profession (https://www.eshg.org/fileadmin/www.eshg.org/documents/committees/LabGenetics/ESHG_Clin_lab_gen_spes_final_EU.pdf) with those of the national guidelines of education. We got already answers from 33 representatives of the 37 national contacts. More information about this process and other developments are summarized.

Talk nach 12

Development of the genetic counselling profession in Europe

Heather Skirton

Plymouth University, United Kingdom

Genetic counsellors have an increasing role to play in the provision of genetic services in Europe. In this presentation, I will make a distinction between genetic counselling as an activity and genetic counselling as a profession. Non-medical genetic counsellors were first employed in the US, but have been working in the UK and the Netherlands for three decades. A recent survey (Cordier et al, 2011) indicated that at present there are approximately 300 genetic counsellors or genetic nurses working in 18 countries in Europe. The majority of genetic counsellors undertake a wide range of tasks in relation to patient care, these include taking family history, discussing genetic testing and communicating test result to patients. Importantly, they also provide psychological support for patients before and after genetic testing (Skirton et al, in press [a]).

The Ad Hoc Genetic Nurse and Counsellor Committee of the European Society of Human Genetics has worked to produce a Code of Ethics and agreed standards of practice and education for these professionals (Skirton et al, 2010). Currently, the European Board of Medical Genetics is working to develop a registration process. A Delphi study was used to produce an agreed Master's programme curriculum against which every Master's degree in Genetic Counselling programme in Europe has been mapped (Skirton et al, in press [b]). We are currently finalising the arrangements for genetic counsellors to apply for European registration. This will entail demonstrating the necessary competences through completion of an examination and production of a portfolio of evidence including a 50 case log, manager references and case studies. A rigorous process of assessment is essential to ensure patient safety.

Genetic counsellors are trained to focus not only on the scientific and informational aspects of a consultation, but to use counselling skills to enable the patient to place the information into the context of their own lives and to consider the psychological, emotional and social aspects of any decision they may take. With the increasing pressure on genetic services, the inclusion of registered genetic counsellors in the specialist team can enhance the patient experience.

References:

Cordier C, Lambert D, Voelckel MA, Hosterey-Ugander U, Skirton H. (2012) A profile of the genetic counsellor and genetic nurse profession in Europe. Journal of Community Genetics. Published online 14 December 2011. DOI 10.1007/s12687-011-0073-x.

Skirton H, Voelckel MA, Patch C. (2010) Using a community of practice to develop standards of practice and education for genetic counsellors in Europe. Journal of Community Genetics 1: 169-173.

Skirton H, O'Connor A, Cordier C, Hosterey-Ugander U, Lambert D, Voelckel MA. (in press [a]) A study of the practice of individual genetic counsellors and genetic nurses in Europe. Accepted for publication in Journal of Community Genetics.

Skirton H, Barnoy S, van Kessel I, Patch C, O'Connor A, Serra C, Stayner B, Voelckel MA. (in press [b]) A Delphi study to determine the European core curriculum for Master programmes in genetic counselling. Accepted for publication in European Journal of Human Genetics.

SEL SELECTED PRESENTATIONS

SEL-01

A misplaced lncRNA in Mendelian disease

Maass PG.[1,2], Rump A.[3], Schulz H.[2], Stricker S.[4], Schulze L.[1,2], Platzer K.[3], Aydin A.[1,2], Tinschert S.[3], Goldring MB.[5], Luft FC.[1,2], Bähring S.[1]

[1]Experimental and Clinical Research Center ECRC - a joint cooperation between the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine MDC, Berlin, Germany; [2]MDC, Berlin, Germany; [3]Institute of Clinical Genetics - Faculty of Medicine Carl Gustav Carus, Technical University, Dresden, Germany; [4]Development and Disease Group, Max-Planck Institute for Molecular Genetics, Berlin, Germany; [5]Hospital for Special Surgery - Laboratory for Cartilage Biology, Weill Cornell Medical College, New York, USA

We investigated two different families with an isolated form of autosomal-dominant inherited Brachydactyly Type E (BDE). We detected the balanced translocations t(4;12)(q13.2-13.2;p11.2) and t(8;12)(q13.3;p11.2) and characterized the chromosomal breakpoints. On chromosome 12p, the breakpoints were 86 kb and 130 kb downstream of the chondrogenesis gene PTHLH (parathyroid hormone like hormone). PTHLH is an important morphogene, acting in a well defined balance with IHH (indian hedgehog) to maintain the pool of proliferating chondrocytes. In chondrogenically induced BDE-patients fiboblasts, we found a chondrogenic tissue-specific PTHLH downregulation, correlating with the BDE.

Our major hypothesis was that the chromosomal translocations disrupted cis-regulatory elements controlling PTHLH. We established a chromosomal conformation capture technique with ChIP and chromatin circularization to identify the yet unknown native PTHLH regulatory elements in the human immortalized chondrocytes C28/I2. An experimental control was the major transcription factor involved in chondrogenesis, SOX9, on chromosome 17q. We identified several cis-regulators within the circular chromatin. Interestingly, we found that SOX9 was in interaction with the very same regulator on chromosome 12q that was found to be interacting with PTHLH. We named the regulator CISTR-ACT because of its in cis and in trans actions. In colocalization FISH experiments, we verified the interactions.

We also detected a long non-coding RNA (lncRNA) within the CISTR-ACT's DNA sequence. We hypothesized that this lncRNA can regulate PTHLH or SOX9. Using siRNA approaches, we observed an expression-dependent network. The lncRNA co-regulated the coding genes PTHLH and SOX9 and vice versa. Moreover, expression profiling experiments detected more mesenchymal, pre-chondrogenesis and chromatin-remodeling genes that might be lncRNA-dependent regulated. In the BDE-patients, the lncRNA was upregulated, due to the disrupted chromosome 12 architecture. In ChIRPs (chromatin isolation by RNA purification), the lncRNA was bound at the PTHLH and SOX9 loci. To verify that the disrupted chromosome 12 led to dysregulation of PTHLH and the lncRNA, we used BDE-patient-derived chromatin and performed ChIRP. The lncRNA enrichment was reduced at the PTHLH locus.

Finally, transgenic mice carrying a CISTR-ACT-lacZ reporter, determined CISTR-ACT as classical enhancer. We observed tissue-specific lacZ-staining of the fore and hind limb buds in different developmental stages. The patterning correlated with the most apparent BDE-regions.

SEL-02

High-resolution molecular characterization of circulating cell-free DNA and circulating tumor cells of patients with metastatic breast cancer

Auer M.[1], Ulz P.[1], Pristauz G.[2], Petru E.[2], Jahn S.[3], Gasch C.[4], Riethdorf S.[4], Pantel K.[4], Geigl JB.[1], Speicher MR.[1], Heitzer E.[1]

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With the increasing number of available predictive biomarkers, clinical management of cancer is becoming more and more reliant on the accurate serial monitoring of tumor genotypes. Recent advances in the understanding of the molecular mechanisms of cancer highlight the need for personalized medicine for both diagnostic purposes and the prediction of prognosis. Serial monitoring of biomarkers may explain why some patients initially respond well to therapy, but ultimately relapse due to treatment resistance. The evaluation of patient blood samples for mutant DNA molecules is an attractive approach since blood samples

are is easily accessible. Over the past years, the identification of somatic mutations and/or genetic polymorphisms has facilitated the detection of disease. On this basis, patients could be stratified according to treatment response.

In the present study, the identification of biomarkers from circulating cell-free (cf) DNA and circulating tumor cells (CTC) of patients with metastatic breast cancer was performed. We addressed whether complex tumor genomes may be identified non-invasively from the peripheral blood samples of cancer patients. 64 patients with metastatic breast cancer, 10 patients in complete remission and 8 healthy controls were enrolled in the evaluation. Cf-DNA concentration was determined and followed by qualitative and quantitative analysis for subsequent high-resolution whole-genome characterization using array-CGH. Regarding CTCs an enumeration with the CellSearch system® (Veridex) and, in some cases, isolation with micromanipulation and further high-resolution characterization with array-CGH was done. A subset of metastatic patients had a biphasic size distribution of cf-DNA fragments which was associated with an increase of CTCs, elevated concentrations of cf-DNA with a high percentage of mutated cf-DNA fragments. With our strategy, we identified in the plasma DNA copy number changes associated with breast cancer, such as ERBB2 amplification. Furthermore, we identified changes, which were not observed in the respective primary tumor and which may reflect tumor evolution. Hence, the techniques used in this study allow serial monitoring of tumor genomes with a simple blood test. The results yield novel insights potentially answering basic research questions, such as processes underlying metastasis and tumor evolution. Furthermore, investigations on the biomarker status may lead to individualization of cancer therapies.

SEL-03

Plastin 3 ameliorates spinal muscular atrophy via delayed axon pruning and improves neuromuscular junction functionality

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F-actin bundling plastin 3 (PLS3) is a fully protective modifier of the neuromuscular disease spinal muscular atrophy (SMA), the most common genetic cause of infant death. The generation of a conditional PLS3 over-expressing mouse and its breeding into an SMA background allowed us to decipher the exact biological mechanism underlying PLS3-mediated SMA protection. We show that PLS3 is a key regulator that restores main processes depending on actin dynamics in SMA motor neurons (MN). MN soma size significantly increased and a higher number of afferent proprioceptive inputs were counted in SMA-PLS3 compared to SMA mice. PLS3 increased presynaptic F-actin amount, rescued synaptic vesicle and active zones content, restored the organization of readily releasable pool vesicles and increased quantal content at the neuromuscular junctions (NMJs). Most remarkably, stabilized axons by PLS3 over-expression delayed axon pruning, counteracting poor axonal connectivity at SMA NMJs. These findings together with the observation of increased endplate and muscle fiber size upon MN-specific PLS3 over-expression suggest that PLS3 significantly improves neurotransmission. Indeed, ubiquitous over-expression improves survival and motor function in SMA mice. As PLS3 seems to act independently of Smn, PLS3 might be a potential therapeutic target not only in SMA but also other MN diseases.

SEL-04

HDAC8 mutations cause a clinically distinct subgroup of Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a rare multisystem genetic disorder characterized by characteristic facies, prenatal onset growth failure, intellectual disability, distal limb anomalies, gastrointestinal and neurological problems. The CdLS phenotype shows a broad range of variability. More than half of the patients show mutations in NIPBL, which encodes a cohesin associated factor. In about 5% of the patients mutations in SMC1A or SMC3, both encoding structural components of the core cohesin

complex, can be detected. Although no clear genotype-phenotype correlation exists, mutations in NIPBL are observed in the majority of the “classical” or severely affected patients. However atypical or probable CdLS cases have a much lower mutation detection rate as shown for mutations in the RAD21 gene recently. Here we report the identification of mutations in the X-linked gene HDAC8 in about 5% of our patient cohort as the cause of a recognizable subgroup of CdLS and CdLS-like phenotype. Most of these identified mutations are missense and de novo. About two-third of the affected cases are heterozygous females and marked skewing of X-inactivation in peripheral blood DNA appears to be common. All hemizygous males identified are more severely affected than the females. The craniofacial appearance of HDAC8 cases has CdLS characteristic but is distinct from classical CdLS, delayed anterior fontanelle closure may be a useful discriminating feature. Limb malformations appear to be rare however postnatal growth failure is less severe than classical CdLS. HDAC8 encodes a lysine deacetylase, in silico and in vitro analysis of the functional consequences of the missense mutations suggest partial or complete loss of enzymatic function. These data suggest that mutations in HDAC8 cause a clinically distinct subgroup of CdLS and that HDAC8 lysine deacetylation as new and relevant molecular mechanism critical for proper cohesin function.

W1 MONOGENIC DISEASE

W1-01

Mutations in SNRPE, which encodes a core protein of the spliceosome, cause autosomal dominant hypotrichosis simplex

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Hypotrichosis simplex (HS) comprises a group of hereditary isolated alopecias that are characterized by a diffuse and progressive loss of hair starting in childhood with a wide phenotypic variability. We mapped an autosomal dominant form of HS to chromosome 1q31.3-1q41 in a Spanish family. By direct sequencing, we identified the heterozygous mutation c.1A>G (p.Met1?) in SNRPE which results in loss of the start codon of the transcript. We identified the same mutation in a simplex HS case from the UK and an additional mutation (c.133G>A, p.Gly45Ser) in a simplex HS case originating from Tunisia. SNRPE encodes a core protein of U snRNPs, the key factors of the pre-mRNA processing spliceosome. The missense mutation c.133G>A leads to a glycine to serine substitution and is predicted to disrupt the structure of SNRPE. Western blot analyses of HEK293T cells expressing SNRPE c.1A>G revealed an N-terminally truncated protein, thus the mutation might result in use of an alternative in frame downstream start codon. Subcellular localization of mutant SNRPE by immunofluorescence analyses as well as incorporation of mutant SNRPE proteins into U snRNPs was found to be normal, suggesting the function of U snRNPs in splicing, rather than their biogenesis is affected. In this report we link a core component of the spliceosome to hair loss, thus adding another specific factor in the complexity of hair growth. Furthermore, our findings extend the range of human phenotypes that are linked to the splicing machinery.

W1-02

MAP4 defect underlines centrosomal organization as a central mechanism in growth regulation

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Shortness of stature is one of the common medical concerns in childhood, since 3 % of the general population present with a body height below -2 SD scores (SDS). After excluding already known defects the underlying cause remains unknown in approximately 80 % of patients.

In a consanguineous family with two affected children with severe growth retardation of -7 SDS and mild microcephaly (-2 SDS) genome wide homozygosity mapping identified 3 overlapping runs of homozygosity (52 Mb) containing 718 refseq genes. Using whole exome sequencing after Agilent sure select 50 Mb enrichment on a SOLiD 5500xl platform in one of the patients, we identified a homozygous missense mutation (c.1117G>A; p.A391T) in the coding region of the MAP4 gene. The mutation was not found in 300 control individuals, the 1000genomes project or ESP5400 and segregated in the family. We performed a quantitative real-time RT-PCR and detected a 70 % reduced MAP4 expression level in the patient compared to healthy controls indicating a reduced stability of the mutant transcript.

MAP4 is a major protein for microtubuli assembly during mitosis. Immunofluorescence studies on a fibroblast cell line of the affected patient using centrosome markers showed an aberrant number of centrosomes in 40 % of mitotic cells. No such anomaly was found in mitotic control fibroblasts. In addition we noted significant overlap of the patient's phenotype with Seckel syndrome and Microcephalic Osteodysplastic Dwarfism type Majewski which are also caused by defects in centrosomal proteins.

Bioinformatic analysis predicts a new phosphorylation site for the Threonin at position 391 which is most likely phosphorylated by important kinases in cell cycle regulation like cdk1. Since phosphorylation of MAP4 at specific positions is important for interaction and organization of microtubuli during mitosis, this novel phosphorylation site can explain the observed aberrant number of centrosomes found in patient fibroblasts.

First results for MAP4 siRNA knockdown analysis in control fibroblasts suggests a similar phenotype of MAP4 knockdown cells as observed in patient cells.

These results illustrates the feasibility of our approach using exome sequencing to identify recessive genes for short stature and confirms centrosomal defects in the pathogenesis of severe short stature with microcephaly.

W1-03

A founder mutation in ULFIN, a new gene on chromosome 16q22.1, in patients with spinocerebellar ataxia type 4 (SCA4)

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Spinocerebellar ataxia type 4 (SCA4) is an adult-onset neurodegenerative disorder with autosomal dominant inheritance, progressive ataxia, dysarthria and peripheral sensory neuropathy. The candidate region was tracked down to an 8 Mb interval at chromosome 16q22.1 in in two large families from Northern Germany. Since classical sequencing approaches of about 140 protein coding genes within this region could not reveal any obvious disease causing mutation we performed whole genome sequencing analyses of two patients from both families respectively.

By this, about 17500 variants per genome could be detected. Approximately 12000 of which were shared by both patients. After exclusion of known variants from dbSNP (Version 132), 1000G and ESP (Exome Sequencing Project), 512 variants were left. Of these, approximately 100 variants were found in public genomes (complete genomics). Due to the relative large number of variants left, we subsequently filtered based on the degree of conservation across species. For this we annotated the data using Annovar software and Phast Cons 46 way alignment. All variants within non-conserved regions were subsequently removed. This approach left nine variants, which were further evaluated based on their regulatory function using the public ENCODE data. Thereby one variant was located within an expressed region. This variant segregates with the movement disorder in both pedigrees and was found in another five unrelated patients with ataxia from Northern Germany but could be excluded in 5000 control probands of same ethnicity.

Interestingly, detailed in silico analyses could reveal five EST-clones, three of which are spliced, within this chromosomal region. By RT-PCR we were able to amplify a hitherto unknown cDNA of 1712 bp, spanning eight exons and encoding a 346 amino acid protein we named ULFIN. The new ULFIN gene codes

for a WD40-repeat protein and is preferentially expressed in fetal and adult brain tissue. Homology searches show high similarities to the transducin-like enhancer binding protein (TLE) family. These TLE proteins are implicated in a variety of functions such as the differentiation and stabilization of neuronal cells. The mutation identified affects a highly conserved amino acid residue within the WD40 repeat domain. Protein modeling and dynamic structure analyses could demonstrate that this missense mutation affects the overall protein conformation and is located within an accessible region of the WD40 domain which is known to exert protein-protein interactions.

In summary, we could identify a mutation in a new gene, ULFIN, which encodes a WD40 repeat domain containing protein. This mutation affects a highly conserved amino acid and is predicted to alter ULFIN protein function. Genetic analysis could reveal co-segregation of the mutation with the disease in both families. Furthermore this specific missense exchange was also found in five additional unelated patients. Since all these patients are from Northern Germany and no additional mutation in ULFIN could be detected at present, strongly indicate a founder effect as genetic cause of SCA4, a likewise frequent ataxia with ancestry in Northern Europe.

W1-04

Impaired ceramide synthesis due to mutations in CERS3 causes autosomal recessive congenital ichthyosis and reveals impact of lipid architecture and ceramide acyl chain length for terminal epidermal differentiation.

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The barrier function of the human epidermis is supposed to be governed by lipid composition and organization in the stratum corneum. Disorders of keratinization, namely ichthyoses, are typically associated with disturbed barrier activity. Using SNP-chip based autozygosity mapping we identified a homozygous interval of 3.4 MB on chromosome 15q26 with a lod score of 6.9. In parallel, exome sequencing with a subset of DNAs from affected individuals revealed homozygous missense mutations in two genes in the linked region in our patients with congenital ichthyosis. Cosegregation analysis pinpointed mutations in CERS3 as underlying the phenotype. The gene encodes ceramide synthase 3 (CerS3), which is exclusively expressed in skin and testis. We demonstrate here that the mutation impairs the activity of CerS3, which synthesizes very long chain ceramides in the skin. We show a specific loss of ceramides with acyl chain lengths from C26 up to C34 in terminally differentiating patient keratinocytes, which is in line with findings from a recent CerS3 deficient mouse model. Reconstructed skin with patient fibroblasts and keratinocytes reveals disturbed epidermal differentiation and a moderate impairment of barrier function. Our findings demonstrate that specific synthesis of very long chain ceramides by CerS3 is a crucial early step for the formation of the epidermal barrier and indicate that disorders characterized by ichthyosis can be attributed to defects in the epidermal metabolism of ceramides and other components of the cornified lipid envelope. With the identification of mutations in CERS3 a missing link between several genes implicated in congenital ichthyosis has been identified, and we propose a single epidermal pathway of the lipid metabolism involving the genes TGM1, ALOX12B, ALOXE3, ABCA12, CYP4F22, LIPN, FALDH, ELOVL4, FATP4, ABDH5, and CERS3.

W1-05

Targeted sequencing of GPI anchor synthesis pathway genes identifies PGAP2 as a new cause of hyperphosphatasia with mental retardation

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Recently, genes involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor came into the spotlight as a new subclass of congenital disorders of glycosylation (CDG) with a characteristic spectrum of clinical features. Up to date mutations in six genes of the GPI-anchor synthesis pathway, PIGA, PIGL, PIGM, PIGN, PIGO, and PIGV have been identified in patients with severe neurological features including seizures, muscular hypotonia, and intellectual disability. We developed a diagnostic gene panel for targeting all known genes of the GPI-anchor synthesis pathway to screen patients matching these features. By this means we detected three missense mutations in PGAP2, c.46C>T, c.380T>C, and c.479C>T in two unrelated patients with hyperphosphatasia mental retardation syndrome (HPMRS). These mutations cosegregated in the investigated families. PGAP2 is a gene coding for an acyltransferase that is involved in fatty acid remodeling of the GPI-anchor that is required for Golgi transport of GPI-linked substrates. Transfection of the mutant constructs p.Arg16Trp, p.Leu127Ser, and p.Thr160Ile into PGAP2-null cells showed only partial restoration of GPI-anchored marker proteins, CD55 and CD59, on the cell surface. In this work we show that also an impairment of GPI-anchor remodeling causes HPMRS and conclude that targeted sequencing of the GPI-anchor pathway genes is an effective diagnostic approach for a subclass of CDGs.

W1-06

Combined NGS approaches identify mutations in the intraflagellar transport gene IFT140 in skeletal ciliopathies with early progressive kidney disease

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Ciliopathies are genetically heterogeneous disorders characterized by variable expressivity and overlaps between different disease entities. This is exemplified by the chondrodysplasias Jeune (JATD), Sensenbrenner and Mainzer-Saldino (MSS) syndrome and other short rib-polydactyly syndromes which are frequently caused by mutations in intraflagellar transport (IFT) genes. In this study, we identified convincing autosomal recessive mutations in the IFT complex A gene IFT140 by whole exome sequencing and targeted next-generation sequencing using a customised ciliopathy panel of 131 cilia-related genes in five unrelated JATD and two MSS families. Further, an enrichment of probably damaging IFT140 alleles occurs in JATD compared to non-ciliopathy diseases, implying modifier effects for certain of the variants identified. All IFT140 patients presented with only mild chest narrowing but consistent end-stage renal failure before the age of seven years accompanied by retinopathy in about half of the patients. This is consistent with the recently reported severe cystic kidney phenotype in conditional Ift140 knockout mice. In line, we detected only weak expression of Ift140 in the mouse skeleton at E15.5 compared to strong expression in the developing kidney and retina. IFT140 is therefore a major cause of cono-renal syndromes (JATD, MSS) and should be prioritised for screening in patients on the skeletal ciliopathy spectrum that have kidney disease with or without retinopathy.

W2 COMPLEX DISEASES

W2-01

Androgenetic alopecia: identification of four new genetic risk loci and evidence for the contribution of WNT-signaling to its etiology

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Androgenetic alopecia (AGA, male pattern baldness) is the most common form of hair loss among humans. It is characterized by a progressive loss of hair from the scalp. Research has established, that the pathogenesis of AGA is driven by androgens based on a genetic predisposition as the major precondition. During the past years, candidate gene and genome-wide association studies have identified single nucleotide polymorphisms (SNPs) at eight different genomic loci to be associated with AGA-development. Despite these recent breakthroughs in the understanding of the genetics of AGA, a significant fraction of the overall heritable risk has yet to be identified. Furthermore, the pathophysiology of AGA is far from being understood in detail and every newly associated locus promises novel insights into contributing biological pathways. In this study, we sought to identify additional AGA risk loci by replicating SNPs at twelve genomic loci that show association with AGA in a recent meta-analysis (P-value< 10-5) but fall beyond the threshold of genome-wide significance (P-value > 5x 10-8). We analyzed a new replication sample comprising 2,759 cases and 2,661 controls of European descent to confirm the association with AGA at these loci. The combined analysis of the replication and the meta-analysis data identified four additional genome-wide significant risk loci for AGA on chr2q35, chr3q25.1, chr5q33.3, and chr12p12.1. The strongest association signal was obtained for rs7349332 (P=3.55x10-15) on chr2q35 located intronically in the wingless-type MMTV integration site family, member 10A gene (WNT10A). WNT10A is a member of the family of WNT-genes that encode for small secreted signaling proteins which play important roles during development and tissue homeostasis. Interestingly, WNT-signaling has also been implicated in the regulation of hair development and hair cycling. This seems to be of particular interest, as changes in hair follicle dynamics are a key feature in AGA. Indeed, genotype-specific expression studies showed the AGA risk allele of rs7349332 to be associated with lower WNT10A-expression in human hair follicle. These changes in expression levels might be of functional relevance in the regulation of hair cycle dynamics. Our present study thus provides the first genetic evidence for an involvement of WNT-signaling to AGA-development. It is hoped that a deeper understanding of the role of WNT-signaling in AGA will provide the basis for the development of new therapeutic options in the future.

W2-02

Genome-wide association study of SIDS predisposing genetic variation identifies association to 15q11 within the PWS/AS region

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In SIDS the contribution of monogenic disorders is established while the importance of complex predispositions is less clear. We conducted a two stage GWAS comparing SIDS cases with population controls. As SIDS exhibits 2:1 male:female sex bias we included Chr.X markers. We also analyzed CNVs which may likewise predispose to SIDS.

Patients: We included 446 cases with both information and biosamples available. 346 originated from the multi-center German study on sudden infant death (GeSID) and 100 cases were recruited in the U.K. As controls we used 1311 population based individuals with genotypes and information available.

Methods: We performed genome-wide SNP genotyping of 320 cases. After rigid quality control, data from 295 individuals were analyzed. Autosomal SNP- and CNV-markers (chr 1-22) were analyzed using an additive model adjusted for sex. Markers on Chr. X were analyzed stratified by sex and then meta analyzed. As age matching is not feasible in SIDS we performed sex matching of controls and geographical adjustment by multidimensional scaling.

Results: In the GWAS markers a signal on Chr.15 was the only one to surpass the genome-wide significance level (p<5x10-8.). The signal includes more than 20 genome-wide significant markers and has at least three statistically indipendent peaks. It is located within the PWS/AS region known to be parentally imprinted. It replicated with similar effect size upon analysis of the entire sample of 446 cases and 1311 controls. We did not observe genome-wide significant association with markers on Chr.X.

Conclusion: The preliminary analysis and follow-up of our GWAS has shown that even samples of about 300 cases can be adequately powered to achieve a genome-wide significant result. Studies of SIDS trios are underway to unravel the influence of parental imprinting on the association signal.

W2-03

Genome-wide association study reveals four new risk loci for bipolar disorder

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Bipolar disorder (BD) is a severe disorder of mood, characterized by recurrent episodes of mania and depression. The genetic factors underlying the etiology of BD are still largely unknown, although the estimates of heritability for BD are high and range between 60% and 80%. Since the first genome-wide association study (GWAS) of BD in 2007, a handful of risk loci at the widely acknowledged formal threshold of genome-wide significance (P<5E-08) could be identified which replicated in adequately sized follow-up studies, notably ANK3, NCAN, CACNA1C, and ODZ4. The first genetic findings for BD together explain only a small proportion of the heritability but experimental data strongly suggests that a substantial portion of the heritability may be explained by hundreds of different risk loci of very small genetic effect. One crucial step towards the identification of additional loci should be amenable by increasing the sample sizes.

In the BMBF-funded MooDS consortium, we generated a second wave of GWAS data from 2,266 BD patients and compared them with 5,488 controls originating from 6 European countries and Australia who have not been investigated in a discovery step for BD before. To further increase the statistical power, we combined our MooDS data with published data from the Psychiatric GWAS consortium, resulting in the currently largest GWAS sample studied in BD (9,747 patients, 14,738 controls). Our analysis in 2.4 million SNPs revealed 47 SNPs reaching genome-wide significance at 7 loci, four of which were novel (2q11.2, 5p15.11, 6q16.1, and 7p21.3). Our findings suggest a role of neurodevelopmental processes, and - for the first time through GWAS - signal transduction at G-protein coupled receptor pathways in the etiology of BD. Our study demonstrates that an increase of GWAS sample size will likely pinpoint many more genetic factors in BD and gradually increase our knowledge about the biological processes involved in this common neuropsychiatric disorder.

W2-04

Association of rare variants in TCF4 gene with schizophrenia

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Schizophrenia is a severe psychiatric disorder with a lifetime prevalence of ~1% and a complex genetic architecture. Genome-wide association studies in very large patient-control cohorts have robustly identified that common single nucleotide polymorphisms of small to moderate effect sizes as well as rare copy number variants of larger effect sizes contribute to disease susceptibility. Association of common variation at the TCF4 locus with schizophrenia was one of the strongest findings of the international Psychiatric Genomics Consortium. Based on this finding, the aim of our study was to explore whether also small rare variants in the TCF4 gene (single nucleotide changes, in/dels), which would not be picked up by the previous SNP-array-based studies, might also contribute to schizophenia susceptibility. We resequenced the protein coding exons and flanking sequences of the TCF4 gene in 191 schizophrenia patients to detect low frequency variants that occur in the patient population and identified 8 low-frequency variantsof them. Information on low frequency variants in controls were extracted from 1000 Genomes data. In order to test for disease association, we genotyped all low frequency variants from patients and controls (n=16) in a large sample of ~1800 schizophrenia patients and ~2250 control individuals. Analysis of the data by specific methods developed for association analysis of rare variants (e.g. Li and Leal 2008, Madsen and Browning 2009, Ionita-Laza et. al 2011) revealed a significant association of rare (minor allele frequency <0.3%) TCF4 variants with schizophrenia. Our study suggests that there is a wide frequency spectrum of susceptibility variants contributing to schizophrenia at the TCF4 locus. The discovery of such rare disease associated variants in complex disorders not only adds to explaining their heritability, but also provides promising targets for functional studies to better understand the relevance of specific gene alterations to disease etiology.

W2-05

Microarray based blood profiling of Parkinson's disease patients with LRRK2 mutation

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What are the molecular mechanisms leading to LRRK2 induced Parkinson's disease (PD) pathology? This is the major question manifesting since 2004 several mutations in the Leucin-rich repeat kinase 2 (LRRK2) gene had been associated with 1-2% idiopathic and 5-6% familiar forms of PD. To respond to this question, this study examines the blood mRNA expression profile of 20 PD patients carrying a LRRK2 mutation in comparison to 20 age and gender matched healthy controls. Among the mutation carriers not only 11 patients with the most common G2019S mutation but also 9 R1441G mutation carriers were analyzed. Overall, 94 genes were differentially regulated with a p-value less than 0.05 and a logFC of ±0.6 between PD patients and controls. These genes fall into different categories such as antigen presentation, cell-to-cell signaling and interaction, molecular transport as well as cell death. One of the known biological functions of LRRK2 is its influence in cytoskeleton signaling. In line with this, we identified cellular movement, cellular growth and cell morphology as differentially regulated functions. At the gene level this is represented by the upregulation of the actin cytoskeleton associated genes ACTA2 (actin, alpha 2, smooth muscle, aorta) and LIMK2 (LIM domain kinase 2) as well as the microtubuli motor protein KIF1B (kinesin family member 1B). Also, the influence in vesicle metabolism is a postulated function of LRRK2 as demonstrated by the upregulation of STX3 (syntaxin 3), STX11 (syntaxin 11) and their interaction partner SNAP23 (synaptosomal-associated protein, 23kDa), all three are important for SNARE complex assembly. The identification of differentially regulated genes will not only help to understand the molecular biological relevance of LRRK2 mutations in the pathogenesis of PD but may also have the potential as blood biomarkers of this neurodegenerative disease.

W2-06

Obesity-associated alterations in the hepatic DNA methylome are remodeled through bariatric surgery

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The contemporary lifestyle in the western civilization is characterized by a combination of a high caloric diet and insufficient exercising. As a consequence a large part of the population of western countries suffers from obesity leading to a metabolic syndrome including type II diabetes, high cholesterol and high blood pressure. Such morbid obesity can result in morphological and physiological changes in hepatocytes accompanied with inflammatory processes finally leading to non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH). Both liver disorders can severely reduce liver function and can finally result in liver cirrhosis or liver cancer.

For morbid adipose patients who fail dieting, bariatric surgery is a treatment choice which has been shown to significantly reduce weight, diabetes risk and overall mortality. During bariatric surgery, parts of the stomach are functionally removed. The metabolic changes induced by bariatric surgery go along with a decline of the inflammatory processes in the liver. In the present study we aimed at determining the hepatic DNA methylation patterns associated with morbid obesity-associated NAFLD and NASH and at investigating whether bariatric surgery affects the DNA methylation of the diseased liver. Therefore, we analyzed DNA methylation signatures of more than 480,000 CpG sites corresponding to more than 20,000 genes in 63 liver samples from controls and morbidly obese patients ranging from normal liver histology to non-alcoholic steatohepatitis using Illumina's HumanMethylation450k BeadChip. In addition, 23 matched follow-up liver samples obtained after bariatric surgery were analyzed by this genome-wide approach. In order to explore the possible existence of epigenetically determined differential transcript expression, gene expression data was collected by an array based approach.

Using DNA methylation as a molecular tracer for disease phylogeny, patterns supporting progression from normal histology through steatosis to non-alcoholic steatohepatitis were identified. Differential methylation specifically associated with the liver disorders were observed for nine genes including IGFBP2 (insulin-like growth factor binding protein 2), IGF1 (insulin-like growth factor 1), PC (pyruvate carboxylase) and ACLY (ATP citrate lyase). Besides one exemption, expression and methylation at these loci were inversely correlated, suggesting differential methylation to be associated with differential expression of these genes in different liver phenotypes. Linking DNA methylation data to transcription factor binding site data in liver cells available from the ENCODE project revealed gene aberrantly methylated in diseased livers to be over 1000-fold enriched for the transcription factors ZNF274, PGC1A, SREBP2 and GRP20, implicating an involvement of these factors in both disease and remodeling.

A paired analysis of DNA methylation before and after bariatric surgery resulted in a total of 113 differentially methylated sites (FDR<0.05). For these sites, a strong correlation of differential methylation between the liver phenotypes (controls versus NASH) and the changes after bariatric surgery was observed ($p< 2.2\times10\text{-}16$, rho= –0.94). The partial reversibility of disease-specific alterations in the DNA methylome after bariatic surgery along with transcription factor binding and gene ontology data of the affected genes provides one of the first examples of epigenetic organ remodeling in humans.

W3 CANCER GENETICS

W3-01

Reliable and effective PMS2 mutation analysis in Lynch syndrome and constitutional mismatch repair deficiency

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Biallelic germline mutations in the mismatch repair gene PMS2 are responsible for more than half of all cases of the recessively inherited childhood cancer syndrome which is termed constitutive mismatch repair deficiency (CMMR-D) with reference to the underlying defect. CMMR-D is still under-diagnosed which is largely attributable to the lack of specific clinical signs and a broad tumour spectrum that overlaps also with other childhood cancer syndromes. Furthermore, heterozygous PMS2 mutations account for a significant proportion of Lynch syndrome (LS) cases that often show reduced penetrance in their family histories. As only the detection of the pathogenic PMS2 mutation(s) can confirm the diagnosis and allow for predictive testing in these cases, effective and reliable PMS2 mutation analysis is needed. However, PMS2 mutation analysis is complicated by the presence of a number of highly homologous pseudogenes. The greatest difficulties arise from the transcribed pseudogene PMS2CL. Sequence transfer between PMS2 and PMS2CL has led to PMS2 hybrid alleles containing PMS2- and PMS2CL-specific sequence variants at the 5'- and 3'-end, respectively, as well as the reciprocal PMS2CL hybrid alleles. We and others have shown that non-pathogenic hybrids can be found in up to 70% of all individuals and, hence, they severely impede all gDNA-based mutation analysis protocols. Therefore, we developed an RNA-based assay that effectively circumvents co-amplification of PMS2 pseudogenes and simultaneously allows for the amplification of hybrid alleles. For comprehensive mutation analysis we complement this direct cDNA sequencing approach with MLPA which we recently refined by the selection of appropriate reference DNAs.

We applied this comprehensive mutation analysis protocol to so far 14 suspected CMMR-D and LS patients with isolated PMS2 expression loss in their tumours. In all cases the underlying PMS2 mutations were uncovered. There were a total of 17 different pathogenic alleles in heterozygous, compound heterozygous or homozygous states. Identified mutations include nine truncating point mutations, five intragenic deletions, one missense and one splice-site mutation as well as one pathogenic PMS2 hybrid allele were identified. Careful evaluation of cDNA sequence data in combination with refined MLPA analysis was required for the detection of a single-exon deletion as well as two different deletions for which a CMMR-D patient was compound heterozygous. These findings highlight the technical difficulties in diagnosing PMS2 mutations.

Several of the new CMMR-D cases in our cohort developed tumours entities and clinical features so far unreported in CMMR-D patients. These features expand the clinical spectrum of CMMR-D in paediatric cancer patients. Effective and reliable mutation analysis in the PMS2 gene will allow more timely diagnosis and help to improve clinical management of these patients and their families.

W3-02

Exome capture identifies recurrent somatic mutations in EIF1AX and SF3B1 anti-correlated in uveal melanoma with disomy 3

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Uveal melanoma (UM) is a rare tumor of the eye. Gene expression profiling (GEP) has shown that there are two classes of UM. Tumors belonging to either of these classes are also distinct by chromosome 3 status and by patients´ prognosis. Specifically, metastatic disease (met), affecting about half of patients, originates from tumors with monosomy 3 (UM-M3). By contrast, most patients with tumors that show disomy 3 (UM-D3) have a good prognosis; only 10% of D3 tumors metastasize (UM-D3-met). Data on prognosis of patients who

have tumors with loss of only parts of chromosome 3 (UM-partM3)are inconsistent and from the chromosome 3 status these tumors can't be assigned to one of the established classes. In order to identify recurrent somatic mutations specific for the classes and clinical behaviour of UM we sequenced the exomes of 22 primary UMs (10 UM-D3 and 12 UM-M3). We identified six genes with protein-altering mutations in more than one tumor sample. Three of these genes – GNAQ, GNA11 and BAP1 – are known targets of recurrent somatic mutations in UM and of these only BAP1 mutations are specific to a UM-class (UM-M3). The three other mutant genes – SF3B1, EPB41L3, and EIF1AX – have not been linked to UM biology so far. SF3B1 and EIF1AX mutations were found in UM-D3 and EPB41L3 mutations in UM-M3 only. Re-sequencing of all coding exons of EPB41L3 in further 35 UM-M3 did not reveal any further mutation suggesting a minor role of this gene in UM biology. We also re-sequenced the recurrently mutated regions of SF3B1 and EIF1AX in a set of 89 primary UM (31 UM-D3, 35 UM-M3, 13 UM-partM3, 10 UM-D3-met). In 23 of 31 UM-D3 we identified somatic mutations of either EIF1AX or SF3B1 at a frequency of 48% (14/31) and 29% (9/31), respectively. As the mutations in these genes were mutually exclusive it is plausible that the roles of the mutant genes in UM-D3 are complementary. All mutations of EIF1AX, which maps to Xp22.12 and codes for an essential translation initiation factor, were missense mutations or short in frame deletions clustered in the region coding for the N-terminal tail (NTT). Mutations of SF3B1, which encodes a subunit of the U2 snRNP splice complex, were all heterozygous missense mutations and affected codon 625 in 9 of 10 samples. Among 35 UM-M3 EIF1AX and SF3B1 mutations were infrequent (2/35, 5.7%) and, consequently, transformation of UM-D3 into UM-M3 is an unlikely route for most UM-M3. Targeted sequencing of 13 UM-partM3, which is associated with good prognosis in our cohort of patients, revealed SF3B1 and EIF1AX mutations in 7 (53%) and 1 (8%) of these samples, respectively. This overlap in molecular alterations suggests that the biology of most UM-partM3 is related to UM-D3. Interestingly, SF3B1, which is located on chromosome 2, is almost exclusively altered in tumors from male patients. If the mutant allele has a trans-effect on splicing of specific genes then the skewed sex-ratio suggests that one of the possible targets is on the Y chromosome. Mutation analysis in UM-D3-met revealed SF3B1 mutations in 3/10 cases and absence of EIF1AX mutations. Notably, all three mutations of SF3B1 in D3-met were outside of codon 625 thus suggesting that genotype-phenotpye correlation with respect to the outcome of disease might exist. In summary we have found that UM classes which are distinct by chromosome 3 status also show distinct gene mutation profiles.

W3-03

Epigenetic profiling of germinal center B-cell lymphomas by whole genome bisulfite sequencing

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Germinal center B-cell (GBC) lymphomas are by far the most frequent malignant lymphomas in children and adults. They are systemic and frequently chronic diseases requiring repeated therapies over extended periods of time. About 30-50% of patients die from the disease. The epigenetic mechanisms, that are taking a central role in the control of GBC lymphoma pathomechanisms, recently have becoming more accessible to analysis thanks to rapid advances in next-generation sequencing technologies.

Within the framework of the ICGC MMML-Seq project we performed whole genome bisulfite sequencing of 28 complete DNA methylomes of germinal center derived B-cell lymphomas and of one normal germinal center B-cell control. This high-resolution approach was complemented by microarray-based DNA-methylation analysis (using Illumina HumanMethylation450K Bead Chips) of an additional 210 lymphoma samples and controls: 129 germinal center derived lymphomas (BL, FL, DLBCL), 18 lymphoma cell lines and 63 normal controls including lymph nodes, cells isolated from tonsils (memory B cells, naive B cells, GC-derived B cells, CD3+ and CD19+ cells), and whole blood samples.

Statistical analysis of the microarray data identified 554 genes that were differentially methylated in Burkitt and non-Burkitt lymphomas (FDR<1x10-4, □/□max>0.6, t-test). 266 of these genes were specifically hypermethylated in Burkitt lymphomas. GO-term enrichment analysis of the differentially methylated genes identified potentially affected cellular processes including positive regulation of leukocyte mediated immunity (enrichment 9.66x; GO:0002705), B cell differentiation (7.65x; GO:0030183), positive regulation of leukocyte mediated cytotoxicity (10.82x; GO:0001912), B cell receptor signaling pathway (10.82x; GO:0050853), negative regulation of interleukin-12 production (18.44x; GO:0032695), positive regulation of immunoglobulin mediated immune response (15.6x; GO:0002891), antigen processing and presentation of exogenous

peptide antigen via MHC class I, TAP-independent (30.05x enrichment; GO:0002480), and lymphocyte homeostasis (10.91x; GO:0002260) among others.

To elucidate the epigenetic regulatory mechanisms of GCB lymphoma we are currently analyzing the whole genome bisulfite sequencing data that was generated in this project. Comparison of microarray and sequencing data derived from the same samples revealed a very high concordance between the microarray and whole methylome sequencing datasets (R-square=0.94). Furthermore, lymphoma-subtype specific differentially methylated regions and partially methylated genome domains have been identified. The current status and most recent results of our analyses will be presented.

W3-04

RNA-Seq identifies differentially expressed genes and mutations in oligodendrogliomas

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Brain tumor patients have a very poor prognosis (survival time often below one year) and targeted therapies are not yet available. Therefore, we started an explorative study for the identification of therapeutic targets in 13 oligodendroglial tumors. Most oligodendrogliomas (O) and also some oligoastrocytomas (OA) are characterized by a unique and typical unbalanced translocation, der(1;19), resulting in a 1p/19q-codeletion. We studied nine Os/OAs with and four Os/OAs without the 1p/19q-codeletion in addition to three normal brain samples obtained commercially.

We combined transcriptome next generation sequencing (RNA-Seq) using a 2x100 nt paired-end approach on the Illumina-HiSeq-platform, with high resolution array CGH using the 400K chip as well as miRNA array, 8x60k expression array and Exon array analyses (Agilent).

Array CGH identified additional aberrations (e. g. -4, -9, -14, -18), which are known to be associated with an unfavorable prognosis. We could also detect additional smaller deletions and duplications, which have not been described so far, such as a gain on chromosome 2 (+2p22.1-pter) involving the MYCN oncogene.

The comparison of the transcriptome data between tumors with and without the 1p/19q-codeletion as well as normal brain samples revealed significantly differentially expressed genes (adjusted p-value <0.05). The average expression level of genes on 1p and 19q was reduced by half in the 1p/19q-codeleted tumors compared to tumors without the codeletion. Consistent with this, most of the down-regulated genes were located on 1p. Interestingly, in normal brain samples, the average expression level of 1p-located genes was found to be placed in between the two tumor groups. Even more surprisingly, the average expression level of the genes on 19q in normal brain samples was as low as in the tumors showing the 19q deletion. These findings were confirmed by the RNA-expression data using Agilent 8x60k Arrays.

Using RNA-Seq we could also identify structural changes, like the known IDH1 c.395A>G mutation and mutations in CIC and FUBP1.

Our ongoing integrative data analysis shows that our comprehensive approach is very promising for identifying the key molecular changes in oligodendrogliomas and we aim at identifying new candidate genes as molecular therapeutic targets in a larger cohort.

W3-05

Large Scale miRNA Profiling Of Germinal Center Derived B-Cell Lymphomas

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Mature microRNAs (miRNAs) are single-stranded RNA molecules of 20- to 23-nucleotide length that control gene expression in many cellular processes. They typically reduce the translation and stability of mRNAs, including those of genes that mediate processes in tumorigenesis, such as inflammation, cell cycle regulation, stress response, differentiation, apoptosis, and invasion.

Germinal center (GC) derived B-cell lymphomas are the most frequent malignant lymphomas with Burkitt lymphoma (BL) being the predominant subtype in children. Within the German BMBF-funded International Cancer Genome Consortium (ICGC) Network Project ICGC MMML-Seq we so far determined sequence-based miRNA profiles of 13 BL, 7 diffuse large B-cell lymphomas (DLBCL) and 9 follicular lymphomas (FL) using Illumina technology. We are recording the classical miRNA fraction (18-35 nucleotides) as well as a larger size fraction (35-90 nucleotides).

Initial differential expression analyses comparing BL against non-BL showed that miR-155, of which high levels are associated with impaired clinical outcome for DLBCL, was highly upregulated in BL. In contrast, miR-17, which has been shown to play a role in the GC immune response as well as being discussed for the development of GC-derived B-cell lymphomas was downregulated in BL.

Further differentially regulated miRNAs include miR-150 (roles in the adaptive immune response, upregulated in cutaneous marginal zone B-cell lymphomas), miR-184 and miR-372, which represent novel targets in lymphomagenesis.

We have also identified four novel candidate miRNAs which are currently being validated in additional patient samples followed by further functional analysis.

Finally, initial mutational analysis of curently five samples showed five miRNAs (miR-1206, miR-1302-3, miR-1324, miR-146a, miR-4273, miR-532, miR-618) to be mutated in every miRNA profile.

Taken together, our initial series of so far 29 analyzed cases from the ICGC MMML-Seq has already given exciting insights into the role of differentially regulated as well as mutated miRNAs in GC-derived B-cell lymphomas. (Supported by BMBF through 01KU1002A-J)

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W3-06

A novel, diffusely infiltrative xenograft model of human anaplastic oligodendroglioma with mutations in FUBP1, CIC, and IDH1

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Background: Oligodendroglioma poses a biological conundrum: it is - as a malignant glioma - incurable, and yet, only a few human tumors grow as cell populations in vitro or as intracranial xenografts in vivo. Their survival, thus, seems not to be an intrinsic property, but one that may emerge only within a specific environmental context.

Methods: To determine the fate of human oligodendroglioma, we established an in vivo model of an anaplastic oligodendroglioma after intracranial implantation into enhanced green fluorescent protein (eGFP) positive NOD/SCID mice and extensively characterized the patient's and xenograft tumors using histopathological, immunhistochemical and genetic methods (including exome next-generation sequencing).

Results: After nearly nine months the tumor not only engrafted, but also retained classic histological and genetic features of human oligodendroglioma: cells with a clear cytoplasm, an infiltrative growth pattern, a 1p/19q-codeletion, and mutations in IDH1 (R132H) and the tumor suppressor genes FUBP1 and CIC. The xenografts were highly invasive and exhibited a distinct migration and growth pattern around neurons (especially in the hippocampus), along white matter tracts of the corpus callosum, and around established vasculature. Although tumors exhibited a high proliferation rate in vivo, neither cells from the original patient tumor nor the xenograft exhibited significant growth in vitro over a six-month period.

Conclusions: Our results indicate that growth of oligodendroglioma cell populations may depend on specific micro-environmental conditions. A description of the growth requirements may lead to new therapeutic avenues for these tumors.

W4 INTELLECTUAL DISABILITY

W4-01

Defects in the genome organizer CTCF cause intellectual disability with microcephaly and growth retardation

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Over the last few years an increasing number of genes involved in chromatin remodeling and epigenetic regulation has been linked to several disease phenotypes, often including intellectual disability (ID). We now for the first time linked the key chromatin organizer CTCF to a human disease phenotype by identifying de novo mutations in patients with ID, microcephaly and growth retardation.

By trio exome sequencing we identified a de novo frameshift mutation in the CTCF gene in a boy with ID, short stature, microcephaly and various other anomalies. Subsequent mutational screening of the CTCF gene revealed another de novo frameshift mutation and a de novo missense mutation in two further ID patients. Additionally one patient with a de novo deletion comprising eight genes including the complete CTCF gene was discovered in the Decipher database. Although no distinct common phenotype could be delineated between the patients, common clinical features included variable degrees of intellectual disability, and head circumference and/or body height in the low normal or below the normal range. CTCF (CCCTC-binding factor) is involved in various chromatin regulation processes such as higher order of chromatin organization and enhancer function as well as nucleosome positioning, X-inactivation, and imprinting. This crucial role in gene regulation prompted us to perform whole transcriptome analyses in blood lymphocytes of three of the patients and eight healthy controls. We found a broad deregulation of genes with a significant overlap between the patients. Down-regulated genes were enriched for genes involved in signal transduction and cell-environment interaction, processes which have been implicated in developmental and cognitive disorders. Together with data from chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of CTCF in lymphocytes and publicly available ChIA-Pet data of CTCF from the related K562 cell line, we found that CTCF is important for enhancer-driven gene activation and that defects in CTCF affect the genomic interaction of enhancers and their regulated gene promoters, which are required for proper developmental processes.

Interestingly, impairment of these fundamental mechanisms results in a rather confined phenotype in humans characterized by intellectual disability, microcephaly and growth retardation.

W4-02

Homozygous mutation in the transcriptional regulator HMG20A causes non-specific intellectual disability: from NGS to pathogenicity

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We examined a large consanguineous family with 4 children (3 males, 1 female) that presented with moderate intellectual disability and epileptic seizures, while motor development, hearing, vision, head circumference, and growth were unremarkable. We postulated an autosomal recessive pattern of inheritance and mapped the causing mutation to a single candidate region of 23 Mb on chromosome 15q23-q26.1. Using DNA enrichment with Agilent 50 Mb kit and Next Generation Sequencing (NGS) on a SOLID 5500xl platform, we sequenced the whole exome of our index patient. We excluded known variants (1000 Genomes Project, Exome Sequencing Project, in house NGS controls) and technical artefacts and prioritized the remaining variants based on exhaustive in silico analyses and screening against 330 ethnically matched controls. Only one missense candidate mutation p.R232G in HMG20A at a highly conserved amino acid position remained and was shown to segregate in the family. HMG20A encodes for the protein iBRAF (inhibitor of BRAF35). iBRAF binds to DNA and plays a role in histone 3 methylation by recruiting the histone methyltransferase MLL. Histone 3 methylation is crucial for brain development and neuronal differentiation. Many genes involved in methylation, like MLL and MLL2, or demethylation, like PHF21A, of histone 3 have been linked to intellectual disability.

To further substantiate the pathogenicity of the identified mutation we undertook a comprehensive bioinformatic analysis at protein level and showed that the structure of the DNA-binding-domain of iBRAF is disturbed due to the amino acid exchange and predicted to strongly impair protein function. Since overexpression of HMG20A has been reported to upregulate voltage-gated sodium channels e. g. SCN3A, we transfected HeLa cells with wildtype and mutant HMG20A and measured expression changes with quantitative PCR. The upregulation of SCN3A following transfection with HMG20A seen with the wild type construct was markedly reduced when transfecting the mutant construct further supporting the pathogenicity of the variant identified. Further experiments in vitro and in Drosophila models are ongoing. Taken together, we add iBRAF to the growing number of transcriptional regulators and chromatin remodelling proteins involved in the aetiology of intellectual disability.

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W4-03

Intellectual Disability And Macrocephaly In Two Families Caused By Novel Mutations In BRWD3

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Mutations in the BRWD3 gene were previously described in three families with X-linked intellectual disability. Additional features that were observed in the majority of affected individuals and therefore considered as characteristic of this entity included macrocephaly, tall stature, speech delay, and muscular hypotonia. Carrier females show skewing of X-inactivation with preferential inactivation of the chromosome carrying the BRWD3 mutation. The protein encoded by BRWD3 is thought to have a chromatin-modifying function, and may thus play a role in the regulation of transcription.

We describe two half-brothers and an unrelated third patient with moderate intellectual disability, speech delay, macrocephaly, and tall stature. Both half-brothers had abnormal EEGs but no clinically apparent seizures were described. The older brother had normal findings on brain imaging whereas the younger brother showed mildly enlarged ventricles. The third patient showed behavioural abnormalities including aggressiveness. All patients were otherwise in good health. No internal malformations were known, and no specific dysmorphic features were present.

In all patients high resolution GTG-banding showed normal karyotypes, molecular testing regarding Fragile-X-syndrome and molecular karyotyping (array-CGH) were unremarkable. Because of the strong evidence for a X-linked form of intellectual disability, but the non-specific findings in the two half-brothers the coding sequence of the X chromosome (X-chromosomal exome) was investigated using NGS-technology. We could identify and confirm by Sanger sequencing a novel missense mutation in the BRWD3 gene in both affected half-brothers. The mutation is predicted to be disease causing using several prediction programs. Further segregation analysis in the family showed that the mother of the two boys carried the BRWD3 mutation, as did the maternal grandmother.

Screening of BRWD3 in a set of 10 patients with intellectual disability, macrocephaly and tall stature revealed a deletion of exon 34 and 35 in the third patient shown here. This deletion of two exons is creating a premature stop-codon in exon 36. We could confirm the deletion with MLPA and breakpoint-spanning sequencing in the patient. Segregation analysis in the family is ongoing.

We speculate that BRWD3 mutations may be more common than it is suggested by the few previous reports. These patients are likely to be considered having Fragile X or even Sotos syndrome. X-exome sequencing is a useful tool to decipher the genetic defect in families with a pedigree suggesting X-linked inheritance.

W4-04

Mutation in EZR influences the Ras/MAP pathway and causes autosomal recessive intellectual disability

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We examined a large consanguineous family with intellectual disability. The family has two sons with very severe non-specific intellectual disability and early epilepsy. Brain CT scan showed enlargement and

deformation of the ventricles, periventricular leukomalacia, cerebral atrophy, dysplasia of corpus callosum, and reduction in the white matter in both hemispheres. We undertook autozygosity mapping and identified three candidate loci on chromosomes 6, (8.1 Mb), 18 (2.7 Mb), and 22 (7.8 Mb). We then enriched the exome of the index patient with Agilent SureSelect Kit 50 Mb and sequenced it on SOLiD 5500XL. We identified two novel homozygous variants in EZR (p.A129T) and MAP3K4 (p.M577V). The mutated alanine in EZR is highly conserved. In silico analysis using three programs predicted a pathogenic effect of the identified variant. Comprehensive bioinformatic analysis on protein level showed that the mutation in EZR buries the hydrophilic threonine in the hydrophobic core and thus destabilizes the protein structure, probably leading to a strong effect on the protein function. EZR encodes ezrin, a member of the ERM (ezrin, radixin and moesin) protein family, which shares the FERM (four point one ERM homology) domain. Ezrin is necessary for a number of cellular processes, such as cell adhesion, motility, morphogenesis and cell signaling. The identified mutation is located in the FERM domain, which has binding sites for many membrane and signaling molecules. Because we recently showed that ezrin is required for the activity control of the small GTPase Ras we measured the effect of the ezrin mutant specifically on Ras. We transfected NIH3T3 cells with the wild type or mutated EZR. NIH3T3 cells expressing mutant ezrin blocked growth factor induced Ras activity. As a consequence of the inhibition of Ras we observed a decrease in proliferation. These in vitro cellular assays show that this mutation has a drastic effect on the ezrin protein and expression of which leads to an abnormal cellular phenotype. We suppose that this effect exists also in neurons and causes the severe phenotype of the examined family. Further experiments are ongoing. Taken together, we were able to identify EZR as a novel gene causing severe autosomal recessive non-specific intellectual disability. The loss of function of the ezrin mutant with the observed defects in Ras signaling is in line with other phenotypes of neurodevelopmental disorders and defects in Ras/MAP pathway.

W4-05

X-linked syndromic intellectual disability type Nascimento is a clinically recognisable, probably underdiagnosed entity

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The X-linked syndromic intellectual disability type Nascimento (MIM #300860) was first described by Nascimento et al. (2006), who found a nonsense mutation in UBE2A in three retarded males of a two generation family. Since then, only two further familial missense mutations (Budny et al., 2010) and four different deletions (De Leeuw et al., 2010, Honda et al., 2010) have been described. The X-linked ID type Nascimento phenotype is mainly characterized by craniofacial dysmorphisms (wide face, synophrys, prominent supraorbital ridges, deep-set, almond-shaped eyes, large mouth with downturned corners), macrocephaly, urogenital anomalies (small penis), skin anomalies (hirsutism, onychodystrophy, myxedematous appearance), moderate to severe intellectual disability (ID), motor delay, impaired/absent speech, and seizures.

We report on three previously undescribed families with UBE2A associated ID. Family 1 is a consanguineous Syrian-Lebanese family with two affected sons and more affected males with similar phenotypes in two other branches of the family. Examination of the two index patients showed facial dysmorphism, hirsutism, severe ID, absent speech and seizures in both and edematous appearance of feet and onychodystrophy of great toe nails in the younger brother. Array analysis (Cytochip v.1.0 180K, BlueGnome) identified a 7.1 kb deletion encompassing the first three exons of UBE2A in both affected brothers (arr[hg19] Xq24(118,706,962-118,714,074)x0), that was confirmed by qPCR and Sanger sequencing. Mother and sister also carried the deletion. All female carriers in the family showed a skewed X-inactivation. In family 2 from Spain, the clinical diagnosis XLMR type Nascimento was established clinically in a male and his maternal uncle because of severe ID and typical facial features. UBE2A mutation analysis revealed a missense mutation in Exon 4 (c.C236G, p.P79R) and therefore confirmed the diagnosis. In family 3, sequencing of the X-exome in the index patient identified a UBE2A mutation in exon 6 (c.387dupG, p.Tyr130Valfs*9). X-inactivation in his mother was not skewed and mutation analysis showed de novo occurrence of the mutation. Evaluation of his clinical data showed overlap of the phenotypic and developmental features (severe global delay, epilepsy) and also of his facial similarities with the UBE2A characteristics.

Based on these three novel families and the families from the literature we discuss the typical phenotype of UBE2A insufficiency caused by mutations or deletions and suggest XLID type Nascimento being a clinically recognisable, probably underdiagnosed entity.

W4-06

NGS-based parallel analysis of X-linked intellectual disability genes detects causative mutations in a high percentage of sporadic male patients

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Intellectual disability (ID) has a prevalence of 1-3 % and is a major reason for consulting a clinical geneticist. Mutations in X-chromosomal genes are estimated to account for approximately 10 % of male ID patients. Apart from fragile X syndrome, which is at cause in about 25 % of X-linked ID (XLID) and which has been part of the routine diagnostic work-up for many years, more than 90 other XLID genes are known to date. The low prevalence of mutations in each individual gene has, however, rendered routine testing of these genes impractical in patients with unspecific clinical features.

The advent of new sequencing technologies has enabled us to establish a platform combining in-solution enrichment of the coding regions of all XLID genes and subsequent next-generation sequencing (NGS). We have employed this XLID panel for analyzing a cohort of more than 100 unselected male ID patients in whom chromosome aberrations and fragile X syndrome had already been excluded. As a result, we found unambiguously disease-causing mutations in genes such as MED12, CUL4B, DLG3, SLC9A6 and UBE2A in more than 5% of the patients, and variants of unclear pathogenicity were present in additional patients. Considering the high recurrence risks for X-linked disorders, XLID panel analysis has thus been shown to be a valuable diagnostic tool in male patients with non-syndromic or atypical syndromic ID.

W5 DISEASE MECHANISMS

W5-01

Endogenous LINE1 Retrotransposon-Mediated Insertional Mutagenesis is Activated During and After Reprogramming Into Human Induced Pluripotent Stem Cells

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Objective: The use of human induced pluripotent stem cells (hiPSCs) holds great therapeutic promise for custom-tailored cell therapy and so-called 'disease modeling', and offers striking advantages over human embryonic stem cells (hESCs). hiPSCs present unlimited capacity for proliferation, differentiate into all cell types of the germ layers, represent a source of autologous cells compatible with the immune system and avoid ethical issues associated with the use of hESCs. However, reprogramming and subsequent cultivation of hiPSCs in vitro can induce genetic and epigenetic abnormalities that can result in tumorigenic iPSCs. Thus, it is unclear if iPSCs or their derivatives are safe for administration. Genomic mutations may undermine their use in regenerative medicine. Activation of the human endogenous mobile retrotransposons LINE-1 (Long Interspersed Element 1, L1), Alu and SVA have the potential to cause such mutations. L1 activity in germ cells and at early stages of the embryonic development was shown to be the cause of various forms of genetic instability and can affect host gene expression. To date, ≥90 cases of genetic disorders and tumor diseases were shown to be the result of L1-mediated mobilization events. We investigated if endogenous L1, Alu and SVA elements mobilize in hiPSCs and contribute to their genomic destabilization.

Methods: To explore if L1 elements are activated in hiPSC lines, we analyzed CpG methylation of the L1 promoter regions by bisulfite sequencing, and L1 expression by qRT-PCR and immunoblot analysis in two somatic parental cell lines and five derived iPSC lines. To map individual mobilization events that occurred during or after reprogramming into these iPSC lines, we used an enhanced version of a novel high-throughput protocol termed retrotransposon capture sequencing (RC-seq). RC-seq utilizes sequence capture to enrich for the junctions between 5' and 3' termini of all members of all functional human retrotransposon families (L1, Alu and SVA) and the surrounding genome, followed by multiplexed, paired-end Illumina sequencing to discriminate already known and novel L1, Alu and SVA insertions. Novel transposition events

that were absent from the parental cells and identified only in the iPSC lines were validated as de novo by insertion site PCR and capillary sequencing.

Results and Conclusions: We show that reprogramming reduced methylation of the L1 promoter on average from 70% to 37%, induced full-length L1 mRNA expression by 30 to 2000-fold relative to parental cells resulting in excessive quantities of the L1 protein machinery which is absent from parental cells. Utilizing RC-Seq, we so far identified a total of 22 novel insertions of L1, Alu and SVA elements in hiPSCs, which were absent from their parental cells. 50 % of all de novo insertions were found in introns of protein-coding genes, including PTPN9 that is coding for a tyrosine phosphatase impairing oncogenic growth and invasion of breast cancer cells. We show that hiPSC lines differ severely in their endogenous mobilization rate from each other, and that L1-mediated mobilization occurs during or after reprogramming into iPSCs and during long-term cultivation. These mobilization events can perturb key protein-coding genes with unknown consequences in differentiated cells, questioning biosafety of hiPSCs and their derivatives.

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W5-02

Compensation for dysfunctional THAP1 by increased expression: Autoregulation of THAP1 expression

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Dystonias represent a group of heterogeneous movement disorders, characterized by involuntary twisting, repetitive movements and abnormal postures. Dystonia 6 (DYT6), a monogenic form of primary (isolated) dystonia, is associated with mutations in the THAP1 gene. THAP1 encodes a transcription factor with a characteristic THAP zinc finger domain at its N-terminus which mediates specific binding of THAP1 to bipartite THAP-binding sequences (THABS) in the promoter region of target genes. The core promoter of TorsinA (TOR1A), the gene mutated in patients with dystonia 1 (DYT1), contains two THABS. Recently, we and others demonstrated that THAP1 specifically binds to and represses the TOR1A gene. This effect was shown to be disturbed by mutations affecting the DNA-binding THAP-domain of the THAP1 protein in luciferase assays. Surprisingly, expression analysis in patient fibroblast cells carrying THAP1 mutations revealed unaltered TOR1A expression levels while THAP1 expression levels were increased. By in-silico analysis, we now identified five THABS within the THAP1 core promoter. Chromatin immunoprecipitation (ChIP) analysis using chromatin of human neuroblastoma cells (SH-SY5Y) and electromobility shift assays (EMSA) were performed to demonstrate specific binding of THAP1 to its core promoter region. Luciferase reporter gene assays revealed strong repression of the THAP1 core promoter activity by wildtype THAP1 while several DYT6-causing mutations in THAP1 perturbed THAP1-mediated repression. For gene expression analysis, we re-programmed fibroblast cells from two THAP1 mutation carriers (p.Arg13His, p.Lys158Asnfs*23) and two controls to induced pluripotent stem (iPS) cells which were subsequently differentiated into neurons. Quantitative PCR in these cells revealed a significant increase of THAP1 expression levels in mutant THAP1 neurons compared to the wildtype neurons pointing towards an autoregulation of THAP1 in vivo.

In summary, our data provides evidence for an autoregulation in THAP1 expression in vivo. This may represent a protective mechanism which leads to a compensation for expressional changes of THAP1 target genes triggered by THAP1 mutations. This hypothesis needs to be further elucidated to better understand the biological function of THAP1 and the pathophysiological disease mechanism of DYT6 dystonia.

W5-03

Aberrant Arp2/Arp3-dependent actin polymerization and formation of lamellipodia & filopodia in patients with intellectual disability and ARID1B mutations

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ARID1B encodes an AT-rich DNA interacting domain-containing protein, which is a component of mammalian SWI/SNF-A chromatin remodeling complex. ARID1B is involved in transcriptional activation and repression of selected genes by chromatin remodeling. We have previously shown that de novo mutations in ARID1B are associated with a syndrome of moderate-severe intellectual disability (ID), speech impairment

and muscular hypotonia. In order to understand which cellular mechanisms lead to this specific phenotype we performed RNAseq whole transcriptome analysis to identify pathways involved. We investigated PAXgene stabilized peripheral blood from 12 controls and 6 patients using Ovation RNAseq system kit on a SOLID 5500XL platform. In total 451 genes showed expression changes >1.5 x or more ($p<0.01$). We validated the RNAseq results in a subset of genes with quantitative Real-Time PCR (qRT-PCR). Analysis with Ingenuity Systems IPA software indicated several significantly deregulated pathways including actin nucleation by ARP-WASP complex signaling pathway ($p=1.44E-04$). This pathway has been shown to be important for cellular motility and thus for neuronal path finding and neurite outgrowth. Arp2/3 complex is a seven subunit complex, which mediates actin polymerization and nucleates the generation of branched actin filament arrays, resulting in formation of lamellipodia. Filopodia are developed by the reorganization of Arp2/3 complex generated actin network in unbranched filaments that bundle together. Four subunits of Arp2/3 complex (ARP2, ARP3, ARPC2 and ARPC5) showed significant reduction in RNA expression in the patients. ARP2 and ARP3 code for the two actin-related proteins of the complex, whereas ARPC2 and ARPC5 for subunits mainly responsible for structural and regulatory functions. Expression changes of ARP2 and ARP3 were validated with qRT-PCR. Immunofluorescence in lymphoplastoid cell lines of 3 ARID1B patients and 2 controls showed significant down-regulation of Arp2 and Arp3 proteins and morphological changes in patient's cell lines. In contrast to the morphology of the majority of control cells, which were either round or with developed lamellipodial protrusions, patient cells formed more elongated and rigid cytoskeletal actin projections that resemble filopodia or filopodia-like structures. The morphology of the cells in patients was variable but always different to controls. Knockdown experiments and migration assays are ongoing to further confirm these findings. Nevertheless, our data already suggest that some of the symptoms in patients with ARID1B mutations are mediated via Arp2/Arp3-dependent actin polymerization and actin-based protrusions formation, indicating its possible role in regulation of general motility functions and neuritogenesis.

W5-04

Mutations in NEK8 link organ size and different ciliopathies with altered Hippo signalling and increased expression of the oncogene c-myc

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Ciliopathies display a broad spectrum of phenotypes ranging from mild manifestations to lethal combinations of multiple severe symptoms and most of them share renal cysts as a common feature. Starting point of this study was a consanguineous pedigree with three affected fetuses showing an early embryonic, multisystemic phenotype with enlarged cystic kidneys, liver and pancreas and developmental heart disease. By genome-wide linkage analysis we mapped the disease locus to chromosome 17q11 and identified a homozygous nonsense mutation in NEK8 which encodes a kinase involved in ciliary dynamics and cell cycle progression. Missense mutations in NEK8/NPHP9 have been identified in juvenile cystic kidney jck mice and a few patients suffering from nephronophthisis (NPH), an autosomal-recessive disease with normal-sized or small kidneys. In contrast to these data, we could confirm a complete loss of NEK8 expression due to nonsense-mediated decay in the affected fetuses with enlarged organs. In cultured fibroblasts derived from these fetuses the expression of prominent polycystic kidney disease genes (PKD1 and PKD2) was decreased while the oncogene c-myc was upregulated, providing potential explanations for the kidney phenotype seen in our patients. Finally, we could link NEK8 with NPHP3, another NPH protein known to cause a very similar phenotype in case of null-mutations. We show that both proteins interact and activate the oncogenic transcriptional Hippo effector TAZ. Binding of NEK8 to TAZ was enhanced in a TAZ mutant that lost its ability to bind 14-3-3 suggesting that 14-3-3 and NEK8 may compete for TAZ binding with 14-3-3 favouring cytoplasmic retention and NEK8 mediating nuclear delivery. Consistent with a role for TAZ in controlling proliferation and tumorigenesis, downregulation of NEK8 inhibited the TAZ-dependent proliferation of Hippo-responsive normal epithelial but also breast cancer cells. As NEK8 has been shown to be upregulated in breast cancer, these data do not only support a critical role for TAZ/Hippo signalling in the

pathogenesis of different cystic kidney diseases and ciliopathies, but may also imply a possible role for NEK8 in TAZ-mediated tumorigenesis. Taken together, our study demonstrates that NEK8 is essential for organ development and that the complete loss of NEK8 perturbs multiple signaling pathways resulting in a severe early-embryonic phenotype.

W5-05

TRAPPC11 deficiency causes a novel form of autosomal-recessive limb girdle muscular dystrophy

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Limb Girdle Muscular Dystrophies (LGMDs) are a heterogeneous group of degenerative myopathies caused by functional alterations e. g. in the dystrophin-glycoprotein complex. Progressive proximal muscle weakness is characteristic of the disease and it may involve the heart and bulbar muscles in a minority of cases. We examined a consanguineous Syrian family with three affected family members in two different branches of the family presenting with a hitherto undescribed form of LGMD characterized by progressive muscle weakness of the proximal limbs, highly elevated serum creatine kinase, hip dysplasia and scoliosis in all three patients. Combination of whole exome-sequencing in the index patient with homozygosity mapping identified a single novel variant, a homozygous missense mutation variant c.2938G>A (p.Gly980Arg) in TRAPPC11. The mutation affects a highly conserved amino acid residue within the gryzun domain. Co-segregation of the mutation within the family was confirmed by Sanger sequencing. It was not found in any current database of human variation. Screening of all coding exons of TRAPPC11 by Sanger sequencing in 32 German single cases of LGMD did not identify a second mutation. TRAPPC11 encodes a component of the multiprotein TRAPP complex involved in early ER-to-Golgi trafficking. We demonstrate that the mutation leads to specific impairment of binding ability to the TRAPP components C2, C13 and C2L, and thereby to disassembly of the TRAPP complex. This in consequence disrupts the architecture of the Golgi apparatus of patient fibroblasts. Moreover, we observed a quantitative reduction of LAMP1 and LAMP2 in patient cells, suggesting a defect in the transport of secretory proteins as the underlying pathomechanism.

W5-06

Mutations in OLFML2B within the QT interval associated region 1q23.3 Disturb Cardiac Repolarization, Predispose to Long-QT Syndrome (LQT) and to Sudden Infant Death (SIDS)

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Several GWAS have mapped the strongest human cardiac repolarization and QT interval modifying QTL to Chr.1q23.3 near NOS1AP (CAPON). Predisposition to repolarization disturbances and its sequelae (e.g. sudden infant death syndrome, SIDS) can be caused by monogenic (Long-QT Syndrome, LQT) as well as complex etiologies (e.g. SCN5A-p.S1103Y). Aim: We investigated regional genes NOS1AP and OLFML2B for rare variants (mutations) with strong effects on cardiac repolarization to potentially cause monogenic LQT or SIDS under monogenic disease models. Results: Both genes are expressed in the human heart. Their

knockdown in zebrafish induces cardiac dilatation and arrhythmia. Among 114 cases of LQT (without mutations in known genes) we identified heterozygous carriers of the OLFML2B missense mutations p.R86W and p.R527Q. In 513 SIDS cases we identified three heterozygous carriers of OLFML2B missense mutations p.P504L, p.G515E and p.Y557H. No mutations were observed in NOS1AP. All five OLFML2B mutations suppressed cellular export of the secreted glycoprotein. Expression of wildtype OLFML2B in Xenopus oocytes reduced current density of the voltage gated IKr potassium channel (KCNH2, Kv11.1) but not of other cardiac ion channels by 8±5% while mutations led to larger reductions (from -18% to -49%). Conclusions: Our findings suggest that OLFML2B mutations can predispose to LQT and to SIDS by decreasing myocardial repolarization reserve. We uncovered a novel monogenic disease gene illuminating its pathomechanism acting through IKr. In addition we identify novel roles and properties of the olfactomedin class of proteins providing an example how GWAS based QTL mapping may enhance our general understanding of pathophysiology.

W6 CLINICAL GENETICS I

W6-01

SHFM Causing Missense Mutation in FNDC3A

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Split hand/split foot malformation (SHFM) is a congenital limb malformation characterized by truncation or loss of central rays of hands and/or feet. It occurs in syndromic- and non-syndromic forms; dominant, recessive and in rare cases X-linked inheritance have been reported. So far, six different loci (SHFM1-6) have been associated with SHFM: point mutations in TP63 and WNT10B, copy number changes in 10q24, 17p13.3 and 7q21 as well as translocations in 7q21; but in a large number of cases the underlying cause remains unresolved.

We studied a large consanguineous family from Syria with four affected individuals affected by non-syndromic SHFM. Mutations in the known loci had been excluded previously. We identified a homozygous mutation in the FNDC3A gene (Fibronectin Domain Containing Protein 3A) by whole exome sequencing in a homozygous run of 4 MB on chromosome 13q14.2 and subsequent confirmation by Sanger sequencing. All affected family members share the homozygous missense mutation in FNDC3A while the nine analyzed non-affected members carry either only a heterozygous mutation or wildtype.

FNDC3A is required for cell-cell adhesion (Obholz et al. 2006) which is critical for the development of different tissues. For the limb, the adhesiveness of mesenchymal cells undergoes spatiotemporal changes during cartilage formation (Wada et al., 2011) and alterations in cell adhesion have been shown to lead to limb malformations such as distal truncations (Yamaguchi et al., 1999). The mutation is located in a highly conserved region of FNDC3A, in the eighth of nine fibronectin domains, and might affect the elasticity of the protein or even interfere with a possible inactivation of the protein by cleavage. We could show by whole mount RNA in situ staining of E9.5 mouse embryos that FNDC3A is expressed in the developing limb bud i.e. the apical ectodermal ridge and/or the underlying progress zone. This region is known to be essential for outgrowth and patterning of the limb and, if misregulated, to cause malformations such as SHFM.

In summary, we suggest FNDC3A as a candidate gene for autosomal recessive SHFM.

W6-02

De novo truncating mutations in ASXL3 cause a novel clinical phenotype

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Molecular diagnostics can resolve locus heterogeneity underlying clinical phenotypes that may otherwise be co-assigned as a specific syndrome based on shared clinical features. Here we describe a novel syndrome which shares characteristics with Bohring-Opitz syndrome (BOS), a disease that is associated

with mutations in ASXL1. Using genome wide sequencing we identified heterozygous, de novo truncating mutations in ASXL3, a

transcriptional repressor related to ASXL1, in four unrelated probands. ASXL3 is a putative Polycomb group (PcG) protein, required to maintain the transcriptionally repressive state of homeotic genes throughout development. We also confirmed the paternal origin of one of the de novo mutations (Q466X) by allelic specific PCR and clone-sequencing. We excluded the possibility of recurrence of this mutation in newborn, by checking its existence in the father's semen.

W6-03

A new face of Borjeson-Forssman-Lehmann syndrome? De novo defects in PHF6 in females with a distinct phenotype

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Borjeson-Forssman-Lehmann syndrome (BFLS) is an X-linked disorder, caused by mutations in the PHF6 gene, and characterized in males by variable intellectual disability, moderate short stature, obesity, gynecomastia, hypogonadism, tapering fingers, short toes, epilepsy, and a typical facial gestalt with coarse features and large ears. Most heterozygous carrier females are reported to have skewed X-inactivation and can either be asymptomatic or show milder clinical features such as learning difficulties, tapering fingers or short toes.

So far only two sporadic females with de novo defects in PHF6 have been reported in literature. One was clinically suspected to have BFLS before, the other was identified by detecting an intragenic PHF6 deletion in routine copy number screening.

We now report on four females with de novo defects in PHF6. A missense mutation was identified by exome sequencing in the first girl with an early-childhood clinical diagnosis of Coffin-Siris syndrome but age-related changing phenotype. Subsequently, three patients with a similar phenotype were identified and screened for PHF6 defects by sequencing, qPCR, and MLPA, which revealed a truncating mutation in one patient and a duplication of exons 4 and 5 in two patients.

All four patients display a distinct, recognizable phenotype in older childhood/early adulthood that overlaps but is not identical with the phenotype observed in male patients with BFLS. All girls/women were mildly to severely intellectually disabled. They showed distinct facial features with bitemporal narrowing, prominent supraorbital ridges, narrow palpebral fissures, mild hypertelorism in the younger girls, a prominent nasal tip and prominent columella, long and slightly backwards rotated ears, and a relatively large mouth. Toes and fingers were short with hypoplastic distal phalanges, syndactyly (one patient), and a pronounced brachyclinodactyly of finger V. Further consistent findings were an abnormal dental status with incomplete and small, irregularly shaped teeth that were prone to caries. Furthermore, linear skin hyperpigmentation was noted.

Interestingly, the characteristic facial phenotype seems to evolve with age, as the first and another of the girls had been suspected to have Coffin-Siris syndrome in early childhood due to hypoplastic distal phalanges, sparse hair, prominent eyebrows and dental anomalies.

X-Inactivation pattern in DNA from lymphocytes was normal in two of the patients and 100% skewed in the two others. Interestingly, in one of the latter, X-inactivation in DNA from fibroblasts was normal, indicating mosaicism for activity of the aberrant allele.

Our findings indicate that de novo defects in PHF6 in females result in a recognizable adult phenotype that might have been under-recognized so far.

W6-04

Delineation of a non lethal variant of Ogden syndrome

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Recently we and others showed that exome sequencing is a powerful method to identify mutations in sporadic cases with unexplained severe, non syndromic intellectual disability. We now report trio exome

sequencing in 2 unrelated patients, a girl and a boy, with unexplained severe global developmental delay. After exome capturing using Agilent 50Mb kit and sequencing on either SOLID 5500xl or Illumina HiSeq platforms we identified in both patients heterozygous de novo missense variants in the NAA10 gene located on Xq28. The girl is heterozygous for variant p.Val107Phe in exon 5 and the boy hemizygous for p.Arg116Trp in exon 6. Both variants are not annotated in dbSNP (build 135), not listed in NHLBI Exome Sequencing Project (ESP) database and absent from in house controls. Both mutations are located in the catalytic N-acetyltransferase domain of the protein and likely to be detrimental. Protein structure-based predictions revealed that the Trp116 mutation most probably hampers CoA binding and reduces the enzymatic activity of NAA10 while Phe107 doesn't fit in the hydrophobic core of the protein and most probably reduces protein stability or enzymatic activity of the protein. NAA10 (N-alpha-acetyltransferase 10), also known as ARD1 (arrest defective protein 1) or ARD1A is part of a major N-alpha-acetyltransferase protein complex (NatA) responsible for alpha-acetylation of proteins and peptides. Although N-terminal acetylation is one of the most common protein modifications and is implicated in several processes like protein degradation and mediator of protein complex formation, little is known about its general cellular and physiological function. Recently Rope et al. (2011) associated a single mutation in NAA10 with Ogden syndrome (OMIM 300855), a lethal X-linked disorder of infancy characterized by an aged appearance, craniofacial anomalies, hypotonia, global developmental delay, cryptorchidism and cardiac arrhythmias. So far, only seven boys from two different families were described who died in early infancy with cardiac arrhythmias. All of them carried the same hypomorphic NAA10 p.Ser37Pro allele located in the dimerisation domain. No affected females were described, so far. Both our patients did not show the characteristic clinical course of Ogden syndrome, but had severe global developmental delay and hypotonia as overlapping features. The 3 years old girl in addition had pulmonary artery stenosis, inguinal hernia and postnatal proportionate short stature with microcephaly and unclear episodes of increased body tension and breath holding . Functional studies are pending but the phenotypic difference with the previously reported boys may be explained by the location of variants in the N-acetyltransferase domain of NAA10 which could thus result in this non lethal variant of Ogden syndrome in male and female patients. Our study adds to the growing evidence that current syndromic descriptions are incomplete and unbiased large-scale sequencing approaches are needed to fully understand the complex relation between genotype and phenotype.

W6-05

Mutations in the ACTB gene cause a severe phenotype consistent with Fryns-Aftimos syndrome.

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Background: Mutations in ACTB and ACTG1 genes have recently been reported to cause Baraitser-Winter syndrome (BRWS) – a rare condition characterized by congenital ptosis, ocular colobomata, anterior neuronal migration disorder (pachygyria, lissencepahly), distinct facial minor anomalies, and intellectual disability. One of the patients carrying an ACTB mutation was previously diagnosed with Fryns-Aftimos syndrome (FAS). The main clinical features of FAS are craniosynostosis, anterior pachygyria and cerebral atrophy, a short webbed neck, limited extension of the joints with pterygia and very specific facial features: arched eyebrows; proptosis; hypertelorism; downslanting palpebral fissures; a broad nasal bridge; macrostomia and dysplastic low set ears.

It was recently suggested that BRWS and FAS are in fact the same condition, and the differences observed in both instances are due to age. However, other studies have countered that FAS and BRWS patients' phenotypes are too different to be one and the same disorder.

ACTB and ACTG1 code for the beta- and gamma isoforms of actin - the nearly identical highly conserved proteins, that differ only by four amino acids.

Results:

Patient 1 (20 years) exhibits craniosynostosis, anterior pachygyria and subependymal nodual heterotopia; bilateral eye colobomas; intestinal malrotation; ectopic kidneys; diastasis recti; preaxial polydactyly; a short and webbed neck; webbing at the axilla and elbows; and extension limitation of the shoulders, elbows, and knees. Moreover she presents with a striking and distinct pattern of facial anomalies: high arched eyebrows; proptosis; hypertelorism; downslanted palpebral fissures; broad nasal bridge; broad nasal tip and columella; anterverted nostrils; macrostomia; very high and narrow palate; crowded teeth; low set, long, dysplastic ears; and a low posterior hairline. The patient has severe ID with lack of speech development.

Patient 2 (8 years) shows heart defects, a bilateral cleft lip, cleft palate, anterior pachygyria, trigonocephaly, a short and webbed neck, axillary webbing and a narrow thorax. Her facial anomalies include high arched eyebrows, hypertelorism, downslanted palpebral fissures, broad nasal bridge, broad nasal tip

and columella, anterverted nostrils, macrostomia, low posterior hairline. The patient has moderate to severe ID with no speech development.

High resolution array CGH analysis revealed normal results in both patients.

Sanger sequencing of ACTB identified the heterozygous missense mutations NM_001101.3:c.359C>T (p.(Thr120Ile) in patient 1 and NM_001101.3:c.220G>A (p.(Gly74Ser) in patient 2. Neither of these mutations has been reported before, not in BRWS patients nor in association with other phenotypes. These mutations are also not listed in dbSNP or NHLBI ESP Exome Variant Server.

The ACTB mutation NM_001101.3:c.359C>T in patient 1 is analogous to the ACTG1 mutation NM_001614.1:c.359C>T previously reported in a patient with BRWS (significantly milder affected than patient 1).

Conclusions

We propose that FAS is a distinct entity with an early-onset, severe phenotype caused by mutations in ACTB.

Despite the structural similarity of beta- and gamma-actins and their expression in the same tissues, mutations in ACTB cause a distinctly more severe phenotype. This confirms the significant difference in the function of the non-muscular actins during development.

Detailed clinical follow up with genotype-phenotype correlation is elaborated on.

W6-06

Homozygous SALL1 Mutation Causes a Novel Multiple Congenital Anomaly-Mental Retardation Syndrome

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We identified 2 female siblings presenting with multiple congenital anomalies, central nervous system defects, cortical blindness, and absence of psychomotor development, ie, a novel recognizable, autosomal recessive MCA-MR. The positional candidate approach led to the identification of a homozygous SALL1 mutation, c.3160C > T (p.R1054*). The mutant SALL1 transcript partially undergoes nonsense-mediated mRNA decay and is present at 43% of the normal transcript level in the fibroblasts of a healthy carrier. Previously heterozygous SALL1 mutations and deletions have been associated with dominantly inherited anal-renal-radial-ear developmental anomalies. We identified an allelic recessive SALL1-related MCA-MR. Our findings imply that quantity and quality of SALL1 transcript are important for SALL1 function and determine phenotype, and mode of inheritance, of allelic SALL1-related disorders. This novel MCA-MR emphasizes SALL1 function as critical for normal central nervous system development and warrants a detailed neurologic investigation in all individuals with SALL1 mutations.

W7 CHROMOSOMAL VARIATIONS IN CANCER AND COMPLEX DISEASES

W7-01

Does Chromothripsis play a role in the Pathogenesis of AML with Complex Aberrant Karyotype?

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Background: It has not yet been evaluated for AML with complex karyotype whether gradual evolution through a sequence of genetic alterations or one catastrophic event generating multiple lesions across the genome in a single step plays the major role. “Chromothripsis” was recently defined as shattering of a single chromosome followed by rejoining resulting in a highly recombined chromosome based on a single catastrophic event (Stephens PJ et al., Cell 2011).

Patients and Methods: We selected 889 AML cases harboring a complex karyotype - defined as 4 or more abnormalities - at diagnosis. These were analyzed by chromosome banding analysis, 24-color-FISH, interphase-FISH, array CGH (n=78) and TP53 mutation analysis (n=195).

Results: In 518/889 (58.3%) cases at least one subclone was observed that showed extra chromosome aberrations, thus demonstrating clonal evolution already at the time point of AML diagnosis. Clonal evolution was more frequent in cases with del(5q) as compared to those without (404/666 (60.7%) vs 117/223 (52.5%); p=0.034) while no association of clonal evolution with loss of 7q, loss of 17p, or TP53 mutation was observed. In 46 cases which evolved from MDS (n=43) or MPN (n=3) chromosome banding analysis had been performed prior to the diagnosis of AML. In 21/46 (45.7%) cases karyotype had not changed while in 25/46 (54.3%) cases clonal evolution had occurred. In 28/57 (49.1%) cases analyzed also at relapse clonal evolution was detected. Additionally, 78/889 cases were evaluated by array CGH. The occurrence of chromothripsis was analyzed following the definition by Rausch et al. (Cell 2012) with at least 10 segmental copy-number changes involving two or three distinct copy-number states on a single chromosome. Evidence of at least one "shattered" chromosome was found in 24/78 (30.8%) cases. In 21 cases only one chromosome fulfilled these criteria, while in 3 cases chromothripsis affected two or more chromosomes. The chromosome most frequently affected by "shattering" was chromosome 11, observed in 18 (85.7%) cases, followed by chromosomes 2 and 21, which were affected in 2 cases each. Chromosomes 1, 5, 7, 13, 15, 16 and 20 showed signs of chromothripsis in single cases only. In 19/24 (79.2%) cases showing evidence of chromothripsis a high level amplification was observed for the MLL gene (11q23) in 17 cases and for the ERG gene (21q22) in 2 cases. Thus, amplifications were more frequent than in cases without chromothripsis (21/54, 38.9%; p=0.001), while no association was observed between chromothripsis and deletions of 5q, 7q or 17p or TP53mut, or presence of clonal evolution. With respect to outcome only TP53 mutations and the presence of 5q deletions were significantly associated with shorter overall survival (relative risk (RR) in TP53mut cases: 3.19, p<0.0001, and in del(5q) cases: 1.61, p=0.006; median OS: TP53mut vs TP53wt cases: 4.6 vs 22.0 months, p<0.0001; del(5q) vs non-del(5q) cases: 5.7 vs 14.4 months, p=0.006), while presence of deletions of 7q, or 17p, chromothripsis and clonal evolution showed no impact on outcome. In multivariable Cox regression analysis only TP53mut had an independent association with shorter OS (RR: 3.12, p=0.001).

Conclusions: In AML with complex karyotype chromothripsis does occur. However, the "shattering" of one chromosome was never observed as the sole abnormality, indicating that chromothripsis and sequential genetic evolution are not alternative but more likely combined mechanisms in AML.

W7-02

Characterization of structural aberrations involving chromosome 1 in germinal center derived B-cell lymphomas by whole-genome sequencing

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In the framework of the ICGC MMML-Seq project we investigate germinal center derived B-cell lymphomas including Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) by whole genome sequencing. Our initial analyses focused on four prototypical Burkitt lymphomas (BL) which besides the IG-MYC translocation showed only few (median 34, range 29 to 42) additional structural variants (SVs, > 50 bp length and translocations). Validation by Sanger sequencing, IG-PCR and FISH of the first four samples confirmed 17 out of 17 tested SVs. Remarkably, two of these BLs showed complex patterns of rearrangements on chromosome 1q (Richter et al., Nat Genet, 2012). Though being one of the most frequent cytogenetic events in these malignancies, the origin and functional impact of 1q aberrations remain unknown. Meanwhile we have extended whole genome sequencing to a cohort of 28 germinal center derived B-cell lymphomas including 12 BL, 8 DLBCL and 8 FL. Based on our findings in the initial BL cases the present evaluation of our ongoing analyses focuses on changes of chromosome 1. Currently, we have identified aberrations on chromosome 1 in additional 8 BL and DLBCL samples. Of these, two had a loss in 1p, one a gain on 1q and five showed a complex pattern of gains and losses affecting both 1p and 1q. Furthermore, reminiscence of chromothripsis (Stephens et al., Cell, 2011) was observed on chromosome 1 in one DLBCL sample that also showed accumulation of other complex rearrangements on other chromosomes. Candidate genes potentially involved in the pathogenesis of germinal center B-cell lymphomas affected by the complex chromosome 1 aberrations have been identified. In summary, by fine analysis of germinal center derived B-cell lymphomas by whole genome sequencing, we could characterize complex chromosomal aberrations of chromosome 1, one of the most frequently altered chromosomes in these malignancies. The possibility to precisely map chromosome aberrations in our ongoing whole genome

analyses might help to explain the mechanisms of cytogenetic tumor evolution and might lead to the identification of new subtype-specific genetic aberrations.

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W7-03

Copy number variation analysis in 222 patients with colorectal polyposis reveals potential new causative candidate genes

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Background: Adenomatous polyposis syndromes are characterized by multiple colorectal adenomas and a high lifetime risk of colorectal cancer. Germline mutations of the APC and MUTYH genes cause the autosomal dominant Familial Adenomatous Polyposis (FAP) and autosomal recessive MUTYH-associated Polyposis (MAP), respectively. However, in up to 50% of families no germline mutation could be identified. Copy number variants (CNVs), in particular heterozygous microdeletions, contribute significantly to the mutation spectrum of hereditary tumor syndromes and thus it can be hypothesized that those heterozygous deletion CNVs might also be the underlying cause in yet unidentified genes responsible for adenomatous polyposis syndromes.

Methods: Genomic DNA from 222 unrelated mutation negative polyposis patients was used for genome-wide SNP genotyping with the HumanOmni1-Quad BeadArray (Illumina). Putative CNVs were identified by the QuantiSNP v2.2 algorithm, filtered according to various criteria by use of the Cartagenia Bench™ software, by in-silico-analysis, and by comparison with 531 healthy controls, and validated by qPCR. The gastrointestinal expression of genes covered by the identified CNVs was examined by cDNA analysis of normal mucosa.

Results: 33 unique deletions (size 13-613 kb) were found in 28 patients (13%) and 64 unique duplications (size 21-532 kb) were found in 50 patients (23%). Each unique CNV except one occurred only once in the patient cohort. The 97 CNVs encompass 151 protein coding genes. Of the 37 deleted genes, 7 were completely and 30 were partly deleted; of the 114 duplicated genes, 42 and 68 were completely and partly duplicated, respectively. After expression analysis and data mining, the number of candidates could be further reduced to 23 heterozygous deletions and 70 duplications. These candidate adenoma genes include protein kinases, transcription factors, and potential tumor suppressors. A few of them have been reported to be associated with CRC phenotypes. The vast majority of cases are sporadic or DNA from affected relatives was not available, however, a deletion in 2q22.1 did not segregate with the phenotype in the family.

Conclusions: By applying stringent filter criteria, we identified a group of rare deletion and duplication CNVs which might contain predisposing genes for adenoma formation. Consistent with other studies, some CNVs might be relevant but low penetrant etiological factors. After prioritization of the included genes according to function, pathway, and literature, present work includes sequencing of the coding regions of the most promising candidates in a large number of patients to identify pathogenic point mutations. However, due to the strict filter criteria, some causative genes might have been overlooked by this approach. The study was supported by the German Cancer Aid.

W7-04

Duplications in RB1CC1 are associated with schizophrenia in large samples from Europe

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Schizophrenia (SCZ) is a severe and debilitating neuropsychiatric disorder with an estimated heritability of ~80%. Large, SNP array-based association studies have identified common SNPs and specific, rare, often recurrent copy number variants (CNVs) as susceptibility factors for SCZ. Recently, studies applying next generation sequencing technology also found an excess of de novo occurring mutations in SCZ patients compared to controls. While this is exciting, it is difficult to pinpoint the specific genes involved in disease etiology as a large number of genes were reported to carry a de novo mutation and secondly the reported mutations have a very low frequency among the patients. Therefore, genetic studies in independent samples are warranted as important support for a specific gene will be provided by the identification of additional gene alterations in patients. It is reasonable that these follow-up studies include the investigation of CNVs as it is known, that in many human disease associated genes both deletions and duplications contribute substantially to the mutational spectrum. Furthermore, CNVs have been successfully implicated as risk factors for SCZ. In the present study, we took advantage of an existing genome-wide SNP array dataset and screened, in a discovery step, 1,637 patients with SCZ or schizoaffective disorder and 1,627 controls for the presence of CNVs in 55 genes reported to carry a de novo mutation. All individuals were genotyped on HumanHap550v3, Human610-Quadv1, or Human660W-Quad arrays (Illumina, CA, USA). CNVs were identified using the two algorithms QuantiSNP and PennCNV. Our top finding was verified using quantitative PCR. Among the 55 genes screened for CNVs, we identified a significant overrepresentation of duplications in the gene RB1CC1 in patients versus controls. This finding was followed-up in large, independent samples from Europe. In the combined analysis, totaling 7,750 patients and 112,007 controls, duplications in RB1CC1 were associated with schizophrenia (P-value = 0.016; odds ratio = 4.65). Our study provides strong evidence that duplications in RB1CC1 are a risk factor for schizophrenia.

W7-05

Genome-wide detection of copy number variants in psoriatic arthritis patients

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Psoriatic arthritis (PsA) is a common complex inflammatory joint disease occurring in about 30% of patients with psoriasis vulgaris (PsV). Although several genetic risk factors have been identified in genome wide association studies (GWAS) as well as by different approaches in candidate genes, the heritability of this complex disease is by far not explained yet. Not only single nucleotide polymorphisms (SNP), but also copy number variants (CNV) contribute to the variability of the human genome and have been shown to act as susceptibility factors for several common complex genetic diseases.

In a genome-wide approach, we performed an association study for CNVs to identify further risk factors for PsA. We applied two different algorithms to data sets of 478 PsA patients and 3798 controls. The

analysis of predefined CNVs (Canary) revealed evidence for significant association at 22 loci, while association at 33 loci could be identified with Birdseye, 4 of them overlapping with the Canary algorithm. Due to power limitations, we focused on 37 common variants (MAF>5%). Further in silico analyses indicated false-positive findings due to erroneous CNV-determination of DNAs processed in the same experiment (="batch effects") at 19 of 37 loci (51%). A known multiallelic CNV encompassing the DEFB4 gene was incorrectly genotyped and therefore also excluded. In silico analyses confirmed association at 13 of the remaining 17 loci (35%). After comparing with two further control cohorts, we exluded 3 further CNVs since they had comparable allele frequencies in one or more cohorts. In order to validate the remaining 10 CNVs, we established an alternative quantitative method, MLPA. Sequence identity up to 99% due to segmental duplication prevented specific probe designs at 4 loci. Differential copy numbers were detected at 5 of 6 remaining CNV loci. Furthermore, 2 CNVs were false-positive due to smaller segments that had escaped the initial filtering process and 2 CNVs did not replicate in a smaller case-control study.For the only remaining and immunologically interesting CNV (TCRB), we investigated the complex sequences and could show a perfect alignment to the hg18 freeze, but not anymore to hg19. In MLPA analyses, evidence for association could not be replicated, either. Overall our study indicates a high rate of false-positive findings which are due to technical artifacts or subtle differences in lab-processing of the same microarray. In conclusion, the current SNP genotyping arrays are no suitable for discovery of common CNVs as risk factors for complex diseases.

In a second approach, 30 published susceptibility loci for PsV were investigated for CNVs as potentially causative variants in regions with linkage disequilibrium to the initially associated SNP. We detected a common CNV at HLA-C, which is probably an artifact due to >99% sequence similarity to HLA-B. No further common CNVs were identified, but 4 rare deletions and 10 rare duplications at 10 susceptibility loci. In MLPA analyses, we could confirm intronic deletions at CSMD1 and RYR2 locus that occurred similarly in controls. Furthermore, single duplication events at the IL12B locus were detected solely in patients: one affecting a neighboring gene and a further one affecting IL12B as well as 3 flanking genes. The screening of larger cohorts and functional studies might help verify the effect of these rare CNVs on the pathogenesis of PsA.

W7-06

An insertion of eight amino acids in HLA-DQB1 and three amino acid substitutions in HLA-DQA1 and HLA-DQB1 confer risk to idiopathic achalasia

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Idiopathic achalasia is a rare esophageal motility disorder with a lifetime prevalence of 1:10,000. It is a neurodegenerative disorder in which the neurons of the myenteric plexus are lost, leading to dysfunction of the lower esophageal sphincter (LES) and to a derangement of esophageal peristalsis. In the final stage of achalasia, esophageal motility is irreversibly impaired, and complications ensue because of the retention of food that is no longer transported into the stomach. The cause of achalasia is mainly unknown, but autoimmune processes appear to be involved in individuals with a genetic susceptibility.

The purpose of the present study was to identify genes which are involved in the disease process of idiopathic achalasia. Therefore, we performed the first large-scale association study study for achalasia using Illumina´s Immunochip (117,550 SNP-markers). The sample consisted of 1,068 cases with idiopathic achalasia and 4,242 population-based controls from Central Europe (Belgium, Germany, and The Netherlands), Italy, and Spain. The association analysis yielded a total of 106 markers reaching genome-

wide significance which are all located within the HLA region on chromosome 6. In order to pinpoint the causal mechanisms underlying the association we used a reference panel of > 5,000 European individuals to impute classical HLA genotypes for HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, and HLA-DRB1, their corresponding amino acid changes and SNPs. In total, we tested 10,899 variants across the HLA region for association with a logistic regression analysis.

Of all included variants, an insertion of 8 amino acids in HLA-DQB1 showed the strongest achalasia association (P = 1.73x10-19, relative risk (RR) = 2.43). Furthermore, the logistic regression analysis revealed that the amino acid lysine at protein position 41 of HLA-DQA1 confers independently achalasia risk (P = 5.60x10-10, RR = 1.89). However, amino acid alanine at HLA-DQA1 position 130 showed the same association, since both positions are in tight LD on the genomic level. Conditioning on the 8 amino acid insertion in HLA-DQB1 and on position 41/130 in HLA-DQA1 revealed a third achalasia association. The amino acid glutamic acid at protein position 45 of HLA-DQB1 was significant more frequent in patients compared to controls (P = 1.20x10-09, RR = 1.25). Conditioning on all four identified risk variants did not reveal any residual association at other HLA-DQ amino acid positions (P > 1x10-04).

Our data emphasise the hypothesis that autoimmune-relevant processes are playing an important role within the disease process of idiopathic achalasia.

W8 GENETIC TESTING AND COUNSELING

W8-01

Clinical utility of exome sequencing

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Exome sequencing has a great potential in genetic diagnostics, particularly if used for strong heterogeneous diseases were the diagnostic yield is currently low. The analysis and interpretation of exome data in diagnostics has specific challenges with respect to quality control, the possibility of incidental findings, and allowing for easy and flexible interpretation. We now have implemented a routine diagnostic exome sequencing workflow.

Six heterogeneous diseases are interrogated by exome sequencing: Intellectual Disability ('de novo' strategy), Blindness, Deafness, Movement disorders, Oncogenetics, and OXPHOS diseases (disease gene packages). The patient and/or legal representative need to sign an informed consent before exome sequencing can be requested by the clinical geneticist. This contains an agreement for sequencing their exome and subsequent reporting of all medically relevant findings, including possible findings not related to the initial enquiry. Sequence variants detected can be analyzed using a diagnostic-oriented user interface that automatically limits genetic findings to the relevant genomic loci for a disease, and allows interpretation using predefined filter schemes.

The results of a cohort of 300 patients (including 100 ID patients) will be presented. QC, cases, and pitfalls will be discussed. Data-analysis shows a median coverage of 67x per exome. With the analysis still ongoing, disease gene package analysis detects on average 378 variants with 2 to 18 private non-synonymous variants per patient. The 'de novo' approach for ID demonstrates its potential with ~25% of the ID-patients having causal mutations identified in known ID-genes.

This represents the first in-use approach to establish a genetic diagnosis of patients by exome sequencing.

W8-02

Array-CGH in routine prenatal diagnosis significantly increases diagnostic power

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Array comparative genomic hybridization (array-CGH) or molecular karyotyping has become a first tier diagnostic tool in the evaluation of pediatric patients with congenital anomalies or intellectual disability unraveling disease causing microaberrations in about 15% of such cases.

Therefore recently the use of array-CGH was also investigated in invasive prenatal diagnostics. After conventional karyotyping, several studies using BAC-, oligonucleotide or targeted arrays showed an

additional diagnostic yield of 7.6-9.1% in fetuses with ultrasound abnormalities, and 0.5% - 1.1% relevant microaberrations in pregnancies referred for advanced maternal age or parental anxiety.

However, reflected by the differing recommendations of different countries, experience with its use for clinical prenatal diagnosis is still relatively limited. We therefore investigated the possible advantages of routine high-resolution array-CGH in prenatal diagnosis by analyzing 260 cytogenetically normal prenatal samples with a combined copy-number-SNP array independently from the clinical indication.

We processed 214 chorionic villi, 44 amniotic fluid and 2 other samples and found an overall rate of apparently disease causing microaberrations in 4.2% of cases. Like in previous studies pathological microaberrations were increased to 9.2% in fetuses with abnormal ultrasound. However, we observed an unexpected high rate of 3.6% of pathological microaberrations in cases with a low pretest probability (advanced maternal age or parental anxiety), which was significantly higher than in previous studies. Additionally, we detected a microdeletion of a known X-linked mental retardation gene in a female fetus which led to the identification of the mother as a carrier with 25% risk for affected boys in future pregnancies. In order to clarify unknown rare variants, we also investigated the parents in 2.7% of all cases and found all but one inherited from a healthy parent. Thus only one de novo variant remained of unknown significance, but this fetus with increased nuchal translucency eventually turned out to carry a PTPN11 mutation associated with a severe Noonan syndrome phenotype. Of note, in one additional case a pathological microdeletion was limited to the placenta, which is to the best of our knowledge the first observation of this phenomenon for subtle copy number changes. Following genetic counseling in 82% of cases with apparent pathological microaberration the parents decided to terminate the pregnancy

In summary we show that prenatal array analysis is a powerful diagnostic tool not only in fetuses with abnormal ultrasound but also in pregnancies with advanced maternal age with an acceptable low rate of unclear results.

W8-03

Array-based determination of DNA methylation in children born small for gestational age

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Abstract

Several genes underlying parental imprinting have been shown to be involved in the regulation of prenatal growth, like the genes involved in Russell-Silver syndrome or transient neonatal diabetes mellitus. A main symptom of both named imprinting disorders is prenatal dystrophy. As imprinting is determined by differential DNA methylation, we hypothesized that prenatal growth restriction as seen in children born small for gestational age (SGA) could also be caused by aberrant DNA methylation. Therefore, we compared the DNA methylation patterns in 98 SGA born children to 20 normal controls. DNA was isolated from peripheral blood cells. Array-based methylation analysis was performed using the HumanMethylation450k BeadChip (Illumina) and findings were corroborated by DNA methylation measurements recently performed by bisulphite pyrosequencing (Bens et al., EJHG, in press).

During the first methylation analyses by bisulphite pyrosequencing we detected one SGA born child with hypomethylation at the MEG3 locus due to an epimutation. The clinical phenotype including short stature, small hands and feet, a prominent forehead, developmental delay and precocious puberty were consistent with this finding and led to the diagnosis of upd(14)mat syndrome (Temple syndrome). Pyrosequencing results were confirmed by HumanMethylation450k BeadChip analysis.

Furthermore, by performing genome-wide methylation analysis, we detected one SGA born child with a methylation defect at multiple loci involved in imprinting. Interestingly, the patient is known to have a bladder exstrophy. These results led to the conclusion, that imprinting defects exist, but are rare events in SGA born children.

In a second approach we compared the cohort of SGA born children with healthy controls on a genome wide level. A global comparison between those two cohorts revealed 188 differentially methylated loci ($p<0.01$; delta.bata>0.15). Gene ontology analyses of the differentially methylated CpGs showed enrichment for genes which are involved in the negative regulation of glucose and glycogen processes. These findings suggest that the condition SGA is associated with methylation defects beyond imprinted loci which might eventually explain the higher risk for metabolic diseases that are associated with this condition.

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W8-04

Validation of the breast cancer genetic risk models BOADICEA, IBIS, BRCAPRO, and Claus for predicting BRCA1/2 mutation carrier probabilities: a study based on 7,352 families from the German Hereditary Breast and Ovarian Cancer Consortium

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Routine genetic screening of BRCA1/2 cannot be offered to all patients presenting with breast or ovarian cancer because of the associated costs and possible adverse psychosocial effects for patients. To identify families with a high BRCA1/2 carrier probability either rule based methods or more sophisticated genetic risk prediction models are used in clinical genetic counselling. Despite their frequent use, the genetic risk models BOADICEA, IBIS, BRCAPRO, and Claus have never been comparatively validated. We present the results of the largest model validation study of its kind which provides robust estimates on the use of such models for referring high-risk individuals for BRCA1/2 screening. Furthermore it is the first one to evaluate the incremental predictive value of incorporating tumor pathology information in the BOADICEA algorithm. Recently, the model has been extended to account for tumour pathology information using independent data from ~5000 families.

Our results suggest that the extended CLAUS model should no longer be used for estimating mutation carrier probabilities in clinical settings. We demonstrate that BRCAPRO and BOADICEA have similar discriminatory ability, and BOADICEA is best calibrated. The use of tumor pathology information in BOADICEA resulted in a significant model improvement across all model performance measures. We will discuss the consequences of policy changes from rule based methods to mutation carrier estimation using a genetic risk model.

W8-05

Validation of the Manchester scoring system for predicting BRCA1/2 mutations in 9,390 families suspected of having hereditary breast and ovarian cancer

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Background: The probability to detect deleterious BRCA1 or BRCA2 mutations in index cases from families suspected of having hereditary breast or ovarian cancer depends on the type, number and age at diagnosis of cancers observed in the family. Accurate estimation of BRCA1/2 mutation probabilities prior to genetic testing is crucial to decide which families should undergo costly genetic testing. In 2004, Evans and colleagues proposed the so called Manchester Scoring System (MSS) for calculating the likelihood of identifying BRCA1 or BRCA2 mutations [1]. The MSS comprises 12 components representing the number of breast, ovarian, pancreatic and prostate cancers found at different ages in the family. In 2009, an updated version of the MSS was published, which additionally considers the breast cancer pathology of the index case [2,3].

Purpose: We aimed to determine the predictive performance of the MSS without (MSS-2004) and with (MSS-2009) using pathology information in a large sample of 9,390 families. Moreover, we aimed to analyse whether all components of the MSS are significantly predictive and if the diagnostic accuracy can be further improved in our sample by modifying the score values of each component.

Patients and Methods: Families were ascertained and registered within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC), using defined clinical criteria. One female member of each family (index patient), who had breast cancer, was searched for a deleterious mutation in BRCA1 and BRCA2. Pathology information comprised histology (lobular, ductal, invasive), grading, and the results of estrogen (ER), progesterone (PR) and Her2neu receptor status testing obtained from the index patient. For each index patient, the Manchester score values and BRCA1/2 mutation probabilities were calculated both for the MSS-2004 and the MSS-2009 as originally published by Evans. The re-calibrated MSS model (MSS-R) was developed using logistic regression analysis. For this analysis, the dataset was randomly split into one part used for model fitting (n=6,261, 67%) and another part used for validation (n=3,129, 33%). Receiver-Operating-Characteristics (ROC) analysis, sensitivities and specificities were used to characterize the performance of all three models.

Results: The area under the ROC curves (AUC) regarding a mutation in any or the two BRCA genes was 0.77 (95%CI 0.75-0.79) for MSS-2004, 0.80 (95%CI 0.78-0.82) for MSS-2009, and 0.82 (95%CI 0.80-0.83)

for MSS-R. Sensitivity at the 10% mutation probability threshold was similar for all three models (MSS-2004 92.2%, MSS-2009 92.2%, MSS-R 90.3%), but specificity of the MSS-R (46.0%) was considerably higher than of the MSS-2004 (25.4%) and the MSS-2009 (32.3%). In the MSS-R model, almost all components of the original MSS proved to be significantly predictive. However, some components were given different weights than originally proposed.

Conclusion: The original MSS performed well in our sample. The use of pathology information increased the predictive performance significantly. Re-calibration improved the specificity considerably without loosing much sensitivity.

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W8-06

Breast Cancer Risk Assessment – which role should nongenetic risk factors play?

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Background: Identifying women at higher risk for breast cancer is critical for determining when to start annual mammography screening and magnetic resonance imaging (MRI). Risk models are often used to identify such women; for example, in the U.S. a model-assigned lifetime risk of 20% or greater is used to identify high risk women. The models vary in how they handle family disease data as well as the genetic and non-genetic risk factors they include, and many clinicians use only models that include family history and genetic data. Understanding whether non-genetic risk factors can help improve risk prediction in women with extensive family histories would be important to effective clinical risk assessment. Patients and Methods: We assessed the performances of two commonly used risk models in a New York City cohort of 1,857 women without breast cancer who were followed on average for 8.1 years, and of whom 80 developed incident breast cancer. Both models include family history and genetic factors, but one model (IBIS) includes nongenetic factors and the other (BOADICEA) does not. We assessed accuracy through the Hosmer-Lemeshow (HL) goodness-of-fit statistic and discrimination by the area under the receiver operating characteristic curve (AUC), as well as sensitivity and specificity associated with the 20% high-risk threshold. Results: For these data, IBIS showed similar discrimination, but was better calibrated than BOADICEA. Agreement between assigned and observed risks was better for IBIS (HL X42 = 6.4, P value 0.17) than BOADICEA (HL X42 = 20.3, P value <0.001). Using the 20% threshold for high risk, 33% of subjects were discordantly classified by the two models. The models differed in sensitivity and specificity: IBIS was more sensitive than BOADICEA (51.2% vs 22.5% true positive rate) but less specific (38.8% versus 8.5% false positive rate). Conclusion: When using clinical risk thresholds to make decisions about screening intensity, it is important to have accurate assessments of women´s absolute breast cancer risks. Most clinicians use prediction models based on family history and genetics alone for women who may be of higher risk. These cohort data suggest that models incorporating nongenetic risk factors may help even in higher risk women to identify those most appropriate for intensive screening strategies.

W9 EPIGENETICS

W9-01

Deciphering the evolutionary origin of the RB1 imprint

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Previously we could show that the RB1 gene is imprinted. Skewed expression in favour of the maternal allele is due to a differentially methylated CpG-island within intron 2 of the RB1 gene. This CpG-island (CpG85) serves as a promoter for an alternative RB1 transcript and is part of a truncated processed

pseudogene (PPP1R26P1), which is derived from the PPP1R26 gene (previously KIAA0649) located on chromosome 9.

We could now narrow down the time interval of this retrotranspositional event by in silico analyses, which revealed that the ancestral gene PPP1R26 is present in all primates, whereas the pseudogene copy within the RB1 gene is only present in higher primates, which comprise Catarrhini (Old World Monkeys, Gibbons, Great Apes and Human) and Platyrrhini (New World Monkeys). Thus, the retrotransposition of PPP1R26 has occurred after the divergence of Strepsirrhini and higher primates, but before the split between Catarrhini and Platyrrhini. Although information for Tarsiidae as distant sister lineage to higher primates is lacking, the retrotransposition of PPP1R26 into the RB1 gene appears to coincide with the retrotranspositional explosion described by Ohshima et al., 2003. Moreover, the in silico analysis revealed that there are additional pseudogene copies on chromosome 22 in human and chimp, which must have been derived from independent retrotransposition events. Only the chimp and the marmoset have another copy on chromosome 8 and chromosome 4, respectively.

For further examination of the evolutionary origin of the RB1 imprint we compared the methylation patterns of the ancestral gene PPP1R26 and its pseudogenes in different primates (human, chimp, rhesus, orangutan and marmoset). Methylation analysis by next generation bisulfite sequencing on the ROCHE/454 GS Junior showed that the pseudogene copy within the RB1 gene is differentially methylated in all primates studied. All other copies are fully methylated except the additional copy on chromosome 4 in the marmoset, which seemed to be differentially methylated. By using an informative SNP for the methylation analysis in 8 individuals from 4 different families we could show that the methylation pattern of the copy on chromosome 4 in the marmoset is not parent-of-origin-specific, but allele-specific. We conclude that the epigenetic fate of a PPP1R26 pseudogene after integration depends on the DNA sequence and selective forces at the integration site.

W9-02

Overexpression of the 5-methylcytosine hydroxylases TET1 and TET3 causes gene expression changes in HEK293 cells

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In mammals, 5-methylcytosine (5mC) predominantly exists in the context of CpG dinucleotides. This DNA modification is often associated with gene repression and essential for normal development. To date, little is known about the mechanisms regulating DNA methylation dynamics. Recently, it has been shown that enzymes of the TET family can convert 5mC into 5-hydroxymethylcytosine (5hmC). 5hmC is found at low levels in the genome and several biological functions of 5hmC have been proposed. On the one hand, it could represent an intermediate product in active or passive DNA demethylation, on the other hand, it might also have direct effects by displacing 5mC-binding proteins or recruiting 5hmC-specific effectors. Apart from the conversion of 5mC into 5hmC it has been shown that Tet1 can also directly modulate transcription by recruiting a co-repressor complex to its target sites.

To further elucidate the functional role of the TET enzymes, we generated stable HEK293 cell lines that inducibly overexpress TET1 and TET3 about 2.5- and 10-fold, respectively. Overexpression was induced in three biological replicates for each gene of interest and verified by qRT-PCR and western blotting. Comparison of Affymetrix U133 Plus 2.0 gene expression profiles of induced versus uninduced cells revealed consistent deregulation of 21 genes in all TET1- and 116 genes in all TET3-overexpressing cells. These changes range from 2.3-fold up- to 2.3-fold downregulation and were exemplarily verified by TaqMan analyses. Interestingly, upregulation of six genes overlapped in all TET1- and in all TET3-overexpressing cell lines.

For correlating the expression changes with DNA methylation levels, we have analyzed the promoter CpG island of three of these genes (WIPI1, ASMTL, TRAPPC6A) in induced and uninduced cells at single-base resolution by next-generation bisulfite sequencing on the ROCHE/454 platform. Furthermore, we will investigate the effect of TET overexpression on the methylation status of Alu and LINE-1 repetitive elements, which are heavily methylated in wildtype cells. Complementary to the overexpression studies, work is in progress to analyze the effect of siRNA-mediated knockdown of TET1, 2 and 3. Our data advances the understanding of the epigenetic control of gene expression.

W9-03

Different DNA methylation of FOXP2 target genes in adult human and chimpanzee cortices - epigenetic aspects of human language development

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The molecular mechanisms governing language development in the human brain are largely unknown. Up to now, mutations in the gene encoding the transcription factor FOXP2 (forkhead box P2) are the only reported monogenic cause of language and speech disorders in humans (Lai et al., 2001, Nature 413:519–523). A recent study analyzing differential gene expression in human neuronal cells overexpressing human or chimpanzee FOXP2 as well as in human and chimpanzee brain provided evidence for human-specific transcriptional regulation of FOXP2 target genes to be implicated in the development and evolution of language circuitry in humans (Konopka et al., 2009). We set out to investigate if the observed differential expression of FOXP2 target genes may be due to differential methylation of their promoter regions. Therefore, we quantified the overall and single CpG methylation levels of 18 differentially expressed target genes and, in addition, the known FOXP2 target gene CNTNAP2 in adult frontal cortices of 13 humans and 6 chimpanzees using bisulphite pyrosequencing. Two genes, NPTX2 and GJA12 (GJC2), displayed mean methylation levels that were significantly different in human and chimpanzee samples ($p < 0.05$). For both genes, the mean methylation levels were higher in the human brains (7.6% for NPTX2 and 37.3% for GJA12) than in the chimpanzee brains (4.7% for NPTX2 and 27.5% for GJA12). The mean methylation levels of the other analyzed genes were not significantly increased or decreased. For the promoter region of the GJA12 gene whose mutations are associated with the Pelizaeus-Merzbacher-like-disease (PMLD) a condition characterised by delayed speech development and motor impairments, we currently confirm these results using a more in-depth methylation analysis of 11 CpG sites by classical bisulphite sequencing. Of the up to now analyzed three human and four chimpanzee brain samples, the human samples (aged between 24 and 40 years) showed mean methylation levels between 45% and 69% whereas three of the four chimpanzee samples (aged between 14 and 43 years) displayed mean methylation levels between 11% and 24%. The 4th chimpanzee sample aged 7 years had a mean methylation level of 50%, similar to those of the human samples. Overall mean methylation levels were 54.89% for the three human samples and 26.27% for the four chimpanzee samples. The observed higher methylation levels in human brains correlate very well with the data of Konopka et al. (2009) that showed a downregulation of GJA12 expression in both neuronal cells overexpressing human FOXP2 and human brain samples. Thus, our findings suggest that differential methylation of regulatory regions is involved in regulating differential expression of at least a subset of FOXP2 target genes in human and chimpanzee brains. Moreover, the methylation level of 50% that was detected in one brain sample from a pubertal chimpanzee at the age of 7 years and similar to those of the adult human brain samples indicates a possible strong influence of age or other factors on GJA12 methylation profiles. Our study provides additional support for the use of comparative methylation analyses as an important method to detect evolutionary changes in gene regulation that have occurred in human-specific brain development and function.

W9-04

Broad DNA Methylation Changes of Spermatogenesis, Inflammation and Immune Response Related Genes in a Subgroup of ART Sperm Samples

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Background: Aberrant sperm DNA methylation patterns, mainly in imprinted genes have been associated with male subfertility and oligospermia. Here we performed a genome-wide methylation analysis in sperm samples representing a wide range of semen parameters.

Methods and Results: Sperm DNA samples of 38 males attending a fertility center were analyzed with Illumina HumanMethylation27 BeadChips, which quantify methylation of >27,000 CpG sites in cis-regulatory regions of almost 15,000 genes. In an unsupervised analysis of the M-values of all analyzed sites the samples clustered into two major groups. When correlating the clusters with semen and clinical parameters, the sperm counts were significantly different between groups with the minor group exhibiting sperm counts in

the low normal range. Linear modeling with Limma identified almost 3,000 CpGs with significant methylation differences between groups. Functional analysis (GO enrichment) revealed a broad gain of methylation in spermatogenesis related genes and a loss of methylation in inflammation and immune-response related genes. Quantitative bisulfite pyrosequencing validated differential methylation in four of 5 significant candidate genes on the array. Methylation profiling (by pyrosequencing) of the spermatogenesis-related genes INSL6, MAEL, SLC25A3, and PIWIL2 was used to classify 57 additional sperm samples into a major and a minor group, consistent with the results of microarray analysis. The quality of this classification did not differ between test and training data sets.

Conclusion: We identified a subgroup of ART sperm samples with sperm counts in the lower range and broad methylation changes (affecting approximately 10% of analyzed CpG sites) in specific pathways, most importantly spermatogenesis-related genes. Epigenetics can supplement traditional semen analysis and has the potential to provide new insights into the etiology of male subfertility.

W9-05

Frequency and Characterization of Multiple Locus Methylation Defects (MLMD) amongst patients with classical Imprinting disorders

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In the year 2006 Mackay et al. (Hum Genet, 2006) described for the first time patients with a maternal hypomethylation syndrome in a cohort of patients with transient neonatal diabetes mellitus. Since then, a number of studies investigated the frequency of multiple locus methylation defects among cohorts of patients with Russell-Silver syndrome and Beckwith-Wiedemann syndrome. On the basis of these reports the BMBF-funded consortium 'Diseases caused by imprinting defects: clinical spectrum and pathogenetic mechanisms' aims at systematically investigating the frequency and characteristics of multiple locus methylation defects among patients with classical imprinting disorders. Since 2009 we included 127 patients with epimutations causing classical imprinting disorders. Among this cohort there were 6 patients with a known methylation defect at more than one locus (3 with a phenotype of Silver-Russell syndrome and one with a phenotype suggestive of pseudohypoparathyroidism). DNA samples derived from peripheral blood of all patients, were screened for DNA methylation values at the imprinted loci PLAGL1, IGF2R, GRB10, H19 3 CTCF, IGF2, MEG3, NDN, SNRPN, NESP and NESPAS by bisulfite pyrosequencing. Normal methylation ranges were calculated on the basis of methylation values obtained from 50 healthy controls (25 males and 25 females) of Caucasian origin. In addition to the six patients with previously known MLMD, we detected aberrant methylation values of multiple loci in further 15 patient samples. However, defining cutoffs for aberrant methylation values turned out to be quite a challenge in the light of frequent mosaic findings of methylation defects in the recent literature. Thus, on the basis of the bisulfite pyrosequencing results we selected 25 cases (6 previously diagnosed patients with MLMD, 13/15 cases with MLMD after pyrosequencing analysis and 6 positive controls with known single locus epimutations) for array-based genome wide DNA methylation analysis using the Infinium HumanMethylation450 BeadChip (Illumina). Diagnosis of MLMD was confirmed for the 6 patients with previously diagnosed MLMD and established for further four of the analyzed patients.

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W9-06

Epigenetic analysis of risk-associated variants in age-related macular degeneration in the human retina.

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Genome wide association studies and meta-analysis of genome wide association studies have identified multiple disease-associated haplotypes influencing risk of developing age-related macular degeneration (AMD). However, a number of those findings are not readily in a context of a protein-coding gene locus and thus lack obvious causal variants. It appears possible though that such intergenic variants influence gene

expression of cis- or trans-localized genes by interfering with transcription factor binding at regulatory regions.

The present study aims to identify potential regulatory regions in the human retina by a genome-wide analysis of epigenetic markers. Therefore, we performed chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) of three healthy human retinas with antibodies against histone H3 lysine 4 monomehtylation (H3K4me1) and histone H3 lysine 27 acetylation (H3K27ac). Regions within 2kb up- or downstream of known transcription start sites were excluded from analysis. Remaining sites that showed a more than threefold enrichment for H3K4me1 or H3K27ac were considered to be potential regulatory hot spots. We show that within these regions, the most abundant motif (found in approx. 30% of all sites) corresponds to the cone-rod homeobox (CRX) binding site. Additionally, we found several transcription factor binding motifs highly similar to the consensus sites for regulatory factors including CTCF, Mef2a/c or NR2E3. We further show that several AMD risk variants lie within those regulatory hot spots and can therefore potentially alter transcription factor binding and hence, influence the function of the regulatory element. The impact of AMD risk associated variants on transcription levels of near-by genes is currently subject to further analysis.

W10 CLINICAL GENETICS II

W10-01

The Coffin-Siris syndrome – clinical phenotypes of 35 previously unreported patients and mutational spectrum of the SWI/SNF complex

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Coffin-Siris syndrome (CSS) is a well-known condition characterized by coarse facial features, sparse scalp hair, hypertrichosis, hypoplastic or absent fifth finger- and toenails and developmental delay. Dominant mutations of SMARCB1, SMARCA4, SMARCA2, SMARCE1, ARID1A and ARID1B encoding proteins of the SWI/SNF chromatin remodeling complex have been identified in patients with CSS (Tsurusaki et al., 2012; Santen et al., 2012).

We collected clinical data of 35 patients with a tentative diagnosis of CSS. To identify the causative genes in CSS we performed exome sequencing (Nimblegen EZ Exome SR, v2) on four parent-patient trios with severely affected patients as well as two single patients with milder clinical features of CSS. We identified four heterozygous mutations in four different genes of the SWI/SNF complex (p.R374Q in SMARCB1, p.Y37S in SMARCE1, p.R1989* in ARID1A and p.R750* in ARID1B). Exome sequencing was suggestive of mosaicism for an ARID1A mutation in the blood of the fourth patient and was confirmed by Sanger sequencing. In the fifth patient, a female, a de novo mutation in the PHF6 gene, causative for Borjeson-Forssman-Lehmann syndrome (BFLS), was found. This gene is not considered to be part of the SWI/SNF complex, but interacts with the nucleosome remodeling and deacetylation (NuRD) complex also implicated in chromatin regulation (Todd et al., 2012). All five mutations were confirmed by Sanger

sequencing and occurred de novo in the patients. In the sixth patient the causative mutation was identified after HaloPlex Target Enrichment (Agilent) followed by next generation sequencing. This patient carried a mutation within exon 1 of ARID1B (p.Q514Pfs*21), which was not captured by exome sequencing. In one additional patient, genotyping analysis using an Affymetrix SNP array revealed a heterozygous deletion containing the ARID1B gene.

In the remaining 28 patients with CSS, we performed mutational analysis of 18 genes encoding proteins of the SWI/SNF complex using the HaloPlex Target Enrichment System followed by next generation sequencing. These analyses revealed only truncating ARID1B mutations in eleven additional patients. In all families available for testing (n=6) the mutation occurred de novo. In addition, Sanger sequencing of PHF6 in eight patients without any mutation within the SWI/SNF complex, revealed one additional causative de novo mutation in this gene. This leads to the assumption that the early phenotype of BFLS closely resembles CSS and therefore is an important differential diagnosis to CSS. No further causative mutations in the remaining 16 patients were identified.

In total, we identified bona fide mutations in 19/35 (54%) patients with CSS, 14 of these (74%) within ARID1B. We will present the mutational spectrum, discuss differential diagnoses and give an update on genotype-phenotype correlation in CSS.

W10-02

Clinical and mutation data in twelve patients with tentative diagnosis of Nager syndrome

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Nager syndrome (MIM #154400) is the best known preaxial acrofacial dysostosis, characterized by craniofacial and preaxial limb anomalies. The craniofacial abnormalities mainly consist of downslanting palpebral fissures, malar hypoplasia, micrognathia, external ear anomalies, and cleft palate. The preaxial upper limb defects are characterized by radial and thumb hypoplasia or aplasia, duplication of thumbs and proximal radioulnar synostosis.

Haploinsufficiency of SF3B4 (MIM *605593), which encodes SAP49, a component of the pre-mRNA spliceosomal complex, has recently been identified as the underlying cause of Nager syndrome. In our study, we performed exome sequencing in two and Sanger sequencing of SF3B4 in ten previously unreported patients with tentative diagnosis of Nager syndrome, including one familial case. We identified heterozygous truncating SF3B4 mutations in seven out of twelve patients. Two of the seven mutations were shown to be de novo; in five cases, parental DNA was not available. No familial mutations were discovered. Three of the mutations were frameshift, c.1147delC (p.His383Metfs*75), c.546dupC (p.Lys183Glnfs*3), and c.737dupC (p.Pro246Profs*240). One was a nonsense mutation affecting the translation start codon, c.2T>C in exon 1, and three further were downstream nonsense mutations, c.382C>T (p.Gln128*), c.574G>T (p.Glu192*), and c.1006C>T (p.Arg336*). Comparing the clinical presentation of patients with and without SF3B4 mutations, cleft palate, hearing loss and thumb aplasia occurred more frequently in mutation positive patients, but not significantly so. The only significant finding was that midface hypoplasia or retrusion were present more frequently in patients with than in those without mutation. The finding that only half of the patients examined have point mutations in SF3B4 can have the following reasons, i) the other patients have SF3B4 mutations which cannot be identified by sequence analysis like intragenic deletions or duplications, ii) Nager syndrome is genetically heterogeneous or, iii) the mutation negative patients have a condition other

than Nager syndrome but with overlapping features. In order to address the possibilities stated above, additional molecular studies like copy number analyses and exome sequencing must be performed.

W10-03

Deficiency for the Ubiquitin Ligase UBE3B in a Blepharophimosis-Ptosis-Intellectual Disability Syndrome

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Ubiquitination plays a crucial role in neurodevelopment as exemplified by Angelman syndrome which is caused by genetic alterations of the ubiquitin ligase-encoding UBE3A gene. While the function of UBE3A has been widely studied, little is known about its paralog UBE3B. Using exome and capillary sequencing, we here identify biallelic UBE3B mutations in four patients from three unrelated families presenting an autosomal-recessive blepharophimosis-ptosis-intellectual disability (BPID) syndrome characterized by developmental delay, growth retardation with a small head circumference, facial dysmorphisms, ectodermal anomalies, and low cholesterol levels. UBE3B encodes an uncharacterized E3 ubiquitin ligase. The identified UBE3B variants include one frameshift and two splice-site mutations as well as a missense substitution affecting a highly conserved residue of the HECT domain. By using in situ hybridisation, we show that the murine ortholog Ube3b is expressed in the specific regions of the central nervous system and in craniofacial structures, an expression pattern that is relevant to the phenotype observed in individuals with the human syndrome. Moreover, we demonstrate that disruption of mouse Ube3b leads to reduced viability and recapitulates key aspects of the human disorder, such as reduced weight and brain size and a downregulation of cholesterol synthesis. We establish that the probable Caenorhabditis elegans ortholog of UBE3B, oxi-1, functions in the ubiquitin/proteasome-system in vivo and is especially required under oxidative stress conditions. Our data reveal the pleiotropic effects of UBE3B deficiency and reinforce the physiological importance of ubiquitination in neuronal development and function in mammals.

W10-04

Genome sequencing reveals a mutation in the TUBB4 gene as the cause of whispering dysphonia (DYT4 dystonia)

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Hereditary "whispering dysphonia", later designated DYT4, was first described in a large Australian family and follows an autosomal dominant mode of inheritance with high penetrance. The phenotype is clinically characterized by generalized dystonia including cranio-cervical dystonia and prominent spasmodic dysphonia and shows variable expressivity. Additional characteristic clinical features beyond the motor phenotype have been reported including a thin face and body habitus.

After exclusion of mutations in other dystonia genes, we carried out a genome-wide linkage analysis in 14 family members from the original DYT4 family. The disease-causing gene was mapped to a 23-cM region

on chromosome 19p13.3-p13.2 with a maximum multipoint LOD score of 5.338 at markers D9S427 and D9S1034. Next, we performed genome sequencing in two distantly related individuals from the family and identified two novel, protein-changing mismatches to the reference sequence in the linked region shared by both patients. Both missense variants in the Tubulin beta-4 (TUBB4; Arg2Gly) and the DOT1-like, histone H3 methyltransferase (DOT1L; Arg908His) gene were present in all affected family members. However, only the mutation in TUBB4 was absent in unaffected family members and 1,000 control chromosomes and thus considered disease-causing. The index patient underwent a detailed neurological follow-up examination including electrophysiological studies and MRI scanning. Biopsies of the skin and olfactory mucosa were obtained. TUBB4 mRNA levels were significantly reduced in this Arg2Gly mutation carrier compared to controls as determined by quantitative real-time PCR in three different cell types including lymphoblasts, fibroblasts, and olfactory cells. Sequencing all exons of TUBB4 in 394 unrelated dystonia patients revealed another missense variant (Ala271Thr) in a woman with segmental dystonia including spasmodic dysphonia.

In conclusion, a mutation in TUBB4 causes DYT4 dystonia in the Australian family with “whispering dysphonia” and other mutations in TUBB4 may contribute to spasmodic dysphonia. Given that TUBB4 is a neuronally expressed tubulin, our results imply abnormal microtubule function as a novel mechanism in the pathophysiology of dystonia.

W10-05

Identification of TRAP1 as a new disease causing gene for isolated CAKUT and the VATER/VACTERL association

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Congenital abnormalities of the kidney and urinary tract (CAKUT) account for 50% of all chronic kidney disease in children. CAKUT also occur as the renal component of the VATER/VACTERL association in up to 60% of patients. The acronym VATER/VACTERL association describes the combination of at least three of the following congenital anomalies: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). Although there are several mouse models of recessive CAKUT, only a few genes are known to cause CAKUT in humans. To identify novel CAKUT genes we combined homozygosity mapping, whole exome capture using NimbleGen’s SeqCaP EZ Exome™ protocol and NextGeneration sequencing using Illumina Genome Analyzer II in 14 CAKUT families (27 patients) including 4 VATER/VACTERL families. We detected a homozygous missense mutation (R469H) in the TNF Receptor Associated Protein 1 (TRAP1) gene in two unrelated families presenting with isolated CAKUT and CAKUT as part of the VATER/VACTERL association. By screening 960 additional CAKUT patients including 196 patients with VATER/VACTERL association using the Fluidigm Acces Array for High Throughput exon resequencing followed by Next generation sequencing (Illumina High Seq2000) we identified one additional isolated CAKUT and one additional patient with VATER/VACTERL association carrying recessive TRAP1 mutations. All mutations were either truncating or highly conserved missense mutations, predicted to be ‘damaging’ by PolyPhen algorithm. None of the mutations had been found to occur homozygous in the human variant databases EVS and 1000 Genomes. An extensive literature search with focus on non-isolated CAKUT identified one more patient with Rubinstein Taybi syndrome and additional CAKUT. Besides the previously described deletion comprising CREBBP and TRAP1 on one allele, we identified a second deletion comprising exon 14-18 of TRAP1 on the other allele. While the deletion of CREBBP caused the Rubinstein Taybi phenotype in the patient, the CAKUT phenotype is explained by the bi-allelic deletion of TRAP1. Finally Immuno Fluorescent staining of rat kidney showed expression of TRAP1 in the mitochondria of proximal renal tubules and collecting ducts. In summary our

results suggest TRAP1 as a recessive disease-causing gene for isolated CAKUT and CAKUT as part of the VATER/VACTERL association.

W10-06

Mosaicism for a deletion of the MEG3-DMR in a healthy mother of two children with upd(14)pat phenotype – Insights in the complex regulation of the 14q32 imprinting region

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Deregulation of imprinting in 14q32.2 results in different phenotypes, currently known as maternal or paternal uniparental disomy 14 phenotypes (upd(14)mat, upd(14)pat). In both syndromes, three types of molecular alterations have been reported, i.e. uniparental disomy, deletions and epimutations. In contrast to uniparental disomy and epimutations, deletions affecting regulatory elements in 14q32.2 on the maternal or the paternal allele are associated with a high recurrence risk. The 14q32.2 imprinted region harbours two differentially methylated regions (DMRs), the intergenic DMR between DLK1 and MEG3(IG-DMR) and the MEG3-DMR. Both DMRs function as imprinting control centers in the body, but a functional hierarchy of the IG-DMR as a regulator of the MEG3-DMR has been postulated based on a maternally inherited deletion in a patient affecting the MEG3 promoter but not the IG-DMR (Kagami et al., 2010).

We here report on a family with a similar deletion of 5.8 kb affecting the MEG3-DMR but not the IG-DMR. The deletion could be detected by MLPA but was too small to be detected by SNP array analysis. The deletion was identified in two sibs (now 5 and ½ year old) exhibiting the upd(14)pat syndrome. It could also be detected in the healthy mother who was mosaic for the deletion. Methylation studies revealed hypomethylation at the MEG3-DMR in the mother, indicating that she has the deletion on her paternal chromosome 14. However, she did not show any clinical signs compatible with upd(14)mat, probably due to the low degree of mosaicism. In contrast, her children with the non-mosaic maternal MEG3-DMR deletion and a hypermethylation at the MEG3-DMR showed typical upd(14)pat syndrome features (e.g. bell-shaped thorax). Interestingly, the phenotype was less severe than reported in the literature. Both sibs show a normal methylation pattern for the IG-DMR as described in the patient reported by Kagami et al. The normal IG-DMR methylation pattern in our patients corroborates the hypothesis of a hierarchic regulation of the DMRs in 14q32.2 and thus allows interesting insights in the complex regulation of other imprinted domains.

W11 BASIC MOLECULAR MECHANISMS

W11-01

A mouse model for distal renal tubular acidosis reveals a previously unrecognized role of the V-ATPase a4 subunit in the proximal tubule.

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The V-ATPase is a multisubunit complex that transports protons across membranes. Recessive mutations in the gene encoding the a4 and the B1 subunit of the V-ATPase lead to distal renal tubular acidosis (dRTA) and hearing loss of variable degree.

In the kidney, the a4 subunit is expressed in intercalated cells of the distal nephron, where the V-ATPase controls acid/base secretion, and in proximal tubule cells, where its role is less clear. Apart from metabolic acidosis, a significant number of dRTA patients also share symptoms with patients suffering from Dent´s disease, which is a disorder of the proximal tubule resulting in nephrocalcinosis, polyuria, dehydration, and hypokalemia.

To study the complex pathogenesis arising from at least two distinct cell populations expressing the a4 subunit, we disrupted the a4-subunit encoding gene Atp6v0a4 in mice. As expected, a4 subunit deficient mice developed severe metabolic acidosis. A more detailed analysis of the distal tubule revealed a reduced number of acid- and base secreting intercalated cells. The reduced numbers in acid- and base-regulating

cells were further accompanied by cellular pathologies, e.g. reduced vesicle numbers, providing further insight into the distal acid secretion defect. Supporting a critical function for the a4 subunit in the proximal tubule a4 deficient mice displayed phosphaturia, proteinuria, and a defect of the degradative pathway of proximal tubule cells with accumulation of lysosomal material. In the inner ear, the a4 subunit co-localized with pendrin at the apical side of epithelial cells lining the endolymphatic sac. As a4 knock out (KO) mice were profoundly deaf and displayed enlarged endolymphatic fluid compartments mirroring the alterations in pendrin KO mice, we propose that pendrin and the proton pump co-operate in endolymph homeostasis.

Overall, the strong phenotype observed in our a4 subunit KO mice is in clear contrast to B1 subunit KO mice, which show only minor symptoms of dRTA. This prompted us to perform a systematic reanalysis of patient data between a4 and B1 related dRTA. Indeed, reanalysis of clinical data revealed that a4 related dRTA patients were more severely affected as compared to patients with mutations of the B1 subunit. Most notably, age of diagnosis, blood pH and dehydration were significantly severe in a4 related dRTA.

Thus, our mouse model gives unique and novel insights into the divergent functions of the V-ATPase and the pathophysiology of a4 related symptoms.

W11-02

SAMHD1 Knock Out Mice Model A Key Feature Of Autoinflammatory Pathogenesis

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Unbalanced release of type I interferon (IFN) is a hallmark of systemic lupus erythematosus (SLE). Also Aicardi-Goutières syndrome (AGS), an early-onset inflammatory encephalopathy which clinically and pathogenetically overlaps with SLE, is characterized by IFN production in the absence of detectable viral infection. The finding that AGS is caused by defects of nucleic acid metabolizing enzymes like Trex1, RnaseH2 and ADAR1 led to a concept of autoimmunity caused by intracellular nucleic acid accumulation, triggering a spontaneous pathogenic antiviral response through the induction of IFN. AGS is also caused by defects of SAMHD1, a phosphohydrolase which cleaves dNTPs and thereby prevents retroviral reverse transcription. Herein we show that also mouse SAMHD1 restricts retroviral replication by lowering the cellular dNTP concentration. More interesting, the absence of SAMHD1 caused spontaneous transcriptional upregulation of type I IFN-induced genes in various cell types indicative of spontaneous IFN production in SAMHD1 deficient mice. This finding strongly suggests that the animals reproduce an important aspect of AGS and SLE pathogenesis.

W11-03

A novel and elegant method for the identification of homozygous causative variants

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Proper and successful filtering of the huge amount of variants detected in exome sequencing data is a new challenge for the identification of variants causing monogenic disorders. To reduce the amount of putative disease causing variants, we designed and performed a new whole-exome sequencing strategy. We mixed equal amounts of DNA of four patients of a consanguineous Turkish family with oto-facial-cervical syndrome clinically characterized by ear anomalies, hearing impairment, bilateral preauricular fistulas, protruding shoulders, winging scapula and mild intellectual disability. We filtered for variants presented in >95% of reads and a coverage >10 reads. Only a single, novel variant fulfilled these criteria, the c.G497T (p.G166V) variant in the PAX1 gene. PAX1 encodes a transcription factor with a critical role in pattern formation during embryogenesis in vertebrates and the mutation is located within the paired box domain resembling the DNA binding motif of the transcription factor which is highly conserved throughout different species. In order to determine the underlying molecular pathogenesis, we performed a dual luciferase reporter assay and observed a significantly reduced transactivation of a regulatory sequence in the Bapx1 promoter region as a direct target of Pax1 transcriptional regulation in HEK293T cells overexpressing Pax1p.G166V in comparison to Pax1WT expressing cells. Our results indicate a reduced DNA binding affinity of the mutant protein.

Taken together, we designed a novel, elegant and cost effective strategy for disease gene identification in consanguineous families. With this new strategy we were able to identify PAX1 as a disease causing gene for the oto-facio-cervical syndrome being part of the EYA-SIX-DACH-PAX network, which is important for early embryogenesis.

W11-04

A Novel Method for Detecting the Load of Somatic Mutations in Next Generation Sequencing Data

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The accumulation of somatic mutations is considered to be one of the most fundamental drivers of both the aging process and cancerogenesis. On the one hand, collectively, such mutations would slowly but inexorably lead to the inability of the cells to produce functional proteins and thus decrease tissue function and vitality, leading to aging. On the other hand, single specific mutations can hit oncogenes or tumorsuppressors, leading to tumor formation, with an increaesd probability depending on the total amount of somatic mutations. In support of these theories, many congenital accelerated-aging and cancer-susceptibility syndromes are caused by mutations in genes involved in the maintenance of genomic stability. Next Generation Sequencing (NGS) data is extensively used in aging and cancer research, for the identification of novel genes involved in familiar and sporadic tumorigenesis and premature and physiological aging. We describe a novel method for the quantitative measurement of the load of somatic mutations in a tissue using NGS technology.

We show that with appropriate filtering, it is possible to detect biologically-relevant low-frequency variants above the noise of technical artifacts introduced by the amplification step, base calling and misalignment. We demonstrate that the predicted severity of somatic mutations correlates with their impact on the clonal expansion of hematopoietic cells. Moreover, we identify clusters of genes, involved in translation and in immune-system functionality, which are especially protected from accumulating severely damaging mutations in leukocytes. Finally, we report on our initial measurement of the load of somatic mutations in blood cells from patients with congenital progeroid syndromes, including cutis laxa with progeroid features, Wiedemann-Rautenstrauch syndrome, Seckel syndrome and an unclassified progeria case.

This new method for the detection of somatic mutations in NGS data increases the usefulness of such technology in aging and cancer research and opens up the possibility of analyzing novel molecular mechanisms underlying aging-associated phenotypes.

W11-05

Higher order chromatin organization provides insights into the propagation of segmental duplications

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Segmental duplications (SDs) are DNA sequences larger than 1 kb, which can be found at least twice in the genome with more than 90% sequence similarity. As such they play an important role in the plasticity of the human genome and represent an important factor in molecular evolution. At the same time, however, SDs endanger individual health as their presence increases the probability for chromosomal rearrangements and thus promotes the emergence of genomic disorders such as the Williams Beuren syndrome. SDs show considerable bias in their intra- and interchromosomal distribution indicating variable susceptibility to SD insertion.

On the example of chromosome 7, which is particular rich in intrachromosomal SDs, we evaluated frequency and sequence similarity of SDs in the context of higher order chromatin and nuclear organization. Taking advantage of public Hi-C data we can demonstrate that SD paralogs, which were split up by the paracentric inversion that distinguishes human chromosome 7 from its gorilla ancestor, are still in close spatial proximity and that this might influence the rate of sequence homogenization by gene conversion. Our analysis further revealed that chromosomal segments separated in the course of evolution retain their spatial proximity through long distance interactions and that this accumulation of long distance interactions

coincides with hot spots of SD insertion. Hi-C data also suggest that the typical deletion leading to Williams Beuren syndrome encompasses a distinct topological domain.

W11-06

The 5-phosphatase OCRL mediates retrograde transport of the mannose 6-phosphate receptor by regulating a Rac1-cofilin signalling module

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Mutations in the OCRL gene encoding the phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) 5-phosphatase OCRL cause Lowe syndrome (LS), which is characterized by intellectual disability, cataracts and selective proximal tubulopathy. OCRL localizes membrane-bound compartments and is implicated in intracellular transport. Comprehensive analysis of clathrin-mediated endocytosis in fibroblasts of patients with LS did not reveal any difference in trafficking of epidermal growth factor, low density lipoprotein or transferrin, compared with normal fibroblasts. However, LS fibroblasts displayed reduced mannose 6-phosphate receptor (MPR)-mediated re-uptake of the lysosomal enzyme arylsulfatase B. In addition, endosome-to-trans Golgi network (TGN) transport of MPRs was decreased significantly, leading to higher levels of cell surface MPRs and their enrichment in enlarged, retromer-positive endosomes in OCRL-depleted HeLa cells. In line with the higher steady-state concentration of MPRs in the endosomal compartment in equilibrium with the cell surface, anterograde transport of the lysosomal enzyme, cathepsin D was impaired. Wild-type OCRL counteracted accumulation of MPR in endosomes in an activity-dependent manner, suggesting that PI(4,5)P2 modulates the activity state of proteins regulated by this phosphoinositide. Indeed, we detected an increased amount of the inactive, phosphorylated form of cofilin and lower levels of the active form of PAK3 upon OCRL depletion. Levels of active Rac1 and RhoA were reduced or enhanced, respectively. Overexpression of Rac1 rescued both enhanced levels of phosphorylated cofilin and MPR accumulation in enlarged endosomes. Our data suggest that PI(4,5)P2 dephosphorylation through OCRL regulates a Rac1-cofilin signalling cascade implicated in MPR trafficking from endosomes to the TGN.

POSTER

P-BASIC MOLECULAR MECHANISMS

P-Basic-001

Morphological characterization of murine neurons exhibiting hyperactivation of the Ras-MAPK signaling pathway

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Constitutional dysregulation of the Ras-mitogen activated protein kinase (MAPK) signaling pathway can lead to Noonan Syndrome, Neurofibromatosis type 1 (NF1), Cardio-Facio-Cutaneous Syndrome (CFCS) or similar disorders, collectively called “Rasopathies”. These disorders are characterized by an overlapping pattern of physical abnormalities and variable cognitive impairment ranging from mild to severe. Their common molecular basis is an overactivation of the Ras-MAPK signaling pathway due to mutations in the genes of components or modulators of this signaling cascade. In animal models, it has exemplarily been shown that mutations in the homologous genes can lead to impaired cognitive function and reduced synaptic plasticity. However, the molecular pathogenesis for the cognitive impairment still remains to be elucidated. This study was aimed at further investigating the consequences of dysregulated Ras-MAPK signaling in neuronal cells.

We used a conditional knock-in mouse model expressing the oncogenic allele Ptpn11D61Y (kindly provided by Benjamin Neel, Ontario Cancer Institute, Toronto) to characterize neuronal morphological changes. The oncogenic Ptpn11D61Y mutation affects the same position as a common mutation found in patients with Noonan syndrome, Ptpn11D61G, but is thought to exert stronger activating effects. With an adapted protocol from Kaech and Banker (2007) we prepared primary cultures of dissociated neurons from newborn Ptpn11D61Y animals and control littermates. We established a lentivirus-mediated cre recombinase system to infect cells with a cre-expressing virus (cre cDNA was kindly provided by Pascal Kaeser, Harvard Medical School, Boston), thereby activating the knock-in mutation.

No major morphological changes were found in axon length and number of branching by Sholl analysis in 4 days in vitro cultures. Further analysis like growth cone size and spine analyses are ongoing. Our preliminary results indicate that neuronal dysfunction in patients might be caused mainly by functional changes in neurons that do not grossly alter axonal growth and branching.

P-Basic-002

Expression analyses in upd(7)mat carriers enlightens the pathoaetiology of Silver-Russell syndrome

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Silver-Russell syndrome (SRS) is characterized by primordial growth retardation, asymmetry, and a typical facial gestalt . So far, two genomic regions are known to be associated with the disease: Nearly 10% of patients show a maternal uniparental disomy (UPD) of chromosome 7 (upd(7)mat), more than 40% of SRS patients carry a hypomethylation of the Imprinting Control Region 1 (ICR1) in 11p15 regulating the expression of the H19 and IGF2 genes. On chromosome 7, several genes are consistently discussed to contribute to the pathophysiology of SRS: GRB10 in 7p13 as well as COPG2 and MEST in 7q32, are discussed because of their imprinting status, their expression patterns and their putative function in the embryonic development delineated from mouse models. In 11p15, in particular the genes H19, IGF2 and CDKN1C are interesting candidates as they exhibit similar properties like the chromosome 7 factors. In addition to these factors localized in the SRS candidate regions, further imprinted genes are conceivable to contribute to the SRS phenotype via a so-called “imprinted genes network”. To enlighten the functional interactions between chromosome 7-encoded factors and further imprinted genes, we performed expression studies on fibroblasts in three patients with complete upd(7)mat and a further carrier of a upd(7q)mat/MEG3

hypomethylation. By qRT-PCR we determined the influence of the observed UPD on the expression of the genes MEST, GRB10 and COPG2 on chromosome 7, H19, IGF2 and CDKN1C in 11p15 and MEG3 in 14q32. Thereby we could confirm that upd(7)mat indeed reduces the expression of MEST, whereas hetereogeneous expression patterns of the other tested factors could be observed. In total, the investigations in of upd(7(q))mat carriers, among them one with an additional MEG3 hypomethylation, allow interesting insights into the complex regulation of parentally imprinted regions and their disturbances.

P-Basic-003

Functional analysis of SHANK2 mutations in neurodevelopmental and psychiatric disorders

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Whole exome sequencing is used to investigate the genetic causes of intellectual disability (ID) and autism spectrum disorder (ASD) and multiple studies identified a large number of rare de novo mutations of unclear significance. Many genes, that have been associated with neurodevelopmental and psychiatric diseases, encode for synaptic proteins (e.g. Neuroligin 3, Neurologin 4, SHANK1, SHANK2, SHANK3, Neurexin1, Grin2A/B). Mutation prediction tools that are based on evolutionary sequence conservation and chemical properties of amino acids are helpful to estimate a functional relevance of mutations on protein level, but can not substitute the functional analysis in a cellular context or in an animal model.

We have focussed on the functional analysis of mutations in the postsynaptic scaffolding protein SHANK2 that were identified in individuals with ID, ASD and schizophrenia. To analyze the functional impact of 7 different mutations, we conducted overexpression and knockdown-rescue experiments in primary hippocampal neurons from rat with the major focus on neuronal morphology. We analyzed different types of mutations (missense, nonsense, a small duplication) that were ether inherited or de novo. Additionally, we modelled haploinsufficiency of SHANK2 in these neurons by reducing Shank2 expression with RNA interference, which resulted in an increase of dendritic branching and in a reduction of glutamatergic synapses. Five analyzed variants significantly lost the potential to increase dendritic spine volume compared to wildtype protein. The de novo nonsense mutation (R462X) revealed a dominant negative effect and showed the strongest loss of function effect of all tested mutants in neurons, whereas the missense variants showed different degrees of functional loss and are partially able to rescue the Shank2 knockdown phenotype in neurons. Finally, we introduced the R462X mutation into the hippocampus of mice by using recombinant adeno-associated viruses and observed an impairment of synaptic transmission and cognitive behaviour.

With these tests the functional relevance of different types of SHANK2 mutations found in individuals with ID, ASD and schizophrenia could be analyzed. We recommend this approach for the analysis of mutations in all SHANK family members and other postsynaptic proteins.

P-Basic-004

GBY (Gonadoblastoma Y) locus expression in the dysgenetic gonads of DSD-XY female patients with and without tumour development

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Individuals with disorders in sexual development (DSD patients) and a Y chromosome in their karyotype have a high risk for the development of germ cell tumours (seminoma, dysgerminoma) and the pre-invasive tumour precursors, Carcinoma in Situ (CIS) and Gonadoblastoma (GB) cells, respectively. This suggested the oncogenic expression of Y genes during the development of germ cells in dysgenetic gonads (for review see: OMIM database: ID: # 424500). Consequently, children born and diagnosed with some putative disorders of gonad development were first analysed for presence of a Y chromosome in their karyotype. Discordance between genotype and gonadal phenotype then usually results in surgically removement of their internal gonads before puberty. Mapping studies of broken Y chromosomes in the karyotype of DSD-XY female patients with complete gonadal dysgenesis and the occurrence of gonadoblastoma cells have mapped these putative oncogenic Y genes to a genomic sequence region in proximal Yp and Yq. It was

designated Gonadoblastoma Y (GBY) locus because expressed only in the germ cells of dysgenetic gonads. However, sequence analysis of the human Y chromosome now has revealed that all GBY candidate genes are in fact also functional during normal male spermatogenesis and involved in the control of pre-meiotic spermatogonia proliferation and differentiation: DDX3Y (DEAD box RNA helicase 3 Y); TSPY (Testis Specific Protein, Y), UTY (Ubiquitous Transcribed Y). To investigate their expression in DSD-XY patients with different gonadal differentiation patterns containing or lacking GB tumour precursor germ cells we performed with serial sections of the same patient parallel immunohistochemical experiments with gene specific antisera and analysed expression of their proteins. Whereas DDX3Y and TSPY expression was found to be comparable in the nuclei and cytoplasm of the germ cells present in these DSD tissue sections, UTY expression was weaker and only found in their nuclei. Most interestingly, we found an overlapping expression profile with the pluripotent germ cell marker OCT3/4 but only in GB tumour cells which mainly wer found in the undifferentiated gonad regions (UDG). In streak gonads, consisting of fibrous stroma and devoid of germ cells, DDX3Y, TSPY and UTY expression was consistently missing like that of OCT3/4. Our data confirm the germ cell specificity of expression of these GBY candidate genes and suggest a role of these cell cycle controlling germ cell genes in the development of the pre-invasive germ cell tumor cells when present in a cellular dysgenetic microenvironment.

P-Basic-005

Gene dosage analyses in PWS and AS patients with an imprinting defect not caused by an imprinting center deletion.

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Angelman- (AS) and Prader-Willi-syndrome (PWS) are neurogenetic diseases caused by genetic or epigenetic alterations affecting the imprinted gene cluster on chromosome 15q11q13. Approximately 1-4% of patients have an imprinting defect. An imprinting defect leads to AS, when the maternal allele carries a paternal imprint so that the maternally expressed UBE3A gene is silenced; or to PWS, when the paternal allele carries a maternal imprint so that the SNORD116 gene cluster and other paternally expressed genes are silenced. Only a very small percentage of these patients has an 15q11q13 imprinting center (IC) deletion. To find out whether other copy number variations occur in the non-IC deletion cases, we analysed peripheral DNA samples from 48 PWS and 63 AS patients on Affymetrix 6.0 SNP arrays In this cohort no recurrent de novo variants exceeding 100 kb in size could be detected. We did detect a low number of different so far unreported copy number variants (n=16), but the functional significance is unclear. None encompasses genes known to be involved in epigenetic regulation. These results suggest that copy number variations, apart from 15q11q13 IC deletions, are not a major cause of an imprinting defect. As this method does not detect very small deletions or pointmutations, further analyses using next generation sequencing will be performed.

P-Basic-006

Upstream open reading frames are regulated by nicotinic acetylcholine receptor subunits associated with smoking and smoking-related disorders

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Nicotine addiction poses a major health problem worldwide and is known to considerably increase the risk for diseases such as cancer and cardiovasculopathies. Nicotine both modulates nicotinic acetylcholine receptor (nAChR) subunit expression in various, mostly still unknown ways and acts as a receptor ligand. The genes coding for nAChRs are therefore suspected to play a key role concerning smoking behaviour and related disorders. Especially post-transcriptionally regulatory mechanisms are considered to be involved in the modulation of nAChR subunit expression by nicotine. Such mechanisms are often caused by cis acting sequence elements like internal ribosomal entry sites, microRNA-binding sites and upstream open reading frames (uORFs) located within the untranslated regions (UTR). Therefore, we performed a systematic search for functionally relevant uORFs in the 5'UTR of the nAChR genes CHRNA3, CHRNA4 isoform 1 and 2, CHRNA5, CHRNA7 and CHRNB3 that are assumed to be linked to nicotine dependence and smoking-related diseases. Reporter gene assays revealed that CHRNA4 isoform 1 and CHRNA5 harbor functional uORFs that are able to significantly downregulate the protein expression of the subsequent gene. qPCR tests ruled out that the results of the luciferase assay were due to a transcriptional effect for both genes. Next, we tested by luciferase assay if the ATG start codon of the uORFs of CHRNA4 isoform 1 and CHRNA5

are able to initiate protein translation, which would be a basic requirement for the actual translation of the uORF itself. Regarding the uORF of CHRNA4 isoform 1, our experiments suggest that the ATG of this uORF is recognized efficiently by the ribosomes to initiate protein translation. Concerning the uORF of CHRNA5, our findings imply that other mechanisms have to be taken into consideration, including stalling of ribosomes at the uORF or leaky scanning. Consequently, our study revealed that two of the major nAChR subunit genes contain functional uORFs that are capable of regulating gene expression.

P-Basic-007

How and why do frequent large deletions in FANCA arise?

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Large genomic rearrangements predominantly arise in genes replete with transposable elements or sharing pseudogenes. Moreover, long distance Alu-mediated recombination took place, has often been recognized as the cause of large genomic deletions (LGDs). In Fanconi anemia (FA) the most frequently affected gene, FANCA, accounts for 60 - 65% of FA cases, with approximately 40 % of its mutations being large deletions that show very different extension. Located on chromosome 16q24.3, FANCA reveals LGDs, spanning from one exon more than the entire gene. Routine exon-scanning-sequencing fails to detect LGDs in the presence of the second allele. Exact deletion borders are not determinable, since FANCA breakpoints lie in the large introns, comprising frequently occurring Alu and other repetitive elements. Therefore, FANCA LGDs were mostly assessed by Multiplex Ligation-dependent Probe Amplification (MLPA). Some studies observed a highly significant correlation between the number of breakpoints in a given intron and the number of Alu repeats therein, suggesting that Alu-mediated recombination may explain the high prevalence of LGDs. Nonetheless, exact FANCA deletion breakpoints have not been investigated nor characterized systematically to date. Therefore, we screened DNA samples from more than 30 FA-A patients of different ethnicities. They were all shown to carry heterozygous, homozygous or compound heterozygous large FANCA deletions as demonstrated via MLPA. To narrow down extention of the deletions and to determine the sites of the breakpoints as closely as possible, we used a high resolution customized NimbleGen 720K comparative genomic hybridization array (array-cgh). Probe coverage merely encompassed intronic FANCA regions, as well as the 3´ and 5´ UTRs. Excess probes were distributed over chromosome 16 and the entire genome. Based on the closest unaffected 3´ and 5´array probe of a deletion, junction fragments were obtained by PCR amplification across the suggested sites of breakpoints and the PCR products were sequenced subsequently. Our systematic survey of FANCA breakpoints revises the previous impression that deletions predominantly occur by long distance Alu-Alu recombination. Basically, we identified three different mechanisms of recombination, with most of the breakpoints being private. Beside long distance Alu-Alu recombination, we predominantly found rerarrangements between microhomology tracts and a mechanism not involving sequence homology, commonly referred to as non-homologous recombination that and frequently results in deletions plus short insertions. The detected deletion sizes range from 2 kb up to 182 kb, well beyond the borders of FANCA. Invariably all FANCA deletion breakpoints outside FANCA were found in regulatory genes or non-coding sequences adjacent to FANCA. SPIRE2, TCF25, ZNF276, SPATA2L and c16orf7 as well as short sequences in-between them, seem to be potential candidates of homologous or non-homologous recombination turning this genomic region into a mutational “hot spot” for rearrangements and resulting in large deletions occurring in FA-A patients.

P-Basic-008

Respiratory distress and early neonatal lethality in hspa4l/hspa4 double mutant mice

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Heat shock protein (HSP)-A4L and HSPA4 are closely related members of the HSP110 family that acts as co-chaperone. We generated and analyzed Hspa4l-/-Hspa4-/- mice to investigate a functional complementarity between HSPA4L and HSPA4 during embryonic development. Hspa4l-/-Hspa4-/- embryos exhibited marked pulmonary hypoplasia, and subsequent neonatal death. Compared to lungs of wild-type, Hspa4l-/-, and Hspa4-/- embryos, Hspa4l-/-Hspa4-/- lungs were characterized by diminished saccular spaces and increased mesenchymal septa that were determined to be due to an increased cell proliferation index and decreased cell death. Accumulation of glycogen and diminished expression of surfactant protein B, prosurfactant protein C, and aquaporin 5 in saccular epithelium suggested impaired maturation of type II and

type I cells in the Hspa4l-/-Hspa4-/- lungs. Further experiments showed a significant accumulation of ubiquitinated proteins in the lungs of Hspa4l-/-Hspa4-/- embryos, indicating an impaired chaperone activity. Our study demonstrates that HSPA4L and HSPA4 collaborate in embryonic lung maturation, which is necessary for adaptation to air breathing at birth.

P-Basic-009

Blood cell mosaicism: Natural gene therapy in Fanconi anemia

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Tissue-specific reversion of constitutional mutations is the hallmark of somatic mosaicism. In Fanconi anemia (FA), a rare heterogeneous recessive, autosomal or X-chromosomal disease, 10-20% of patients develop mosaicism in the hematopoietic system, which appears to counteract progressive bone marrow failure. Those patients may have a better prognosis regarding long-term stability of blood cell counts in a way that can be interpreted as “natural gene therapy”. Better understanding of this process will be facilitated by studies that show in which blood cell lineage(s) mosaics occur and how their manifestation varies over time. Here we report the case of an eighteen year-old patient who was diagnosed with FA in 1989 by an increased level of spontaneous cell cycle arrest of cultured lymphocytes (∑G2/GF 0.415). In 1999, he was assigned to subtype FA-A. In 2002 he came to our attention as a potential mosaic patient due to increasing blood cell counts and lesser sensitivity of lymphocytes to MMC. We found only borderline spontaneous cell cycle arrest in the G2 phase (∑G2/GF 0.274) compared to the control population. In the following years we observed further stabilization of the hematological situation. The ∑G2/GF values ranged between 0.282 and 0.346 which is at the lower limit of the FA patient level (2004: 0.346; 2006: 0.306; 2007: 0.323; 2008: 0.284; 2009: 0.282). Only in 2010 we found higher levels of G2 phase arrest (∑G2/GF 0.371 and 0.469). Immunoblot analysis showed a restored FANCD2 monoubiquitination as a further clue to functional reversion of one FANCA mutational allele. The patient was compound heterozygous for the splice mutation c.824-1G>T in intron 9 and the missense mutation c.971T>C in exon 11 (p.L324R) of FANCA in fibroblasts. On sequencing we observed a decrease of c.971T>C compared to the other allele with wild type (wt) sequence at that site. In 2006 that mutation was found in 6.0% of gDNA from blood mononuclear cells and in 2007 in approximate 5.5%. Subsequent studies between 2009 and 2011 showed c.971T>C at a level of 5.8% of DNA corresponding to 10-20% non-reverted cells. We also analyzed gDNA from B lymphocytes (CD19+), T lymphocytes (CD3+), monocytes (CD14+) and granulocytes (CD15+) isolated by magnetic activated cell sorting (MACS). Their purity was CD3+=99.3%, CD19+ =91.2%, CD15+ =98.6% and CD14+ =93.0%. c.971T>C could not be detected any more in CD14+, CD15+ and CD19+ cells. Only in CD3+ cells a fraction of mutated DNA was retained corresponding to a reversion of 10-15% of cells. This relatively small proportion of reverted T lymphocytes may be responsible for the lack of a characteristic bimodal distribution of breaks per metaphase on chromosomal breakage analysis. It may also be accountable for still increased spontaneous G2 phase arrest (∑G2/GF 0.375) on the most recent flow cytometric analysis of cultured blood lymphocytes. Our investigations suggest that somatic mosaicism occurred at the level of blood stem cells since it is prevalent in all blood lineages, that this mosaicism in the hematopoietic system has been stable over many years albeit it did not completely prevail, and that it is least prevalent in the most long-lived blood cell type, T lymphocytes. More information may be gained by future studies of this patient. Moreover, prospective investigations of a greater number of mosaic FA patients are required to understand the natural history of this interesting phenomenon.

P-Basic-010

Human torsinA is able to rescue the lethal phenotype of homozygous DYT1-knockout-mice

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Objective: Generation of transgenic mice, which express human Tor1A in a homozygous Dyt1-knockout background (“rescue-mice”) in order to analyze whether the human torsinA protein is able to compensate the loss of murine Tor1A.

Background: DYT1 Dystonia is an autosomal dominant inherited movement disorder caused by a 3 base pair deletion (delGAGTorA) in the DYT1 gene encoding torsinA.

Mouse models lacking torsinA protein (knockout) or exclusively expressing human mutant torsinA (knockin) die shortly after birth and show an exclusively neuronal cellular phenotype. In contrast, a mouse model overexpressing human wildtype torsinA protein driven by the human prp promoter was viable and did

not show major abnormalities. Murine torsinA has about 95% similarity to human torsinA. This motivated us to investigate whether the human protein expressed in the mouse knockout background is able to rescue the lethal phenotype of the homozygous DYT1 knockout mouse model.

Methods: “Rescue mice” were generated through crossings of DYT1-knockout mice with mice overexpressing human Tor1A through the human Tor1A promoter. The characterization of the model consists of SHIPRPA, motor phenotyping (beamwalk, catwalk, rotarod) and immunohistochemistry.

Results: “Rescue mice” were viable and healthy and did not show any abnormalities in the SHIRPA test. Immunohistochemistry revealed a strong expression of the human torsinA protein in the rescue mice in the pons, brain stem, and cerebellum as well as corpus callosum, an intermediate expression in the striatum and the hippocampus, and low expression levels in the cortex. We did not see cellular abnormalities in different neuronal populations as described for knockout mouse models and overexpressing torsinA mouse models.

Motor behavior analysis did not reveal any abnormalities when compared to control mice.

Conclusion: The phenotype of the homozygous DYT1-knockout mice was reversed through the expression of the human Tor1A. Since the human prp-promoter drives the expression of the human torsinA protein in selective brain regions we have now evidence that torsinA expression in these brain regions seems to be sufficient to provide a normal development of the mouse brain.

P-Basic-011

Neuronal analysis and motor-phenotypical characterization of a transgenic rat model for DYT1 dystonia

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Objective: neuronal analysis and motor-phenotypical characterization of a transgenic rat model for DYT1 dystonia to study torsinA pathology

Background: DYT-1-dystonia is an inherited autosomal-dominant disease characterized by involuntary movements due to a dysfunction of the central nervous system involving selective regions implicated in movement control and is mainly caused by a three base-pair deletion (ΔGAG) in the Tor1A gene. The pathophysiological features of this mutation are still not well understood. To analyze the cellular mechanism underlying this disease a new transgenic rat model, harbouring the full length human mutant and wild type TorsinA gene including promoter and regulatory elements was analyzed.

Methods: We performed neuronal analysis of different brain regions by immunohistochemistry and electron microscopy. Brains of young rats (postnatal day 0 and 14) where analyzed to follow up the onset of neuropathology.

Results: We were able to replicate key features such as nuclear envelope (NE) pathology. Furthermore, we find that membrane abnormalities, such as discontinuous NE membranes and enlarged perinuclear space, are already present at the age of P0 and that there is an increase of NE disintegrity correlating with age. Moreover, we have evidence, that the rat TorsinB expression compensates the effects of human ΔGAG protein overexpression in subregions of the brain. For the first time, we could show an abnormality of Purkinje cells in the cerebellum.

Conclusion: We suggest that the mutated TorsinA causes NE pathology at early postnatal stages with a progressive neuronal phenotype and abnormality of Purkinje cells in the cerebellum, assuming that the cerebellum could be involved in the onset of dystonic symptoms.

P-Basic-012

Systemic involvement in TREX1-associated familial chilblain lupus

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Familial chilblain lupus is an autosomal dominant form of cutaneous lupus erythematosus manifesting in early childhood with cold-induced livoid inflammatory infiltrates at acral sites. While some patients develop antinuclear antibodies or arthalgia, systemic involvement has not been reported. Familial chilblain lupus is caused by heterozygous mutations in TREX1 (3´repair exonuclease) or the phosphohydrolase SAMHD1 (sterile alpha motif domain and HD domain containing protein 1). Rare variants of the TREX1 gene are also associated with an increased risk of developing sporadic systemic lupus erythematosus.

We describe a 20 year old patient with familial chilblain lupus carrying a heterozygous mutation in TREX1 (D18N) affecting a highly conserved amino acid residue within the catalytic centre. Since early childhood he suffered from painful chilblain lesions on this fingers, toes and ears. Histological analysis of a skin biopsy confirmed the diagnosis of chilblain lupus. During the cold season, lesions ulcerated and led to mutilating tissue destruction of the helix of the left ear. His mother and grandmother were affected by similar symptoms. In addition, the patient complained of fever episodes and pain in his knee and shoulder. Radiographs were consistent with non-erosive arthritis. Antinuclear antibodies in a fine speckled pattern and a titer of 1:160 were detected in serum. A heterozygous de novo D18N mutation has been described as a rare cause of Aicardi-Goutières syndrome, a severe inflammatory encephalopathy manifesting in early childhood. In the majority of cases, Aicardi-Goutières syndrome is a recessive disorder caused by biallelic mutations in TREX1. In the patient described here, neurological symptoms were absent. However, laboratory testing was remarkable for mild leukopenia, thrombocytopenia, anemia and hypergammaglobulinemia.

These findings demonstrate that the clinical picture of familial chilblain lupus is not restricted to cutaneous involvement, but also includes systemic manifestations of lupus erythematosus. These findings support the association of heterozygous mutations in TREX1 with systemic lupus erythematosus and expand the clinical spectrum of familial chilblain lupus.

P-Basic-013

Determination of DNA Methylation Patterns in Cardiac Tissue from Hypoplastic Left Heart Syndrome

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Hypoplastic Left Heart Syndrome (HLHS) is a congenital heart defect (CHD) characterised by severe underdevelopment of the left side of the heart, interfering with the ability to support the systemic circulation after birth. This complex cardiac malformation displays merely an incidence of 0.8 – 1% of all CHDs, but, if untreated, HLHS is invariably lethal. Despite good progress in surgical treatment, the etiology of HLHS remains unknown. Displaying 8% sibling recurrence risk, several studies assume a genetic cause for HLHS. However, the pathobiological state of this CHD still needs to be investigated.

Epigenetic mechanisms and particularly DNA methylation are well known to be involved in gene regulation and fetal development. Differential expression of genes through epigenetic factors could disturb cardiogenesis resulting in heart malformations, such as HLHS. Thus, we aimed here to investigate whether cardiac tissue from HLHS shows a distinct DNA methylation pattern.

In this study we included genomic DNA extracted from heart tissue, specifically interatrial septum (IAS) samples of 25 HLHS patients and 10 patients with right heart obstructive lesions as control. RNA of 13 HLHS and 5 control samples of these tissues was subjected to custom transcriptome profiling using RNAseq. DNA methylation was assessed using Illumina's HumanMethylation450K BeadChips. To discover genes aberrantly methylated in HLHS, we compared the HLHS samples to those from the right heart obstructive lesions using the Qlucore Omics Explorer 2.3 software.

T-test revealed 119 CpG loci corresponding to 101 genes differentially methylated in HLHS (FDR < 0.05). Overall, the DNA methylation patterns of both groups, HLHS and control, displayed strong heterogeneity which could also be confirmed in unsupervised analysis. The majority of CpG loci differentially methylated in the IAS between HLHS and right heart obstructive lesions showed methylation differences of less than 4%, with only two loci showing differences of more than 10%. Comparison with RNAseq data revealed 9 of the differentially methylated genes to be also differentially expressed. Our results indicated that the DNA methylation differences in IAS between HLHS and right heart obstructive lesions differ only marginally. As this could be due to the fact that IAS might not be representative for the overall cardiac DNA methylation, we currently analyse DNA methylation patterns of different heart regions. (Supported through DZHK (German Centre for Cardiovascular Research) partner site Hamburg/Kiel/Lübeck, in the WP NCCR3.3 Heart Failure)

P-Basic-014

Next Generation Sequencing of TAAD genes using DNA isolated from paraffin embedded tissue

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Thoracic Aortic Aneurysms and Dissections (TAAD) is a condition that can occur isolated or as part of a syndrome, like Marfan syndrome or Loeys-Dietz syndrome. Mutations in several different genes are known to cause TAAD. Because TAAD is not always recognized during lifetime and has a high morbidity, the diagnosis TAAD is often made during autopsy. In many cases, paraffin embedded tissue is available for post-mortem genetic testing of the TAAD genes. However, DNA extracted from this tissue is often degraded and only short fragments (often only approximately 300 bp) can be produced by PCR. Conventional genetic testing of TAAD is based on DNA extraction from blood, PCR and Sanger sequencing of PCR products larger than 300 bp. Testing of DNA from paraffin-tissue requires extra primer sets and is more costly and time-consuming.

Next Generation Sequencing with the Illumina MiSeq platform requires DNA fragments around 300 base pairs. Therefore it should be possible to analyse DNA isolated from paraffin embedded tissue with this platform.

We isolated DNA from paraffin embedded tissue from six deceased patients who died due to TAAD. Library preparation was carried out using a customized TruSeq Custom Amplicon Kit from Illumina. Sequencing was performed with the Illumina MiSeq.

185 exons of the genes ACTA2, COL3A1, FBN1, MYH11, SLC2A10, SMAD3, TGFBR1, and TGFBR2 have been analysed. The results showed that the quality of the sequences depended directly on the quality of the isolated DNA. When the fixation of the tissue was poor (e.g. unbuffered formalin, no homogenous fixation), the coverage of the exons was very low or the exon could not be sequenced at all. But if the fixation has been done well, the result of the sequencing resembles that of DNA isolated from blood. Between 5% (very poor fixation of the tissue) and 91% (good fixation/DNA from blood) of the 185 exons had a coverage greater than the threshold that we set at 50x.

In the six analysed patients we found some new sequence variations with yet unclear relevance.

In conclusion, DNA from paraffin embedded tissue can be analysed without any difficulties using Next Generation Sequencing, when the fixation has been done properly.

P-Basic-015

Analysing the Fanconi anemia candidate gene FAAP20 in unclassified FA cell lines

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Fanconi anemia (FA) is a rare recessive genetic disorder characterized by bone marrow failure, congenital developmental defects and cancer predisposition. To date 15 genes have been reported to cause FA in the case of biallelic mutations. The products of these genes are members of the FA/BRCA pathway for genomic maintenance. One additional member of the FA core complex is FAAP20 (c1orf86) which binds directly to FANCA and is required for the stability of the complex. Depletion of FAAP20 in HeLa and chicken DT40 cells results in reduced levels of monoubiquitinated FANCD2 which is comparable to other cells with defects in the FA core complex. No FA patients with mutations in FAAP20 have been identified so far. Therefore we screened 23 FA cell lines of unknown complementation group for FAAP20 mutations by Sanger sequencing. We repeatedly detected two different common SNPs in exon three and in the 3'-untranslated region which are registered in the NCBI dbSNP database. We failed to amplify exon one both in FA and control cell lines because of the very high GC content (85%) of this exon. As alternative techniques we used whole exome sequencing (WES) and target enrichment of FA disease and candidate genes following next-generation sequencing to screen FA cell lines for mutations. With an average coverage of 100x FAAP20 exon one was completely covered by WES whereas using target enrichment a coverage less than ten was sufficient. Our data show that WES and target enrichment are appropriate methods for screening gene regions which have a very high GC content where standard methods such as Sanger sequencing fail. To date, we could assign 12 of the 23 FA cell lines to known FA complementation groups and by this exclude those lines from FAAP20 exon one mutations. FAAP20 did not qualify as a new FA gene in this group of patients. Nevertheless, exon one of FAAP20 has not yet been sequenced in eleven FA cell lines of still unknown complementation group. Based on the crucial role of FAAP20 in the FA/BRCA pathway this gene is still a FA candidate.

P-Basic-016

Functional analysis of a duplication (p.E63_D69dup) in the switch II region of HRAS: new aspects of the molecular pathogenesis underlying Costello syndrome

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Costello syndrome is a congenital disorder comprising a characteristic face, severe feeding difficulties, skeletal, cardiac and skin abnormalities, intellectual disability and predisposition to malignancies. It is caused by heterozygous germline *HRAS* mutations mostly affecting Gly^{12} or Gly^{13}, which impair HRAS-GTPase activity and result in increased downstream signal flow independent of incoming signals. Functional analyses of rarer *HRAS* mutations identified in individuals with attenuated Costello syndrome phenotypes revealed altered GDP/GTP nucleotide affinities (p.K117R) and inefficient effector binding (p.E37dup). Thus, both phenotypic and functional variability associated with *HRAS* mutations is evident. Here we report on a novel heterozygous *HRAS* germline mutation (c.187_207dup, p.E63_D69dup) in a girl presenting with a phenotype at the milder end of the Costello syndrome spectrum. The p.E63_D69dup mutation impaired co-precipitation of recombinant HRAS with NF1-GAP (GTPase-activating protein) suggesting constitutive $HRAS^{E63\_D69dup}$ activation due to GAP insensitivity. Indeed, we identified strongly augmented active $HRAS^{E63\_D69dup}$ which co-precipitated with effectors RAF1, RALGDS and PLCE1. However, we could not pull down active $HRAS^{E63\_D69dup}$ by using the target protein PIK3CA, indicating a compromised association between active $HRAS^{E63\_D69dup}$ and PIK3CA. Accordingly, overexpression of $HRAS^{E63\_D69dup}$ increased steady-state phosphorylation of MEK1/2 and ERK1/2 downstream of RAF, whereas AKT phosphorylation downstream of PI3K was not enhanced. By analyzing signaling dynamics, we found that $HRAS^{E63\_D69dup}$ has impaired reagibility to stimuli resulting in reduced and disrupted capacity to transduce incoming signals to the RAF-MAPK and PI3K-AKT cascade, respectively. We suggest that disrupted HRAS reagibility, as we demonstrate for the p.E63_D69dup mutation, is a previously unappreciated molecular pathomechanism underlying Costello syndrome. In conclusion, our data support the idea that insensitivity to physiological stimuli (i.e. reduced reagibility) is a common mechanism in pathological premature ageing syndromes, such as Hutchinson-Gilford progeria, Werner syndrome and Costello syndrome.

P-Basic-017

Chromosome 14 subtelomeric deletion and duplication of 14q32.3q32.33: clinical and molecular cytogenetic findings

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We report on a child with a combined microdeletion of the subtelomeric region of chromosome 14 and partial duplication of the long arm of chromosome 14. The boy is the second child of non-consanguineous healthy parents (aged 35 and 30 years). Apart from intrauterine growth retardation, pregnancy and delivery were uncomplicated. No invasive prenatal diagnosis was performed. The boy presented with heart defect, neonatal cholestasis, hypoplasia of the corpus callosum, polymicrogyria, idiopathic pulmonal hypertonia, growth retardation and several dysmorphic features, like secondary microcephaly, hypertelorism, anisocoria and contractures of knees. At the age of one year signs of developmental delay were already apparent. The family history was uneventful. Cytogenetic analysis revealed a numerical normal but structural aberrant male karyotype with an elongated chromosome 14. Molecular cytogenetic analysis with chromosome 14 painting and subtelomeric probes identified a deletion of the subtelomeric region on the aberrant chromosome 14. This was further characterized by array-CGH analysis. A duplication of 7.1 Mb with breakpoints in 14q32.2 and 14q32.33 was identified. The size of the subtelomeric deletion was determined to be 1.78 Mb. Both parents showed normal karyotypes. Clinical features like pre- and postnatal growth retardation, mental retardation, microcephaly and congenital heart defects have been described in patients with terminal deletion and proximal duplication of chromosome 14, while contractures of knees are not commonly reported in these cases. An irregular polygyria was reported by Masada et al. (1989) in a boy with terminal deletion [(del(14)(q32.11)]. The polymicrogyria seen in our patient might help to further narrow down the critical region on the long arm of chromosome 14.

P-Basic-018

Laboratory management of Multiple Endocrine Neoplasia Type 1 genetic test: results and considerations of 3 years of activity

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Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant disorder that is due to mutations in the tumor suppressor gene MEN1. MEN 1 syndrome is characterized by the occurrence of parathyroid (HPT) (82-100%), gastroenteropancreatic (GEP) (60-80%) and anterior pituitary tumors (PIT) (30-50%). Some patients may also develop carcinoid tumors, adrenocortical tumors, meningiomas, facial angiofibromas, collagenomas, and lipomas. The most common endocrine tumors are parathyroid tumors that cause hyperparathyroidism and hypercalcemia.

Since 2009 Labor Lademannbogen/Praxis für Humangenetik, Dr. Kaminsky has performed mutation analysis of the MEN1-gene, by PCR-based sequencing of coding region (exon 2-10) and intron-exon junctions and by MLPA-analysis. We analyzed 139 patients and 84 family members. Most of the patients were referred to our laboratory for persistent primary hyperparathyroidism. We identified 29 different mutations: 16 frame shift (55.2%), 6 missense (20.7%), 4 nonsense (13.8%), 2 large deletions (6.9%) and 1 splicing site (3.5%).

9 mutations were new and were not described in the literature so far. No correlation between genotype and phenotype has been found, in our series, for any mutation. Recognition of the syndrome is important both for treatment and for evaluation of family members.

P-Basic-019

Fine scale characterization of the breakpoints of a large POU3F4 deletion in a patient with X-linked hearing loss

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Most male patients with X-linked deafness present with a profound sensorineural hearing loss and may also show a conductive component which is sometimes masked by the sensorineural loss. Common features are fixed stapes and temporal bone anomalies (including a dilatation of the internal auditory canal, detectable by computed tomography (CT)) leading to a perilymphatic gusher following stapedectomy or during cochlear implantation. This mixed type of X-linked hearing loss is caused by mutations in the single exon gene POU3F4 which is located on chromosome Xq21.1 and encodes a 361 aa POU domain transcription factor mainly expressed in the inner ear and central nervous system. Besides intragenic mutations, deletions of the POU3F4 gene itself as well as of the upstream region have been reported in X-linked deafness patients.

We extracted DNA from the blood sample of a patient with a characteristic temporal bone CT. Since the PCR amplification of the POU3F4 gene failed, an MLPA analysis was performed that indicated a deletion involving at least the single coding exon of POU3F4. To define the approximate deletion size we subsequently performed a custom high resolution array CGH (e-array, Agilent) covering 77 Mb to 88 Mb around the POU3F4 gene with a density of one probe per 300 bp. Based on these results, a breakpoint-spanning PCR followed by bidirectional sequencing was performed by which the deletion could be determined to be 8,740 bp in size, comprising the complete POU3F4 gene as well as 4,544 bp and 2,689 bp of the upstream and downstream sequences, respectively.

Our study shows that high resolution array CGH and subsequent direct sequencing is suitable for the breakpoint determination of large deletions primary detected by MLPA analysis.

P-Basic-020

MLPA assay for HEXB and GM2A

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Background: GM2 gangliosidoses comprise a group of autosomal recessive inherited, lysosomal storage diseases resulting from a deficiency of beta-N-hexosaminidase (HEX) A and/or B activity. The disease has a

wide variety of clinical presentations, from severe infantile forms, which are fatal before the age of four, to late-onset forms which allow survival into childhood or even longer. GM2 gangliosidoses are caused by point mutations and small or large deletions which have been described in HEXA (Tay Sachs), HEXB (Sandhoff) and GM2A (Tay Sachs variant AB). To date multiplex ligation dependent probe amplification (MLPA) analysis kit is commercially available only for Tay Sachs disease. Hence we established MLPA assays also for HEXB and GM2A.

Methods: 19 synthetic MLPA probes, 88-168 nucleotides in length, were tested to amplify the coding exons of the HEXB (15 probes) and GM2 gene (4 probes). Fragment and data analyses were performed by Beckman CEQ. For probe-specific calculation of copy numbers, an adapted Excel spreadsheet was used (Schouten et al. 2002).

Results and discussion: In total, 35 samples were screened for copy number variations in HEXB and GM2A. No deletion or duplication was detected in ten cases of Sandhoff disease and eleven cases of Tay Sachs variant AB. In the cohort of patients with Sandhoff disease, one copy number variation was observed. This deletion was always associated with a previously identified nucleotide exchange c.1614-14C>A. The deletion encompasses HEXB exons 1-5. Break point analysis revealed that the deletion is similar to the one described by Neote et al. (1990). For GM2A no deletion was detected.

Conclusion: The MLPA method resolves deletions. These assays complement routine diagnostics for Sandhoff disease and Tay Sachs variant AB.

P-Basic-021

Implication of interleukin-6 gene polymorphism in susceptibility to acute myocardial infarction

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Purpose: Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis. Genes coding for cytokines such as interleukin-6 (IL-6) are candidates for predisposing to the risk of coronary artery disease. The aim of this study was to investigate whether molecular polymorphism of the IL-6 gene is involved in the predisposition to acute myocardial infarction (AMI).

METHODS: Genomic DNA and peripheral blood mononuclear cells of patients with AMI and controls were extracted. IL-6 gene variations were evaluated by polymerase chain reaction followed by restriction enzyme analysis. The mRNA expression of IL-6 gene as well as plasma levels of IL-6 and C-reactive protein (CRP) were analyzed.

RESULTS: The prevalence of 'C' allele in -174 G/C variation was higher in patients with AMI than in controls. The IL-6 -174 'C' allele is associated with high levels of IL-6 in the patients, of which the patients with CC and GC genotypes significantly have higher IL-6 concentrations, respectively. Increased CRP concentrations were associated with -174 G/C variation in the patients compared with controls. The mRNA expression levels of IL-6 were significantly higher in the patient compared with controls ($P<0.001$).

CONCLUSION: The findings of this study indicate the relationship between IL-6 gene polymorphism and the risk of AMI, which suggests that genetic polymorphism in IL-6 gene, might be helpful for determining susceptibility to AMI in Iranian patients. In addition, susceptibility to AMI might be related to IL-6 gene expression, which affects its plasma levels. CRP plasma levels also were associated with IL-6 gene variation in the patients.

P-Basic-022

Genome-wide expression studies in 14 patients with microdeletion 5q14.3 syndrome - A novel tool for the systematic study of functional interactions and pathways

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Microdeletions identified in patients with intellectual disability/developmental delay (ID/DD) by genome-wide copy-number analyses have been instrumental in identifying many novel candidate genes for ID/DD. However, our understanding of the etiological mechanisms for these deletions remains unclear. In several cases, dysregulated (i.e., reduced) expression of deleted candidate genes has been demonstrated as evidence for their haploinsufficiency. Additionally, very few functional interactions between such newly identified genes and known ID/DD genes have been found in targeted expression experiments, such as MEF2C, which was identified as the main causative gene in 5q14.3 microdeletion syndrome, and the well-established ID/DD gene MECP2. However, genome-wide expression analyses to study functional interactions and pathways systematically has to our knowledge not yet been applied to microdeletion syndromes.

Here, we performed genome-wide expression studies on 14 patients with non-recurrent microdeletions in 5q14.3-q15 using an Illumina HT12 expression array and whole blood RNA. By adapting an established analysis protocol for non-recurrent deletions, we grouped the patients according to the extent of their deletions and compared all transcripts with a detectable signal (detection p-value <0.01) between patients and healthy controls. TRABD expression was significantly reduced in patients, and this result was verified by quantitative PCR. With this analysis protocol there is a risk of excluding those transcripts that have lower signal intensities due to the 5q14.3-q15 microdeletion. Thus, we developed and applied a secondary analysis strategy to identify transcripts that met the above-mentioned detection p-value threshold in the controls but not in the patients, and vice versa. By this approach we identified several additional genes, which qPCR verification could be performed only for COX7B due to limited RNA. By qPCR, we also detected a significant correlation between the transcript levels of MEF2C, TRABD, and COX7B both in patients and controls. This correlation is therefore independent of the phenotype.

To our knowledge, this is the first attempt to apply genome-wide expression studies to a cohort of patients with non-recurrent microdeletions. Both the established and secondary analysis protocols yielded verifiable results: significantly altered expression of TRABD and COX7B genes, which had not previously been connected to 5q14.3 microdeletion syndrome. Thus, we could demonstrate the utility of both strategies, although only ~4,000 of the original 47,231 transcripts fulfilled the quality criteria (excluding MEF2C). This limitation may be addressed by using RNA from more homogeneous cell populations, such as lymphoblastoid cell lines. Furthermore, the expression of TRABD and COX7B correlates with the expression of MEF2C, the causative gene for 5q14.3 microdeletion syndrome. Finally, we performed a network analysis based on the KEGG database and a literature review. By this analysis, TRABD and COX7B were connected to MEF2C over only two "nodes", pointing to a biological significance of the interaction. Thus, our proof of principle study demonstrates that genome-wide expression studies are applicable to non-recurrent microdeletions and yield biologically significant results.

P-CANCER GENETICS

P-CancG-023

RAD51C and RAD51D mutation screening in breast and ovarian cancer families

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Most currently known breast cancer susceptibility genes play a role in DNA repair. Recently, germline mutations in the genes RAD51C and RAD51D were identified as a predisposing factor for breast and ovarian cancer. Rare mutations in these genes were shown to confer a high risk for both breast and ovarian tumors. To confirm this we conducted a full mutation screen of RAD51C in 83 probands and of RAD51D in 41 probands from high-risk German breast and/or ovarian cancer families that do not carry BRCA1 or BRCA2 mutations. All coding exons and intron-exon boundaries of RAD51C and RAD51D were screened for mutations in genomic DNA from affected family probands by direct DNA sequencing. Furthermore, multiplex ligation-dependent probe amplification (MLPA) was applied to test for genomic rearrangements in RAD51C. In our cohort we found no RAD51C or RAD51D mutations.

In line with published results from similar follow-up studies it seems that RAD51C and RAD51D mutations are rare events among high-risk breast cancer and breast/ovarian cancer families. Further large population based studies will be needed to reliably assess the relevance of pathogenic mutations in RAD51C and RAD51D.

P-CancG-024

Mismatch Repair Cancer Syndrome: Case report of a patient with two germline PMS2 mutations.

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Introduction:

Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC) is an autosomal dominant disorder caused by heterozygous germline mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6, or PMS2. Patients with Lynch syndrome have a high risk of developing colorectal carcinoma and endometrial carcinoma, typically in the fourth or fifth decade of life. In contrast, compound heterozygous or homozygous germline mutations in MMR genes cause the very rare Mismatch Repair Cancer Syndrome (MMRCS, MIM #276300). MMRCS patients may develop colorectal polyposis, primary tumors of the central nervous system, early-onset hematologic malignancies, and cafe-au-lait spots. Here, we report on a patient diagnosed with adenocarcinoma of the rectum at age 22, multiple colonic polyps, and endometrial carcinoma at age 36. Immunohistochemistry showed complete loss of PMS2 staining in the tumor and in normal tissue.

Methods:

All coding regions of MLH1, MSH2, MSH6 and PMS2 were amplified with the multiplex PCR Kit HNPCC-MASTR v1 (Multiplicom). Next generation sequencing was performed using the 454 sequencing kit on a GS Junior (Roche). Data were analysed by NEXTGENe software (Softgenetics). Sequence variations were confirmed by Sanger sequencing. MLPA analysis of PMS2 was performed using SALSA MLPA Kit P008 (MRC-Holland).

Results:

Sequencing detected a pathogenic missense mutation in exon 15 of the PMS2 gene. MLPA analysis revealed a heterozygous deletion of exon 8 of PMS2.

Conclusions:

The compound heterozygosity for two inactivating PMS2 mutations could explain the complete loss of PMS2 expression in tumor and even in normal tissue. Further analysis is under way in order to check for bi-allelic inheritance, a formal requirement of MMRCS.

P-CancG-025

Gain of 1q21 is the first independent cytogenetic adverse prognostic factor in light chain amyloidosis patients treated with melphalan / dexamethasone

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Purpose: In multiple myeloma cytogenetic aberrations detected by interphase FISH (iFISH) are well established as pathogenetic and prognostic factors. Light chain (AL) amyloidosis displays a similar pattern of cytogenetic aberrations, but so far their prognostic implication is unclear. Therefore, the aim of this study was to identify prognostic cytogenetic risk factors in AL amyloidosis.

Patients and Methods: We assessed a comprehensive panel of FISH probes in a series of 93 consecutive AL amyloidosis patients treated uniformly with melphalan / dexamethasone.

Results: Gain of 1q21 emerged as an adverse prognostic factor in AL amyloidosis. Detection of gain of 1q21 was predictive for a poor overall survival (OS) (median OS 10.9 versus 38.2 months, p<0.001), an inferior hematologic event free survival (hem EFS) (median 4.6 versus 9.3 months, p=0.08) and inferior hematologic remission rates (VGPR plus CR rate 11% versus 31%, p=0.08). In a multivariate analysis the adverse OS prognosis carried by gain of 1q21 was retained as an independent prognostic factor (p = 0.007), along with younger age, male gender, poor Karnofsky index and high NT-proBNP values as other statistically significant adverse factors. Patients with t(11;14) had a longer median OS with 42.3 months versus 17.5 months, though no statistical significance was reached (p=0.15). Deletion 13q14 and hyperdiploidy turned out to be prognostically neutral.

Conclusion: Gain of 1q21, which is known as a progression marker and a high risk factor in multiple myeloma, emerged as an independent adverse prognostic factor also in AL amyloidosis patients treated with a standard first-line chemotherapy.

P-CancG-026

Methylation of PCDH10, SPARC, and UCHL1 predicts survival and in stage II colorectal cancer patients

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Colorectal cancer (CRC) is one of the most common cancers and a leading cause of cancer-related deaths worldwide. Surgical excision at early clinical stages is highly effective but 20-30% of patients still relapse. Therefore, it is of clinical relevance to identify patients at high-risk of disease recurrence, who would benefit from adjuvant chemotherapy. Aberrant promoter methylation represents a hallmark of colorectal cancer and, therefore, detection of promoter methylation could be a suitable marker to identify such high-risk patients. The overall objective of this study was to identify prognostic and/or predictive methylation markers in stage II CRC patients.

We performed a Methyl Profiler Assay (Qiagen) of 48 gene promoters followed by expression analysis of 16 methylated promoters after treatment with the demethylating agent 5-Aza-2´-dCytidine (5-Aza). Six genes including FZD9, PCHD19, SFRP2, SPARC, UCHL1, and WIF1 that showed an increase in expression (>10-fold) after demethylation, were selected for further analysis in primary tumors (FFPE) of colorectal cancer patients (n=143) who were enrolled in a prospective randomized phase III trial of the Austrian Breast and Colorectal Cancer Study Group. Patients were randomized to either adjuvant chemotherapy with 5-fluorouracil (5-FU) and leucovorin (LV) or surveillance only.

Methylation frequencies of the six genes analysed in all patients were 32.9% for WIF1, 55.2% for SFRP2, 68.6% for SPARC, 72.7% for UCHL1, 94.4% for PCDH10 and 95.8% for FZD9.. Methylation status was not significantly different between the two treatment arms. Survival analyses revealed that combined evaluation of three promoters (PCHDH10, SPARC, and UCHL1) showed differential effects with regard to

progression-free survival (PFS) and overall survival (OS) in the two treatment groups. In the chemotherapy arm, a statistically non-significant trend for patients without methylation towards longer survival was observed (P=0.07 for PFS and P=0.14 for OS). In contrast, patients in the surveillance arm with no methylation in their tumors had a significantly shorter PFS and OS (P=0.03 for PFS; P=0.003 for OS), indicating a predictive effect of methylation in this group (test for interaction, P=0.006 for PFS; P=0.018 for OS). These results indicate that promoter methylation status of PCHDH10, SPARC, and UCHL1 may be used as a prognostic molecular marker for CRC patients and, therefore, may facilitate treatment decisions for stage II CRC.

P-CancG-027

Three gangliogliomas: results of GTG-banding, SKY, genome-wide high resolution SNP- array, and review of the literature

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According to the World Health Organization gangliogliomas are classified as well differentiated, slowly growing rare neuroepithelial tumors, composed of neoplastic mature ganglion and glial cells. This is the most frequent tumor entity observed in patients with long-term epilepsy. Comprehensive cytogenetic and molecular cytogenetic analyses including high- resolution genomic profiling (SNP- array) of gangliogliomas are not currently found in the literature but are necessary to better understand this tumor entity. The literature lists 10 gangliogliomas investigated by GTG and sometimes additionally by spectral karyotyping (SKY) and 64 studies applying comparative genomic hybridization (CGH). By CGH, frequent chromosomal aberrations are (partial) losses of chromosomes 9, 10, 13, 16, 17, 18, and 22 and (partial) gains of chromosomes 5, 7, 8, and 12. For detailed characterization at the single cell and cell population levels, we analyzed genomic alterations of gangliogliomas using SNP- array, combined with GTG-banding, SKY, and locus-specific FISH. By GTG and SKY, we could confirm frequently detected chromosomal aberrations (losses within chromosomes 10, 13, and 22; gains within chromosomes 5, 7, 8, and 12), and identified not previously described genetic aberrations. Interestingly, we could verify a second case of ganglioglioma with ringchromosome 1. Using the presented methods, we detected a not previously documented unbalanced non reciprocal translocation t(18;1)(q21;?) in 4/40 metaphases of one ganglioglioma. Analyses of SNP- array data from two of the tumors and respective germline DNA (peripheral blood) identified a number of copy neutral regions with loss of heterozygosity (LOH) in germline as well as in tumor tissue. In comparison to germline DNA, tumor tissues did not show substantial regions with significant losses or gains or with LOH. Gene expression analyses are in progress. We described rare cases of three gangliogliomas with overlapping but also distinct and not previously described genetic aberrations.

P-CancG-028

Evaluating the performance of clinical criteria to predict mismatch repair gene mutations in Lynch syndrome: A comprehensive analysis of 3671 families

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Introduction

Lynch syndrome is an autosomal dominant tumour predisposition syndrome caused by mutations in one of four mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2). Carriers of MMR gene mutations have a high lifetime risk for colorectal (CRC) and endometrial cancer as well as other malignancies. As mutation analysis is costly and time consuming, clinical criteria and tumour tissue analysis are used as

prescreening methods. The commonly applied clinical criteria are the Amsterdam criteria and the (revised) Bethesda guidelines. The aim of our study was to evaluate the performance of different clinical criteria to predict MMR gene mutations.

Patients and methods

We included 3671 families from the German HNPCC Registry fulfilling the Amsterdam I or II criteria or the original or revised Bethesda guidelines. The families were divided into nine mutually exclusive groups. Groups 1 and 2a-c comprised families with single affected individuals. Groups 3a-b, 4 and 5 are composed of families with a history of Lynch syndrome related cancer and group 6 consists of families with at least one colorectal adenoma under the age of 40 years without family history of cancer.

Results

A total of 680 families (18.5%) were found to have a pathogenic MMR gene mutation. Among all 1284 families with MSI-H the overall mutation detection rate was 53.0%. Mutation frequencies and spectrum were significantly different between clinical groups ($p<0.001$). The highest frequencies were found in families fulfilling the Amsterdam criteria (45.5% in group 3a and 53.8% in group 3b). Families with loss of MSH2 expression had a higher mutation detection rate (69.5%) than families with loss of MLH1 expression (43.1%). In the clinical groups with a positive family history as well as young age of onset (groups 3 and 4) mutations in MLH1 and MSH2 were predominant, while the proportion of MSH6 and PMS2 mutations was increased in isolated tumour cases (group 2) and families with a higher age of onset (group 5). MMR mutations were found significantly more often in families with at least one MSI-H small bowel cancer ($p<0.001$). No MMR mutations were found in group 6 (colorectal adenoma under 40 years of age).

Conclusions

Familial clustering of Lynch syndrome related tumours, early age of onset, a positive MSI finding and familial occurrence of small bowel cancer are good predictors for Lynch syndrome.

P-CancG-029

The chromosomal abnormalities deletion 17p13, t(4;14), and gain 1q21 predict progression from smoldering to symptomatic multiple myeloma

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Background: Smoldering Multiple Myeloma (SMM) is a plasma cell disorder defined by the presence of ≥10% plasma cells in bone marrow and/or a monoclonal protein level of ≥3 g/dl in serum without organ damage. The aim of the study was to analyze the prognostic impact of chromosomal aberrations on time to progression (TTP) from SMM to symptomatic MM.

Design and Methods: For selection of the patients, we used the same criteria as previously described by Kyle (Kyle et al., NEJM, 2007). We analyzed the prognostic value of 5 chromosomal abnormalities and hyper-/non-hyperdiploidy (HD and NHD, respectively) in a series of 231 patients with SMM by interphase fluorescence in situ hybridization (FISH). Gains of at least 2 of the 3 chromosomes 5, 9, and 15 defined HD status.

Results: FISH analysis on CD138-enriched plasma cells detected gains of chromosomes 1q21 (29.4%) as well as deletions of chromosomes 13q14 (19.3%) and 17p13 (6.1%). Furthermore, the IgH-translocations t(4;14) and t(11;14) were observed in a frequency of 9.2% and 22.3%, respectively. The presence of t(4;14) was correlated with the serum heavy chain IgA ($p<0.001$). For the entire group, the median TTP was 4.9 years (95% CI, 3.9 – NA). Of all analyzed chromosomal abnormalities, del(17p13), t(4;14), and +1q21 showed a significant impact on TTP, whereas the presence of t(11;14) and del(13q14) was of no statistical significance. The median TTP for patients with del(17p13) was 2.7 years (vs. 4.9 years without, $p=0.019$), with t(4;14) 2.9 years (vs. 5.2 years without, $p=0.021$), and with +1q21 3.7 years (vs. 5.3 years without, $p=0.013$), respectively. In addition, HD was associated with a statistically shorter median TTP of 3.9 vs. 5.7 years in patients with NHD, respectively ($p=0.036$). A multivariate analysis identified t(4;14), +1q21, HD, reduction of uninvolved immunoglobulins, and the risk score defined by Kyle et al. as independent factors for adverse outcome.

Conclusions: The study shows that the overall risk of progression in SMM is significantly influenced by markers for tumor burden (i.e. Kyle risk score) as well as the presence of the chromosomal aberrations del(17p13), t(4;14), and +1q21. Our findings provide evidence that specific chromosomal aberrations are not only associated with early tumor progression and drug resistance in patients with overt MM but also drive transition from asymptomatic into symptomatic stage of disease.

P-CancG-030

Sequencing of new candidate genes for Lynch syndrome

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Introduction:

Lynch syndrome (Hereditary non-polyposis colorectal cancer / HNPCC) is the most common form of hereditary colorectal cancer and accounts for 3-5% of all colorectal cancer cases. So far, germline mutations in one of four DNA-Mismatch-repair-(MMR) genes (MLH1, MSH2, MSH6 and PMS2) or the EPCAM gene upstream of MSH2 have been found to cause Lynch syndrome. Tumour tissue of these patients typically shows signs of MMR defect (high microsatellite instability and loss of at least one MMR protein in immunohistochemical staining). Only about 60 % of all patients who fulfil the clinical Bethesda criteria and harbour signs of MMR defect in their tumour tissue carry a pathogenic mutation in one of the known MMR genes. Therefore, we assume that there might be other still unknown causative genes for Lynch syndrome.

Several genes like PRKCZ and MTOR (FRAP1) have been described to regulate MSH2 degradation. Hence we hypothesize that mutations in these genes might lead to an increased degradation and therefore malfunction of MSH2.

Patients and methods:

To identify new causative mutations, we analysed leukocyte DNA samples of 5 non-related patients with strong clinical evidence for Lynch syndrome and loss of MSH2 protein in their tumour tissue, but without a proven mutation in the MSH2 or EPCAM gene. We performed Sanger-sequencing of the whole coding region of the PRKCZ and MTOR (FRAP1) gene.

Results:

We detected the missense mutation c.520G>A;p.Gly174Ser in one patient and the mutation c.1779+127C>A in the 3´UTR of the PRKCZ gene in another patient. Both mutations were not found in any of the genomic variant databases and were predicted to be non-pathogenic by the MutationTaster programme. We did not find any truncating mutations in PRKCZ. Only common polymorphisms were detected in the MTOR gene.

Discussion:

We did not find any clearly pathogenic mutations in the MTOR or PRKCZ genes. Therefore we cannot declare these genes as causative for Lynch syndrome. We intend to do a segregation analysis of the identified PRKCZ variants in the respective families to get a better assessment of pathogenicity.

P-CancG-031

Extended replication of a GWAS for breast cancer in BRCA2 mutation carriers

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Population-based genome-wide association studies have identified several common genetic variants that also contribute to the observed variation in breast cancer risk for BRCA2 mutation carriers. To search systematically for genetic modifiers of breast cancer risk in BRCA2 mutation carriers, a GWAS has been carried out that led to the identification of an additional susceptibility locus.

Based on the results of stage 1 of the GWAS we selected 19,029 SNPs for inclusion on a custom genotyping array which we genotyped in female BRCA2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2. Associations with breast cancer risk were evaluated within a survival framework using a score test statistic based on modelling the retrospective likelihood of the observed genotypes given the disease phenotypes. Analyses were stratified by country of residence and Ashkenazi Jewish ancestry.

After quality control filtering, data from 18,086 SNPs were available for analysis in 3,881 BRCA2 mutation carriers diagnosed with breast cancer and 4,330 unaffected BRCA2 mutation carriers. We confirmed previously reported genetic modifiers of breast cancer risk including SNPs in FGFR2 and TOX3. For six regions previously shown to be associated with breast cancer risk for BRCA2 mutation carriers, including FGFR2 and PTHLH, we found SNPs with p-values smaller than those previously reported. We

identified a novel locus that was associated with breast cancer risk at genome-wide significance level (per allele HR=0.85, 95% CI 0.80-0.90, P=3.9x10-8). There was no evidence of association of this locus with breast cancer risk either in the general population, based on 42,599 breast cancer cases and 46,451 controls from the Breast Cancer Association Consortium (OR=1.00, 95%CI: 0.98-1.02, P=0.74), or breast cancer risk for BRCA1 mutation carriers (HR=0.99, 95%CI: 0.94-1.04, P=0.75), based on 5,920 BRCA1 carriers with breast cancer and 5,783 without from CIMBA. The 6p24 locus lies within a region containing TFAP2A, which encodes a transcriptional activation protein that interacts with tumour suppressor genes.

Our results suggest that 6p24 is the first modifier locus that is associated with breast cancer risk specifically in BRCA2 mutation carriers. This locus might provide further insight into the biology of breast cancer development for these women.

P-CancG-032

The loss of chromosome 4 - a correlation with the long-term survival and the recurrence rate after R0 resection of colorectal liver metastasis

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Most sporadic colorectal cancer (sCRC) deaths are caused by metastatic dissemination of the primary tumor. Multimodale therapy and progress in surgical techniques increased the long-term survival rate after liver metastasis resection. Nevertheles there is a high recurrence rate of >50%. Accurate staging of CRC with clinical and pathological parameters is important for predicting prognosis and guiding treatment. At the moment only some genomic aberrations are known which allow differentiation between prognostic adverse and favourable metastases in primary colorectal cancer (Bruin SC et al., BMC Cancer, 2010 December 2; Munoz-Bellvis L et al., Mod Pathol. 2012 Apr;25). For years, the genetics of metastatic colorectal cancer (CRC) have been studied using a variety of techniques. However, most of the approaches employed so far have a relatively limited resolution which hampers detailed characterization of the common recurrent chromosomal breakpoints as well as the identification of small regions carrying genetic changes and the genes involved. Array-based comparative genomic hybridization (aCGH) was used to investigate the association of DNA copy number alterations in liver metastases with outcome in patients with colorectal liver metastasis. For this pilot-study 20 probes from 257 paraffin-embedded tissues of R0-resected colorectal liver metastases were randomly selected (probes from two distinctive patient groups with upper and lower 5 year median survival rate). DNA was isolated and applied on a 180K oligonucleotide array (Agilent, protocol, version 6.2.1, February 2010) with male control DNA (Promega). We used the 'Fischer-Test' for detection of differences in distribution of amplification and deletion detected in the patient material and the 'log-Rank-Test' to detect differences in survival and recurrence rate. Distribution of age, gender, tumour stage, grading of primary tumour, number and distribution of liver metastases, 'Nordlinger-Score' and 'Fong-Score' showed no significant correlation with a monosomy 4 except the two distinctive prognosis groups. Patients with monosomy 4 had a lower recurrence rate and a higher long-term survival compared to patients with disomy 4. The loss of chromosome 4 in liver metastases has not only association with primary tumour progression (Diep et al., Genes Chromosom and Cancer 45,31-41) but also with long term survival and cumulative recurrence rate after R0 resection of colorectal liver metastasis. In the present study we provide a detailed map of the genetic abnormalities of liver metastasis from CRC patients.

P-CancG-033

BRCA1 and BRCA2 testing in the era of next generation sequencing

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Thus far, Sanger sequencing and MLPA analysis is routinely used for mutation screening in patients with an increased risk for breast and ovarian cancer. However, the full analysis of the genes BRCA1 and BRCA2 as well as additional genes with minor contribution to disease is extensive and cost and labor intensive. Available state-of-the-art technologies for high-throughput DNA analysis such as massively parallel sequencing (also known as next generation sequencing, NGS) can address those difficulties and thus are currently tested for its application in routine DNA testing.

We have established a flexible platform based on the Ion Torrent personal genome sequencer, a semiconductor-based technology that detects a sequence dependent incorporation of nucleotides due to subsequent release of protons. In an initial step, we analyzed specificity and sensitivity of the NGS method in comparison to Sanger sequencing of all exons and immediately flanking intronic sequences of BRCA1 and

BRCA2 in 16 patient samples by using the BRCA MASTR v2 amplification kit (Mulitplicom, Belgium) and barcoding of samples. By Sanger sequencing, polymorphic variants and probable disease-associated mutations were identified in the 16 patient samples. Five patients carried an insertion or deletion of one to five nucleotides (BRCA1: c.1961delA; c.3628insA; BRCA2: c.3847_3848delGT; c.6405_6409delCTTAA) or an insertion/deletion (BRCA1: c.5564_5572delinsAACCCAGT) while one patient was a carrier of a large deletion including BRCA2 exon 24. For Ion Torrent analysis, patient samples were amplified according to the manufacture's protocol and run on the 314 Chip with 6 patient samples analyzed simultaneously with a barcoding approach. Evaluation of raw data was performed on the CLC Genomics Workbench (Aarhus, Denmark). Genescan profiles, providing a fragment analysis of Multiplicom-based fragments, were run on an ABI 3130. In BRCA1 and BRCA2 a total of 108 heterozygous polymorphic variants in the 16 probands were detected by Sanger as well as by Ion Torrent sequencing. All probable disease-associated variants but one were replicated by the Ion Torrent. Only a one-nucleotide deletion (c.1961delA) in a homopolymeric stretch of eight adenines was missed although this deletion was readily obvious by Genescan profiling. Average minimum coverage per nucleotide in BRCA1 and BRCA2 was 121 and 145, respectively. No additional sequence variants were detected by Ion Torrent which had not been found by Sanger sequencing. In a first resume, parallel sequencing (e.g. with the Ion Torrent) revealed specificity and sensitivity comparable to Sanger sequencing but at greatly reduced costs and an unparalleled speed.

P-CancG-034

Unraveling the methylome of thyroid cancer at single C resolution: From screening to clinical diagnostics

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Thyroid nodules are widely spread and approximately 20% of the people develop a palpable nodule during live and even up to 70% of the adults have nodules detectable by sonography or autopsy. Since 5% of these nodules are malignant, all thyroid nodules have to be clinically evaluated to discern benign from malignant cases. The current method of choice for diagnosis is based on fine needle aspiration (FNA) followed by cytological evaluation. Despite many advances in the diagnosis and treatment of thyroid nodules this method is far away from perfect in terms of specificity and sensitivity and results in a huge number of indeterminate cases. Patients with an indeterminate diagnosis will undergo surgery. The consequence is an extensive overtreatment of patients, as only approximately 20% of the indeterminate cases will be identified as malignant at surgery.

In the present study we addressed the call for minimally invasive diagnostics based on state of the art molecular techniques to clearly discriminate between benign and malignant thyroid entities. Therefore we employed 48 thyroid nodules, consisting of follicular thyroid adenomas/carcinomas (FTA/FTC), papillary thyroid carcinomas (PTC) and struma nodosa (SN) to a whole genome methylation screening using Illuminas Infinium HumanMethylation450 BeadArrays covering more than 485000 single CpGs dinucleotides. Upon statistical evaluation we identified the most informative genomic regions, suitable for discerning benign from malignant cases. Those genomic regions were subjected to microfluidic qPCR validation experiments using Fluidigm's Biomark. The results from the screening experiment were technical as well as biological confirmed by the microfluidic qPCR. For the biological validation an independent sample set consisting of 76 different thyroid entities was used. Based on the results from the biological validation a classifier containing a minimal set of genes was constructed which allows the discrimination between benign and malignant cases with high sensitivity and specificity.

The next steps comprise a test phase of the classifier using DNA derived from FNAs as well as sera. After confirming the suitability of the classifier on those sample material a test phase at the clinics is scheduled. For that test phase an easy to use assay will be developed which allows fast and reliable diagnostics of thyroid nodules in the clinical all day routine.

A future approach is the identification of DNA methylation patterns in DNA derived from saliva to advance minimal invasive methods.

P-CancG-035

Update of the genetic monitoring of the LE-MON-5 study

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Introduction: LE-MON-5 is a multicenter German phase-II study to verify the safety of a monotherapy with Lenalidomide in MDS patients with IPSS low or Int-I risk and isolated del(5q). We report our cytogenetic results after a monitoring period of 30 months since start of the trial.Methods: For sequential and frequent survey of Lenalidomide treated patients we applied FISH on enriched CD34+ stem cells from peripheral blood every 2-3 months using panels of 8 to 13 probes. Karyotyping and FISH on bone marrow aspirates was performed at initial screening and every six months. The median number of analyzed metaphases is 25 (4-30) and FISH analyzing is based on 200 interphase nuclei. Results: We have already screened 127 patients and could confirm isolated del(5q) in 102 (81%). Due to our cytogenetic results demonstrating additional changes in 25 (19%) patients, these where registered as screening failures and thus excluded from the study. Until now cytogenetic follow-up data for 53 patients are available. After a median follow-up of 14 (2-30) months we have observed a significant impact of Lenalidomide on the reduction of the clone size ($p < 0.05$) by FISH-monitoring on CD34+ pb cells. Based on molecular-cytogenetic remission, we have divided the study patients into three groups: "Fast responders" showed a very rapid cytogenetic response to treatment with >50% reduction of the 5q- clone size within 2 months (38.5%). In the second group, "Slow responders", we observed >50% reduction in clone size (46.1%) after more than 2 months. However, 5 patients (13%) showed a resurgence of the 5q- clone after 12-21 months. In the third group "non responders" (15.4%) we observed no cytogenetic response as yet. In 6 cases (12%) of the second group we found a reduction of the 5q-clone during the follow-up, but the occurrence of additional aberrations was observed: trisomy 8 (6%), trisomy 4 (2%) and Y-loss (4%). All new secondary abnormalities occurred in independent small clones. In two further cases a new secondary abnormality arose in the 5q-clone after 4 and 24 months of treatment respectively. Interestingly in both cases chromosome 20 was involved. Conclusion: FISH analysis of CD34+ pb cells allows a reliable, frequent and relevant genetic monitoring of treatment response to Lenalidomide. Our results confirm the positive and rapid effect of Lenalidomide on clones with del(5q): Thus, already after 2 months we could observe up to 90% reduction in the 5q- clone size in 38.5% of patients. We suspect that trisomy 4, 8 and Y-loss are fluctuant mini clones without any clinical relevance. The course in 2 patients with additional 20q deletions/chromosome 20 aberration remains to be further followed. To assess the clinical efficacy of Lenalidomide and a correlation with the CD34-FISH data, the hematologic and clinical parameters are not yet available.

P-CancG-036

Comparative gene expression profiles of a monozygotic twin pair discordant for somatic partial RSPO3 deletion in mosaic state, childhood cancer and secondary cancer

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Despite their genetic concordance monozygotic twins may differ in susceptibilities to diseases, even in those with genetic involvement, as for example cancer. Besides acquired epigenetic differences, rare somatic mosaicism in one sibling is thought to cause phenotypic variability of twins. Such mosaics arise from genetic mutations after the twinning event, which are then propagated to subsets of cells of only one twin.

We report on a monozygotic twin pair discordant for childhood leukemia and secondary malignancy of the thyroid. To identify alterations on the genomic and gene expression level, potentially modulating cancer proneness in the twin siblings, we performed genome-wide SNP arrays and gene expression arrays on primary fibroblasts of the individuals.

The SNP array detected a heterozygous 181 kb deletion on chromosome 6q22.33 affecting the promoter region and first exon of the gene RSPO3 (R-spondin 3) in the 2-cancer twin. FISH analysis and genomic qPCR confirmed the deletion and showed its occurrence in a mosaic state.

The gene expression array analysis revealed a set of differentially expressed genes between twins, mainly representing components of the extracellular matrix, in particular collagens. Furthermore, differences were observed in genes that play a role in the WNT/β-catenin signaling and ECM-related pathways, as integrin signaling.

To enable a more detailed analysis we resolved the mosaic state of the affected twin by fibroblast single cell cloning and established 91 single cell clone cultures. 14 out of 23 clones, analyzed up to date, carry the partial heterozygous RSPO3 deletion, whereas the remaining 9 clones have two complete gene copies, as determined by genomic quantitative PCR.

Expression profiling of established single cell clones will enable us to further analyse the consequences of the partial RSPO3 deletion for molecular pathways and processes connected to cancer susceptibility. Furthermore, the obtained single cell clones facilitate the detailed characterisation of the deletion on the genomic and chromosomal level, respectively. Therefore, we plan to use cytogenetic methods and genomic walking technologies to identify possible genomic rearrangements.

This monozygotic twin pair represents an impressive example of somatic mosaicism, connected to differences in cancer predisposition and the first report of a RSPO3 aberration in human non-tumor material. RSPO3 is an emerging regulator of WNT signaling, that to date has mainly been investigated in the field of embryonic development in murine and Xenopus models. Recently, recurrent RSPO3 gene fusions and expression changes were identified in human colon tumors, illustrating its role in formation of WNT-related cancer types.

We hypothesise that discordance for cancer proneness of the here reported twin pair may be connected to a WNT deregulation, triggered by RSPO3 disruption. As a result, changes of the extracellular matrix composition occur, that may enhance cell proliferation and migration and therefore promote tumor formation, invasion and metastasis.

P-CancG-037

Expression of microRNAs miR-371-3 in testicular tumors

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Testicular cancer represents the most frequent malignancy among men aged 18 - 42 years. Clinical management of the testicular cancer is substantially based on the measurement of serum concentrations of the established tumor markers beta human chorionic gonadotropin (bHCG), alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH). Nevertheless, unfortunately only about 50-60% of all germ cell tumors exhibit an increase of these markers. This is particularly disadvantageous in classical seminoma, where only less than 20% of the patients have a beta-HCG increase.

microRNAs are a novel class of small, non-coding RNA molecules, which play an essential role in the post transcriptional gene regulation. These genetically defined RNA molecules show a high specificity for certain tissues and tumors.

We determined the expression profiles of the miRNAs miR-371a-3p, miR 372, and miR-373-3p in patients with testicular tumor before and after Ablatio testis as well as after chemotherapy and/or radiotherapy. For this study, serum samples of adult patients with seminomas, pure and mixed non-seminomas, and Leydig cell tumors were used. The expression profiles of the circulating miRNAs in serum were quantified using real-time PCR. Aged-matched serum samples of healthy men served as controls.

Statistical analyses of the miRNA-expression showed much higher serum levels of miRNAs-371-3 in the stage 1 patients than in controls and a postoperative decrease ($p<0.01$). Also, in those cases with more advanced cancers levels decreased to the normal level after surgery and chemotherapy, respectively. The expression levels of miRNAs-371-3 in patients with Leydig cell tumors before surgery were similar to controls. Our results show that the expression levels of miR-371-3 in serum appear to be a useful biomarker for patients with testicular tumors.

P-CancG-038

Mutation screening of the candidate genes BUB1B and PTPRJ in 85 unrelated patients with unexplained colorectal adenomatous polyposis

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Background. Adenomatous polyposis syndromes are hereditary tumour predispositions characterised by the appearance of numerous colorectal adenomas, which, if not detected early and removed, inevitably

result in colorectal cancer (CRC). In up to 50% of the patients no germline mutation in the currently known genes – APC and MUTYH – can be identified.

BUB1B and PTPRJ are promising new candidate genes: BUB1B is involved in the regulation of the spindle-assembly checkpoint and interacts with APC; mutations in both genes can cause chromosomal instability. A patient with multiple adenomatous polyps and adenocarcinomas of the colon and stomach was reported to harbour a homozygous germline BUB1B splice mutation. PTPRJ is regarded as tumour suppressor gene; loss of heterozygosity was observed in early adenoma formation even before APC mutations. In addition, a germline loss-of-function duplication was identified in a patient with early onset CRC.

Methods. To screen for pathogenic germline mutations we performed a systematic analysis of the BUB1B and PTPRJ genes by sequencing all coding regions in a cohort of 85 apparently unrelated and clinically well characterised adenomatous polyposis patients without APC or MUTYH germline mutations (69 sporadic cases, 16 familial cases). Screening for large deletions and duplications of the BUB1B and PTPRJ regions was performed by CNV analysis using SNP array data (HumanOmni1-Quad BeadArray, Illumina).

Results. In none of the patients a truncating mutation or CNV could be identified in either of the two genes. The number and frequency of common SNPs was in accordance to literature. Altogether, 12 different heterozygous rare variants (reported MAF < 0.006) were identified in 14 patients (11 sporadic, 3 familial). The rare variants include one in-frame deletion, one missense, one silent, one intronic and one variant located in the 5'UTR (BUB1B) and five missense, one silent and one intronic variant (PTPRJ), respectively. The in-frame deletion in BUB1B and three of the PTPRJ variants are not listed in public variation databases (dbSNP, 1000Genomes, Exome Variant Server). In silico analysis using various tools (MutationTaster, PolyPhen2, NNSPLICE 0.9) revealed no consistent deleterious effect in nine of the novel or rare variants. The in-frame deletion in BUB1B and two missense PTPRJ mutations were classified as potentially damaging. All three mutations were identified in sporadic cases.

Conclusions. In conclusion, we could not identify clear pathogenic alterations in BUB1B and PTPRJ in a large cohort of colorectal polyposis patients indicating that germline mutations in these genes seem not to be a frequent cause of adenomatous polyposis.

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P-CancG-039

A low frequency missense variant in SLX4 is associated with early-onset breast cancer and reduced DNA repair capacity

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Background: Genes conferring high or medium risk for breast cancer have been identified by linkage analysis or sequencing of candidate genes in families, while a considerable number of low risk genetic variants was found in genome wide association studies. However, only a minority of familial or sporadic breast cancer cases can be explained by these genes, and the unexplained risk may be attributable to low frequency genetic variants with medium risk.

Methods: Seventeen missense variants with a minor allele frequency of 1-15% were selected in ten genes involved in the repair of DNA double strand breaks. They were tested for association with breast cancer (N=435, controls N=1189) and the result confirmed in a verification cohort (cases N=1013, controls N=1404). This association was characterized by genotyping additional SNPs, imputation of SNPs in the interval, and haplotype analysis. In a subset of the detection cohort (cases N=135, controls N=299) repair of DNA double strand breaks was assessed by the micronucleus test (MNT), and was tested for association to the variant as well as to breast cancer.

Results: In the candidate gene approach only one SNP (MAF=0.048) in the SLX4 gene yielded significant results for cases ? 60 years (OR=2.3; 95%CI 1.5-3.5; P=4.9x10-5; Bonferroni corrected for 51 tests p=0.0014). The association was confirmed in the verification cohort for cases ? 40 years (p=0.0096). The combined cohorts yielded OR=1.26 (1.03-1.54), p=0.025 for all cases, OR=1.35 (1.06-1.72), p=0.013 for age ? 60 years, and OR=2.19 (1.45-3.30) p=0.00012) for age ?40 years. The imputed SNPs revealed a haplotype with similar associations. The same applied to DNA repair (increase from 100 to 137 MN per allele, beta=37.8, p=0.00053). DNA repair was also associated with breast cancer (in patients ? 60 years: OR=3.58 (2.07-6.18) p<0.0001) and this association depended on the genotype. Calculating ROC curves for

this association revealed that the AUC (area under the curve) increased from AUC=0.65(0.55?0.74) to AUC=0.94 (0.86?1.0) when restricted to carriers of the risk allele.

P-CancG-040

Significant differences in the DNA-methylation profile between EBV-positive and EBV-negative Burkitt Lymphoma cell lines

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Burkitt lymphomas belong to the group of mature aggressive B-cell lymphomas which derive from germinal center B-cells. Besides translocations of the MYC gene with one of the immunoglobulin (IG) loci, which are the cytogenetic hallmark of Burkitt lymphomas, several other genes like TP53 and CCND3 are also recurrently mutated in this tumor entity. Recently, the ID3 gene has been discovered being recurrently mutated in more than 60% of Burkitt lymphomas.

Besides somatic mutations in the genome, epigenetic alterations are distinctive for cancer cells. However, although epimutations in single loci like INK4α/ARF are long known, the impact of epigenetic alterations for the progression of Burkitt lymphoma is yet still largely unknown.

In the present study we determined the DNA-methylation profiles of eleven IG-MYC positive Burkitt lymphoma cell lines: BL-2 (EBV-neg.), BL-41 (EBV-neg.), BL-70 (EBV-neg.), BLUE-1 (EBV-neg.), CA-46 (EBV-neg.), Daudi (EBV-pos.), DG75(EBV-neg.), EB-1 (EBV-pos.), Namalwa (EBV-pos.), Raji (EBV-pos.) and U698-M (EBV-neg.). For DNA-methylation profiling we used the HumanMethylation 450k BeadChip (Illumina). This array allows the parallel analysis of more than 450,000 CpG loci. The cell lines were classified according to the epidemiologic subtype of the respective Burkitt lymphoma (endemic vs. sporadic), EBV status, and the mutation status of ID3 and TP53. Subsequently, differential DNA methylation between the resulting groups was determined.

Comparisons between the Burkitt lymphoma subgroups defined by mutation status resulted only in minor differences in DNA-methylation. In contrast, comparison of EBV-positive and EBV-negative Burkitt lymphoma cell lines revealed more than 869 differentially methylated CpG loci. Of these, the overwhelming majority (n=819; 94%) corresponding to 435 genes was found hypermethylated in EBV-positive as compared to EBV-negative cell lines (FDR<0.05 and Δbeta> +/- 0.3). These findings are in line with the results of studies of other groups e.g. in gastric cancer which showed differential DNA methylation upon EBV infection.

In summary, we show here that dependent on the EBV status there is a considerable difference in the DNA-methylation profile in Burkitt lymphoma cell lines.

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P-CancG-041

Detection of Mosaic RB1 Mutations in Patients with Isolated Unilateral Retinoblastoma using Deep Sequencing

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Retinoblastoma is a rare malignant intraocular childhood tumor caused by two mutations, each affecting one of the RB1 alleles. Children with sporadic bilateral retinoblastoma are mostly heterozygous (>95%) for a mutated allele that was inherited from the parents or occurred de novo in the germ line of one of the parents (heritable retinoblastoma). Children with sporadic unilateral retinoblastoma in some cases (<10%) also have the heritable heterozygous form, but in most cases (>90%) the causative somatic mutations of the RB1 gene occurred de novo in somatic cells. In a few of these cases, the first mutation occurs during the embryonic phase, resulting in mutational mosaicism.

In patients with sporadic unilateral retinoblastoma, the planning of control examinations of the remaining healthy eye and the risk assessment for relatives depend largely on whether the patient has the heritable form of retinoblastoma. This is commonly diagnosed by comparing the mutations found in tumor samples to those found in blood DNA. Due to the advances in therapy, an enucleation can be avoided in more and more of these patients, reducing the availability of tumor samples. This creates the requirement for alternative strategies to determine the causative mutations in patients with unilateral retinoblastoma. The heterozygous mutations are readily detectable in blood samples with conventional methods. Mutational mosaics, however, can only be detected if the proportion of the mutant allele is large enough to give a clear signal in sequencing, unless the location of the mutation is known from the tumor. If only blood samples are available,

it is not possible to determine the causative mutation in more than 85% of sporadic isolated retinoblastoma using conventional techniques. Improving the detection of low-dose mutational mosaics in blood would increase the proportion of patients with sporadic unilateral retinoblastoma for whom the causative mutation can be determined.

We have developed a deep sequencing protocol (>10,000-fold coverage) to determine the causative mutations in DNA from blood of patients with unilateral retinoblastoma. Our approach is based on the finding that the number of mutational mosaics in patients with unilateral retinoblastoma has been underestimated so far due to the incapability of the conventional techniques to detect low-dose mutational mosaics. The method was tested on blood samples from patients with unilateral retinoblastoma with known mosaic mutations as determined by Sanger sequencing. Here, we use this method to verify low-dose mosaic mutations in the RB1 gene in blood DNA from 100 sporadic unilateral retinoblastoma patients with a known mutational status of the tumor. Our aim is to optimize this method so that the causative mutation can be determined in DNA from blood even when no tumor sample is available.

P-CancG-042

Sequence Variants of the Genomic Caretaker RAD9A in Primary Fibroblasts of Individuals with Childhood and Independent Second Cancer showing a reduced RAD9A mRNA and Protein Expression

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The etiology of secondary cancer in childhood cancer survivors is largely unclear. Exposure of normal somatic cells to radiation and/or chemotherapy can damage DNA and if not all DNA lesions are properly fixed, the misrepair may lead to pathological consequences. It is plausible to assume that genetic differences, i.e. in pathways responsible for cell cycle control and DNA repair, play a critical role in the development of secondary cancer. To identify factors that may influence the susceptibility for second cancer formation, we compared 20 individuals who survived a childhood malignancy and then developed a second cancer to 20 carefully matched control individuals with childhood malignancy but without a second cancer.

During this study we detected reduced mRNA and protein expression of the genomic caretaker RAD9A in the primary fibroblasts of the two-cancer individuals compared to those of the one-cancer individuals (Weis et al., PloS ONE 2011; 6(10): e25750). The RAD9A gene, located on Chromosome 11q13 with two known isoforms (isoform 1: NM_004584.2, isoform 2: NM_001243224.1), is evolutionarily highly conserved and acts in multiple pathways including base excision, homologous recombination and mismatch repair as well as cell cycle checkpoint control and apoptosis. To further support the idea that modulation of RAD9A and other cell cycle arrest and DNA repair proteins contributes to the risk of developing a second malignancy in childhood cancer patients, we performed mutation analysis of the RAD9A gene in all patients of the study. Using direct PCR Sanger sequencing we detected five sequence variants of RAD9A, four intronic and one coding, in the fibroblast cells of two-cancer patients. Two of the intronic variants (c.234+32C>T het; c.235-34G>A het) have not yet been recorded in the databases (ensembl, NCBI, UCSC). Of particular interest is one sequence variant in intron 3-4 (c.235-6A>G het, rs2066496 in isoform 1; c.1A>G het, p.Met1Val in isoform 2), which causes the change of the initiation codon from methionine to valine in RAD9A isoform 2. In addition, the patients displaying the Met>Val replacement at the initiation codon of isoform 2, show a coding variant in exon 10 (c.1014T>C het, p.Gly338Gly, rs872110), which is a silent mutation not leading to an amino acid change. These variants may have adverse effects on RAD9A RNA structure and splicing as well as RAD9A protein structure.

Further analyses on mRNA, protein and epigenetic level are required to clarify if the reduced RAD9A expression level in the two-cancer patients results in a higher predisposition to develop second cancer.

P-CancG-043

Constitutional karyotype or acquired aberrations?

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Cytogenetic analysis of blood cancer is dealing with many parameters that influence critically its success. Crucial may be the lack of precise indication at the time of culturing, receiving the required amount of cells or banding resolution or the right tissue to analyse by sampling or stimulation.

If some chromosomal features are found in all analysed cells another delicate issue is to differentiate between normal variants, constitutional aberrations and acquired, clonal abnormalities. Sometimes a combination of different methods is needed to draw the right conclusions towards proper diagnosis and to estimate the necessity of additional genetic counselling.

Here, we present exemplary cases of CLL, multiple myeloma, CML and AML to show some of the pitfalls that have to be avoided on that journey.

P-CLINICAL GENETICS

P-ClinG-044

Exome sequencing identifies a homozygous frameshift mutation in AP4B1 in two siblings with severe intellectual disability, progressive spastic tetraplegia and microcephaly

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Intellectual disability (ID) is an extremely heterogeneous disorder. Genetic alterations underlying ID vary from mutations in single genes to large chromosomal abnormalities, which result in a wide phenotypic diversity. Up to date, about 450 disease genes for both syndromic and non-syndromic ID are known; however, for autosomal recessive ID (ARID) the genetic cause remains unknown in the majority of affected cases. Here we report two siblings from a non-consanguineous couple who presented with severe ID, microcephaly, growth retardation, inability to walk and absent speech. Both showed progressive spasticity and became tetraplegic and therefore wheelchair-bound. They presented with mild facial dysmorphism including large ears, protruding tongue, widely spaced teeth and strabismus. The siblings had an anxious and shy character. Pelvis X-ray revealed valgosity of hips with bilateral subluxation and acetabular dysplasia in both. Hearing and eye examinations were normal. Brain MRI did not show any abnormality in the elder sister, however, mild thinning of the corpus callosum in the dorsal region of the splenium was observed in her brother. Conventional chromosome analysis and array CGH did not reveal any alteration of clinical importance in both patients. By exome sequencing, we identified the underlying genetic cause in the two affected siblings. DNA libraries from both patients were generated and exomes were enriched using the Illumina TruSeq kits. Deep sequencing was performed on the Illumina Genome Analyzer IIx platform. We used BWA to align reads against the hg19 reference sequence and applied SAMtools and GATK to detect single nucleotide variants and small insertions/deletions (indels). Finally, we found a 2-bp deletion in exon 7 of the AP4B1 gene in the siblings. We confirmed the frameshift mutation c.1159_1160delCA (p.Thr387Argfs*30) in both patients in the homozygous and in the parents in the heterozygous state by Sanger sequencing. AP4B1 encodes the B1 subunit of the AP4 adaptor protein complex that comprises the four subunits B1, E1, M1 and S1. The AP4 complex is involved in vesicle formation, trafficking and sorting processes in neurons. Recently, mutations in any of the four subunits have been described in few families with the AP4-deficiency syndrome which is characterized by severe ID, growth retardation, microcephaly, delayed or lack of speech, inability to walk and progressive spasticity. The clinical features of our two patients carrying the c.1159_1160delCA mutation in AP4B1 fit well with the described AP4-deficiency syndrome. Thus, our and data of others show that the phenotype underlying AP4 deficiency is clinically recognizable and should prompt testing of the four genes encoding the AP4 adaptor complex.

P-ClinG-045

NGS goes diagnostics: Multiplex analysis of genes for limb-girdle muscular dystrophies

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A main focus of our diagnostic laboratory lies in the identification of mutations causing muscular diseases. Besides classical Sanger sequencing we are now establishing next generation sequencing (NGS)

applications for single large genes and groups of genes e.g. those causing limb-girdle muscular dystrophies (LGMDs). Clinically, LGMDs can be classified into juvenile and adult onset and association with high and low creatine kinase (CK) levels, respectively. Inheritance is autosomal recessive or dominant and more than 25 genes are known to date as causative for the various subtypes of LGMDs.

The autosomal recessive LGMD types 2A, 2B and 2L have a similar clinical presentation and are associated with very high CK levels. Since they are at the same time the most prevalent subtypes in Central Europe, we have established a NGS panel for the three genes CAPN3, DYSF and ANO5. Target enrichment is performed with the Access Array™ System of Fluidigm which allows parallel amplification of 48 target regions for up to 48 samples in one single PCR setup. The assay comprises 98 primer pairs covering all exons of the three genes. The resulting libraries are sequenced using the GS Junior System of Roche.

In a first step, CAPN3, DYSF and ANO5 have been resequenced in a total of 18 patients with known mutations and SNPs in at least one of the three genes. All 50 known variants could be retrieved providing a validation sensitivity estimate of more than 94 % of this NGS panel. As expected, short indels or duplications of single nucleotides in homopolymeric regions could not be reliably identified in the NGS data and have to be resequenced in parallel with Sanger sequencing. Since then, more than 28 LGMD patients have been analysed by this panel. The number of patients analysed per run varies between 8 and 12. With an average of 70,000 reads per run on a GS Junior, this leads to a calculated coverage of 60x - 90x per amplicon. In fact, coverage was not homogenous across all amplicons but in most cases at least 15-fold, i.e. suitable for reliable analyses. Each variant suspected to be causal was confirmed by Sanger sequencing. Up to now, we identified causative mutations in about 50 % of LGMD patients using this NGS panel with no false-positive results.

The combination of Access Array System and GS Junior sequencing has proven to be a reliable and practical NGS method for diagnostic analyses of the three LGMD candidate genes CAPN3, DYSF and ANO5. Compared to classical PCR and Sanger sequencing, the NGS application has proven to be a time and cost efficient diagnostic approach.

NGS panels for other groups of muscle disease genes are under development.

P-ClinG-046

Targeted next-generation sequencing (NGS) significantly improves genetic diagnostics in Bardet-Biedl syndrome and related disorders

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Bardet-Biedl syndrome (BBS) is often called a model ciliopathy due to its pleiotropic character. Clinical hallmarks comprise retinal dystrophy, obesity, polydactyly, renal abnormalities, learning difficulties and hypogonadism. Various additional features can be observed in a subset of patients such as cardiac abnormalities, hearing defects, anosmia, dental anomalies, diabetes mellitus and further endocrinologic manifestations. So far, mutations in 17 BBS genes have been described without convincing genotype-phenotype correlations. Moreover, there is considerable clinical and genetic overlap with other cilia-related disorders, especially Alström syndrome, nephronophthisis, and Joubert syndrome. Extensive heterogeneity has been a major challenge in molecular diagnostics and genetic counselling. We established an NGS (next-generation sequencing) based panel targeting all BBS genes and other genes known or hypothesized to cause ciliopathies (currently, in total 258 genes). We present our molecular and clinical data of more than 100 unrelated families with Bardet-Biedl syndrome. Underlying mutations could be clearly identified in the great majority of cases. We will also comment on the aspect of triallelic and oligogenic inheritance. Conclusively, NGS-based testing for Bardet-Biedl syndrome and related disorders is highly efficient and significantly improves genetic diagnostics.

P-ClinG-047

DNA methylation levels of the PAX8 promoter

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Congenital hypothyroidism is a relatively common disease of the thyroid. 80% of the cases are due to thyroid dysgenesis, which means the thyroid is not at all, hypoplastically or ectopically developed. Thyroid dysgenesis can be caused by mutations in the coding region of different transcription factors that play an

important role during normal thyroid development (PAX8, TTF1, TTF2, NKX2.5). But in the genome of most patients diagnosed with congenital hypothyroidism no mutations in these genes were found. We therefore concentrated on the regulatory promoter regions of one of the transcription factors (PAX8) that we have recently characterized in vitro. PAX8 is important for the early embryonic development of the thyroid as well as for the activation of downstream targets like the thyroid peroxidase (TPO), the sodium iodide symporter (NIS) and the thyroglobulin (TG) genes. We recently identified base pair changes in the promoter region of the PAX8 gene that diminish PAX8 gene expression. Thus we asked, whether other modifications of non-coding regions in the PAX8 gene would have an influence on PAX8 gene expression. Methylation of CpGs that do not change the DNA sequence itself, for example, can inactivate a gene. The effect of DNA methylation of the non-coding regions of PAX8 has not been studied so far.

The aim of our study is to determine the methylation status of the PAX8 promoter region in tissues where PAX8 is expressed and as a control for inactivation of gene expression where PAX8 is not expressed. Thus, bisulfite treatment of genomic DNA of 26 individuals that was isolated from leukocytes was performed to detect the methylated CpGs. Primers for amplification of the 5'-region and the first exon of PAX8 were tested. The PCR products were purified prior to subcloning into a PCR-cloning vector and transformed into DH5α cells. Ten single clones were sequenced per test sample.

Analysis of the sequences results in a certain methylation pattern. As expected for leukocytes where PAX8 is not expressed the CpGs in the PAX8 promoter region were predominantly (>84%) methylated except for three striking CpGs with a reduced methylation level ranging from 45% to 63%. This pattern was observed in all tested individuals, no matter whether they were diagnosed to have congenital hypothyroidism or not. This pattern was also independent from age and gender. In spite of the three clearly less methylated CpGs one can assume that the PAX8 gene is inactive in leukocytes due to the overall predominant methylation.

Further studies are currently under investigation involving the analysis of the DNA methylation level in thyroid tissues derived from normal controls as well as patients with congenital hypothyroidism. The expression level of PAX8 itself as well as its known downstream targets are in progress. Preliminary data reveal a markedly different methylation pattern in several tissues derived from samples diagnosed with different diseases of the thyroid. Thus, methylation of PAX8 might play a role in thyroid disease.

P-ClinG-048

Gene dosis study in DYT1 dystonia. The importance of copy number variations in DYT1, DYT6 and DYT12 in sporadic dystonia patients

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Dystonia is a neurological movement disorder, in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures. Dystonic syndromes include a large group of diseases that have been classified into various aetiological categories, such as primary, dystonia-plus, heredodegenerative, and secondary.

Inherited dystonia designated by DYT locus symbols can be separated into three broad phenotypic categories: primary torsion dystonia (PTD), where dystonia is the only clinical sign (except for tremor) (DYT1, 2, 4, 6, 7, 13, 17, and 21); dystonia plus loci, where other phenotypes in addition to dystonia, including parkinsonism or myoclonus, are present (DYT3, 5/14, 11, 12, 15, and 16); and paroxysmal forms of dystonia/dyskinesia (DYT8, 9, 10, 18, 19, and 20). Mutations in nine genes have been involved, almost all of them are point mutations or small deletions / insertions.

Large duplications or insertions in the genes known to be involved in dystonic symptoms have not been investigated so far.

In order to determine whether copy number variations might be a cause for dystonia syndromes we use the MLPA Kit P059-A1 (MRC Holland) searching for large deletions / insertions in the genes DYT6 (THAP1), DYT1 (TorA) and DYT12 in large cohort of sporadic dystonia patients suffering from different types of dystonia.

Results: We did not identify any copy number variation in DYT12 and DYT1 in this cohort of dystonia patients. However we found one large deletion of exon 2 and 3 of the THAP1 gene in a patient presenting with segmental dystonia.

Conclusions: Large insertions/ deletions in DYT1, DYT6 and DYT12 are a rare cause for dystonia in patients with sporadic forms of dystonia. Whether copy number variations in these genes might explain a significant fraction of familiar cases has to be determined.

P-ClinG-049

Low level mosaicism for Phelan-McDermid Syndrome (Microdeletion 22q13.3) in a five year old girl – a case for clinical phenotyping and FISH testing

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A five year old girl was referred for a genetics opinion by a paediatrician because of speech delay, poor expressive speech, difficult behaviour and soft dysmorphic features. She was an only child and there was no relevant family history. There was no history of early hypotonia and she has normal intelligence. The karyotype was normal , fragile X syndrome was excluded and an MLPA for Smith-Magenis syndrome gave a normal result. Phelan-McDermid syndrome was also considered in the genetics clinic and the relevant FISH test was requested. Results showed 3% mosaicism for the 22q13.3 microdeletion in blood and 7% in a buccal smear. This is the lowest level of mosaicism for Phelan-McDermid syndrome reported in the literature to date, and the low level mosaicism probably explains the relatively mild clinical features. This diagnosis would probably have been missed with Array-CGH and NGS and shows the importance of clinical phenotyping and confirmatory FISH testing.

P-ClinG-050

Phenotype guided approach to finding the proper cause for sudden unexplained death in the young using a typical example of our clinic

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Background: In about 50 % of young patients suffering from sudden unexplained death (SUDS) an inherited arrhythmia syndrome or the coincidence of multiple arrhythmia syndromes can be found. Thorough clinical examination of surviving relatives and postmortem genetic testing in the deceased, if appropriate, can lead to the proper diagnosis.

Case: A young woman of 25 years suffering from syncope with seizure was found by her partner in the early morning in bed. The ECG performed by the emergency doctor showed ventricular fibrillation. After repeated external defibrillation a preliminary conversion in sinus rhythm occurred, but shortly after arriving at the intensive care unit of the next hospital she died. There was no known cardiac disease and autopsy did not reveal any specific findings. A thorough clinical examination of the first degree relatives was performed. Her mother showed a prolonged QTc-interval in the ECG and her father developed multiple premature ventricular contractions during exercise stress test without any obvious structural heart disease, raising the suspicion for long QT-syndrome (LQTS) in her mother and catecholaminergic polymorphic ventricular tachycardia (CPVT) in her father. Betablocker therapy was initiated in both.

Hence, phenotype guided genetic testing for a mutation in the 5 most frequently affected LQTS disease genes and for a mutation the RYR2-gene, leading to CPVT was performed in the deceased.

A heterozygous mutation in KCNH2, causing LQTS type 2 and a heterozygous mutation in RYR2, causing CPVT was found. Her mother was carrier of the KCNH2 mutation and her father suffered from the RYR2-mutation, confirming cosegregation of genotype and phenotype.

Conclusion: Genetic analysis can be useful to identify the molecular substrate leading to sudden cardiac death in the young. However, random genetic testing without adequate clinical evaluation and appropriate counselling of surviving relatives should be discouraged because of the high rate of false positive genetic test results due to variants of uncertain significance (1). Hence, genetic testing in this setting must be phenotype directed. Furthermore, the sensitivity of genetic testing in inherited arrhythmia syndromes is limited reaching from 70% in the LQTS to 25% in Brugada syndrome.

In our case genetic testing confirmed the two clinical diagnoses and facilitates targeted screening of relatives for those highly treatable diseases.

(1) Ackerman MJ, et al., HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies, Heart Rhythm. 2011 Aug;8(8):1308-39

P-ClinG-051

Novel autosomal-recessive syndrome with short stature, distinct facial appearance, myopia, retinitis pigmentosa, bilateral hearing loss, and mild intellectual disability

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Background:

Retinal anomalies in combination with the progressive hearing loss accompany a variety of genetic syndromes, e.g. Usher syndrome, Alstrom syndrome, Cockayne syndrome, Refsum syndrome etc. We report on three patients with retinitis pigmentosa, bilateral hearing loss, peculiar facial phenotype and an unusual combination of other clinical findings incompatible with any of the known genetic conditions.

Case reports:

Patient 1 (3 years) presents with short stature (-2, 5 SDS), distinct combination of minor facial anomalies (prominent forehead, short palpebral fissures, deep-set eyes, bulbous nasal tip with broad columella, thin upper lip with accentuated cupid's bow), brachydactyly, high myopia (-3dp) and progressive bilateral hearing loss. Her motor development is mildly delayed.

Patient 2 (40 years) is the paternal aunt of patient 1. Her brother, the father of patient 1, is unaffected. Like her niece patient 2 shows a short stature (-3, 5 SDS), brachydactyly and strikingly similar facial anomalies. She has progressive bilateral hearing loss with deafness at the age of 35 years. The complex eye involvement includes progressive myopia, retinitis pigmentosa (onset in the 2nd decade), glaucoma and corneal dystrophy (both started in the 4th decade). Patient 2 attended regular school but needs support in her daily life. Both patients 1 and 2 have normal findings in their brain MRI.

Patient 3 (28 years) is a male patient, not related to patients 1 and 2. His family history is unremarkable. He presented with short stature, brachydactyly, progressive bilateral hearing loss, high myopia and retinitis pigmentosa (onset in the 2nd decade). His facial minor anomalies are remarkably identical to patients 1 and 2. The only additional clinical finding not present in the first family is the alopecia areata of the scalp. Patient 3 shows mild intellectual disability.

Comprehensive genetic tests (conventional and molecular karyotyping in all patients; sequencing of LTBP2, ADAMSTS17 and ADAMTS10 in patient 2, and ERCC8 and ERCC6 in patient 3) were all normal.

Conclusion:

Taken together we report on an apparently new genetic syndrome associated with short stature, distinct facial anomalies, myopia, retinitis pigmentosa, progressive hearing loss and mild intellectual disability. Based on the pedigrees we suggest an autosomal recessive inheritance. This condition is distinctive from Usher syndrome by the presence of short stature, facial minor anomalies and a complex ophthalmological phenotype. Weill-Marchesani syndrome can also be excluded by the presence of the specific facial phenotype, progressive hearing loss and retinal abnormalities. Exome sequencing is ongoing to clarify the molecular cause of the disease.

P-ClinG-052

Rare Malformation Syndromes (Orphan Diseases) in Archived Fetal Specimens - Beginning of the Study

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Background

The first anatomical collection of human specimens in history was founded in 1756 at Viennese Medical Faculty, and in 1796 it became a Pathologic-anatomical Museum. Since then it has accumulated a remarkable variety of exhibits, including a rich collection of birth defects. While between the 18th-20th centuries the diagnoses of birth defects were only descriptive, nowadays, with progress of molecular methods, many of them are syndromologically classifiable. Thus it makes sense to undertake a syndrome-oriented reevaluation of the museum specimens, and if possible, a laboratory work-up.

Aim

Our aim is to reveal the nature of developmental anomalies in some specified series of the Viennese Pathologic-anatomic Collection. This diagnostic work-up could help providing extraordinary learn-samples for health professionals dealing with birth defects/syndromes and considering the rareness and age of this material, the work would be of a great international importance as well.

Material and Methods

Preliminary phase included an overall visual inspection of all fetal/pediatric exhibits of the museum (together circa 1000), in parallel with a key words-search in the museum´s electronic database, and selection of a few basic "working groups" - according to the leading type of external defect. Consequently, a few exceptional exhibits of each group underwent detailed objective evaluation and documentation.

Preliminary Results

The first overall inspection allowed us to select 5 main "working groups": 1. Orofacial clefts, non-midline, 2. Middle-line facial defects, 3. Disorders of extremities and skeletal dysplasias, 4. Congenital tumors, 5. Miscellaneous syndromes. The number of the selected exhibits counted circa 140. Already within this first phase we were able to clinically specify some interesting, more or less rare developmental pathologies that we present here as the initial findings, among them e.g. Rosenak-, Piepcorn-, Roberts-, OFD2-, Delleman-, Beckwith-Wiedemann-, Smith-Lemli-Opitz-, Meckel-syndromes and others.

Conclusion - Plans

In further investigations all selected exhibits will undergo a detailed objective reevaluation and documentation, including X-ray, autopsies, histological and DNA analysis to identify or confirm a suspected chromosomal, microdeletion or monogenic syndrome.

P-ClinG-053

1q44 microdeletion in a boy with intellectual disability but without agenesis/hypogenesis of corpus callosum

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Background: Microscopically visible 1q deletions go along with intellectual disability, growth restriction, microcephaly and distinct facial features (upslanting palpebral fissures, broad nasal bridge, high palate). 1q44 microdeletions exhibit similar but milder symptoms. A typical feature is the corpus callosum agenesis (ACC) or hypogenesis (HCC). Boland2 et al nominated the AKT3 gene on 1q44 as candidate for ACC/HCC. However, a further study by van Bon3 et al localized the critical region for ACC/HCC distal to AKT3 to a 0.36 Mb region involving 4 genes (chr1:242.582.560-242.938.957; NCBI36/hg18; genes: ADSS, C1orf100, C1orf101, C1orf121).

Case report and results We saw a 19 year old boy with moderate intellectual and motor disability, epilepsy and unilateral renal agenesis. Furthermore, he has an intestinal malrotation, club feet and autism. Clinical examination at age 19 years revealed normal body measurements and minor facial anomalies (broad, arched eyebrows, downslanting palpebral fissures, broad nasal bridge, high palate). Several brain magnetic resonance images from age 1 till 17 did not reveal any corpus callosum abnormalities but showed only discrete periventricular linear alterations.

Using Molecular Karyotyping we observed a 1.6 Mb interstitial deletion of chromosome band 1q44 (chr1:242.582.508-244.232.167; hg 18).

Conclusions The propositus shows typical clinical features of a microdeletion 1q44 including moderate intellectual disability, epilepsy and minor facial anomalies. The detected 1.6 Mb 1q44 microdeletion overlaps with the ACC/HCC critical region by van Bon but excludes AKT3. As the patient lacks corpus callosum abnormalities this observation supports AKT3 as possible candidate gene for ACC/HCC and requires the reevaluation of the 1q44 critical region published by van Bon.

2 Boland E et al: Mapping of deletion and translocation breakpoints in 1q44 implicates the serine/threonine kinase AKT3 in postnatal microcephaly and agenesis of the corpus callosum. Am J Hum Genet. 2007 Aug;81(2):292-303.

3 van Bon BW et al: Clinical and molecular characteristics of 1qter microdeletion syndrome: delineating a critical region for corpus callosum agenesis/hypogenesis. J Med Genet. 2008 Jun;45(6):346-54.

P-ClinG-054

Pontocerebellar hypoplasia type 2 caused by compound heterozygosity for two novel TSEN2 mutations

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Pontocerebellar hypoplasias (PCH) represent a heterogeneous group of autosomal recessive neurodegenerative disorders characterized by hypoplasia of the cerebellum and pons, variable cerebral involvement, microcephaly, severe delay in cognitive and motor development, and seizures. Seven different subtypes have been reported (PCH1-7) and mutations in three genes, TSEN2, TSEN34 and TSEN54 encoding three of four subunits of the tRNA splicing endonuclease complex have been found to underlie

PCH2, PCH4 and PCH5. PCH2 is characterized by cerebellar hypoplasia affecting the hemispheres more severely than the vermis, progressive cerebral atrophy and microcephaly, dyskinesia, seizures, and death in early childhood. We describe a male patient with progressive microcephaly, severe muscle hypotonia, and myoclonic-tonic seizures. Brain MRI confirmed microcephaly with simplified cortical gyration and revealed hypoplasia of the brainstem, cerebellum and cerebellar vermis. Sequencing of the TSEN2 gene detected the novel missense mutation c.934G>A (p.G312R) on one allele and the first nonsense mutation c.691C>T (p.Q231*) on the second allele. As five different transcript variants are expressed from the TSEN2 gene, the cytosine-to-thymine transition could represent a splice site mutation (c.517-3C>T) in variant 4. Although we did not identify mRNAs representing TSEN2 transcript variant 4 in leukocyte-derived RNA of the patient, the ratio of missense mutant to nonsense mutant transcripts was close to 1:1, suggesting that TSEN2 mRNAs with the premature stop codon are not efficiently degraded. Overall, the clinical phenotype of the patient was consistent with PCH2. However, we noticed decreased cerebral volume with increased extra-axial cerebrospinal fluid spaces and wide-open Sylvian fissures indicating cerebral immaturity that might be associated with the TSEN2 null allele. To establish a possible genotype-phenotype correlation, more individuals with biallelic TSEN2 mutations need to be investigated.

P-ClinG-055

Shprintzen syndrome-like phenotype in a boy with a microdeletion on chromosome 7p22.1

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We report on a 7 year old boy referred with suspected diagnosis of Shprintzen syndrome. He presented with developmental delay, short stature, mild hypotonia, submucous cleft palate, rhinolalia aperta and facial dysmorphisms.

Conventional chromosome analysis (G-banding) on peripheral blood cells and FISH analysis for microdeletion 22q11.2 and microdeletion 10p14 revealed inconspicuous results.

Array CGH using an Agilent 180k chip demonstrated a 1.8 Mb interstitial deletion on chromosome band 7p22.1. This deletion encompasses approximately 30 genes of which 19 have been annotated in the OMIM database.

Terminal and interstitial deletions of the short arm of chromosome 7 are associated with a wide range of clinical features, frequently including craniosynostosis, cardiac malformations, psychomotor deficits, and dysmorphic features.

The development of craniosynostosis has been attributed to mutations in the TWIST1 gene on 7p21 and the GLI3 gene on 7p13, individuals with deletions involving 7p22 commonly show cardiac malformations.

The clinical phenotype of our patient which does not include craniosynostosis or cardiac malformation can be explained by the location and extent of the deleted segment of 7p. Details of our cytogenetic and clinical findings are discussed in the context of the literature.

P-ClinG-056

PHACES syndrome – a case with Moya-Moya-like vasculopathy

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PHACE (Posterior fossa defects, Hemangiomas, Arterial anomalies, Cardiac defects/ Coarctation of the aorta, Eye anomalies) syndrome is a neurocutaneous syndrome with vascular and cardiac involvement of unknown origin. An S is added to the acronym when in addition, sternal clefting and/or supraumbilical raphe is present. More than 300 cases have been reported worldwide, all of them sporadic. Diagnostic criteria were defined by Metry et al 2009: In most patients, a diagnosis of PHACE syndrome includes a segmental hemangioma of >5cm in diameter of the head, neck or face, and one extracutaneous anomaly. In general, at least 20% of children with large segmental facial hemangiomas have extracutaneous anomalies associated with PHACE syndrome. Increased awareness and rigorous screening of at-risk children has broadened the clinical spectrum of PHACE syndrome. Cerebral vascular anomalies appear to be the most common extracutaneous manifestation of PHACE and include congenital non-progressive anomalies as well as progressive vascular dysplasia and occlusive arterial disease.

We report on a female patient who was born with a sternal cleft, pericardial defect and supraumbilical raphe and developed hemangiomas on the face and upper thorax within the first week of life. A diagnosis of PHACES syndrome was made. Cranial magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) at age 1,5 years showed dysplasia of the cerebellum and an occlusive Moya-Moya-like vasculopathy of the intracerebral arteries. Caliber irregularities of the large cerebral arteries affected predominantly the right internal carotid artery showing narrowing, aberrant course and aneurysmatic dilatation. Serial MRA of the asymptomatic patient revealed progression of vascular changes. Neurosurgical interventions were planned which are the treatment of choice. This case demonstrates the need for thorough neuroimaging in case of a possible and definite PHACE syndrome and for repeat MRA when cerebrovascular anomalies are present.

P-ClinG-057

A familiar duplication over three generations: Fine characterisation with self-designed two-colour FISH probes and array-CGH

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Duplications existing within the long arm of chromosome 10, more precisely in 10q22-q23 are a rarely seen genomic change. The architecture of the described region consists of a complex set of low-copy repeats (LCR`s 3 and 4), so it can give rise to various genomic modifications due to homologues recombination (Dasouki et al. 2011). Patients with a 10q22-q23 duplication described in the literature showed wide variety of features including developmental and speech delay, facial dysmorphisms, congenital heart defects, microcephaly, seizures, autism, hyperactivity, attention deficit (van Bon et al. 2011).

Here we report about a three generations pedigree. First, the reason for genetic counselling was a 10 years old girl with mental retardation, obesity and facial dysmorphisms. Cytogenetic analysis and array-CGH revealed a 7,17Mb large duplication in 10q22.3-q23.2. Further investigation showed the same duplication in her mother, their 32 years old half-sister and their son. All had mild to moderate mental disability, facial dysmorphisms and behavioural problems. Duplications of the LCR3-LCR4 region are associated with variable phenotypic penetrance, but do not always lead to an abnormal phenotype, because a sibpair had a 7Mb duplication inherited of a healthy mother (van Bon et al. 2011).

For exact characterisation of the duplicated segment we established new self-designed two-colour FISH probes. Therefore, we amplified two genes from proximal and distal part of the duplication by Long Range PCR. We produced a 9,7kb fragment of the gene ANXA11 localised in 10q22.3 (MIM 602572) and a 15,2kb fragment of the gene WAPAL in 10q23.2 (MIM 610754). After fluorescent labelling with different colours we hybridised the FISH probes on patients metaphase spreads. FISH analysis revealed a tandem duplication due to specific signal pattern.

In conclusion, to our knowledge, we are first to describe the exact genomic orientation of the duplicated 10q segment with specific two-colour FISH probes.

P-ClinG-058

Duplication 5q35.2-q35.3 Encompassing NSD1: Is It Really A Reversed Sotos Syndrome?

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Background: Sotos syndrome (Sos), which is characterized by overgrowth, macrocephaly, characteristic facies and variable intellectual disability (ID) is caused by loss-of-function mutations in NSD1 and 5q35 microdeletions encompassing NSD1. A microduplication of 5q35.2-q35.3 including NSD1 has been reported in only five patients so far and described as a reversed Sos resulting from a hypothetical gene dosage effect of NSD1. Clinical report: We report on 9 patients from 5 families with interstitial duplication 5q35 including NSD1 detected by molecular karyotyping. The clinical features of all 14 individuals are reviewed. Discussion: In Patients with microduplications including NSD1, in addition to short stature, microcephaly and ID, a consistent phenotype with recognizable facial features emerges. It consists of periorbital fullness, short

palpebral fissures, and a long nose with broad or long nasal tip, a smooth philtrum and a thin upper lip. Behavioural problems, ocular and minor hand anomalies may be associated. As presented in two familial cases, the phenotype is variable and facial features may be mild, the condition may therefore be underdiagnosed. Patients with fetal alcohol spectrum disorders (FASD) share with patients with microduplication of 5q35.2-q35.3 some facial features, variable microcephaly and short stature and behavioural problems. Microarray analysis should be considered in patients with facial features suggestive for FASD, even if the body measurements and the intelligence are within a low normal range. Based on data of a patient with a partial duplication of NSD1, we discuss that a gene dosage effect of NSD1 as cause of the duplication phenotype is possible but so far unproven. The term “reversed Sos”, however, appears not to be appropriate because of the absence of “reversed” facial features and the presence of unspecific or common features in both groups.

P-ClinG-059

An incompletely penetrant novel MAFB (p.Ser56Phe) variant in autosomal dominant multicentric carpotarsal osteolysis syndrome

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Background: Multicentric carpotarsal osteolysis syndrome (MCTO) is a rare autosomal dominant skeletal dysplasia usually presenting in early childhood with variable phenotypic features and course. Clinical manifestations comprise aggressive osteolysis of especially the carpal and tarsal bones, an often progressive nephropathy leading to end-stage renal disease, craniofacial anomalies, and mental impairment. Recently, heterozygous missense mutations in the MAFB (v-maf musculoaponeurotic fibrosarcoma oncogene homolog B [avian]) gene have been causally related to MCTO in 11 simplex cases (de novo events) and two unrelated multiplex families.

Material and Methods: The German index patient was referred to the institute at the age of 19. He presented with irregular and dysplastic, partially missing carpalia, and dysplastic changes of the tarsalia but with normal renal function. No other family member showed signs of MCTO. Sequence analysis of the MAFB gene was performed by standard procedures.

Results: MAFB analysis revealed a single heterozygous c.167C>T nucleotide transition (p.Ser56Phe) in the patient. Contrary to the recent findings suggesting complete penetrance, we identified the novel MAFB variant also in the unaffected mother, sister and maternal grandmother. However, several clues point to a critical role for serine 56 located in the amino-terminal transcriptional activation domain of MAFB: First, this transition has not been deposited in the databases which argues against the possibility that this substitution is just a rare benign variant. In addition, the amino acid exchange substitutes a polar residue for a nonpolar one and pathogenicity prediction with several algorithms predicted it to be disease-causing or at least possibly damaging. Finally, Ser56 is absolutely conserved at its corresponding position in species orthologues as part of the transactivation motif at least as far down as X. laevis.

Conclusions: Our observation implies an incomplete penetrance for some MAFB mutations and suggests that modifier genes, epigenetic mechanisms or environmental factors could modulate the MCTO phenotype. This should be considered in diagnosis and genetic counseling.

P-ClinG-060

Case Report: Anosmia and hypogenitalism due to a novel duplication within the FGFR1 gene

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Introduction:

Kallmann syndrome (KS) is a clinically and genetically heterozygous disorder characterized by hypogonadotropic hypogonadism with anosmia/hyposmia.

One of the causative genes for KS is the fibroblast growth factor receptor 1 (FGFR1). Mutations in FGFR1 underlie an autosomal dominant form with incomplete penetrance, although some of the mutations found in FGFR1 were de novo mutations. Pathogenic changes in FGFR1 have been detected in approximately 10% of the KS patients.

Patient:

We present a 11-year-old male patient from Germany with micropenis and anosmia. His family history showed no other case of hypogonadism or anosmia.

The physical examination revealed: height: 142 cm, weight: 37 kg, micropenis, and no sense of smell. Laboratory testing revealed low stimulation of LH/FSH by GnRH, evaluated as prepubertal or as first hint of the diagnosis of hypogonadotropic hypogonadism.

Methods:

Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding regions of the FGFR1 gene including corresponding exon-intron boundaries. PCR products were sequenced directly. Multiplex Ligation-Dependent Probe Amplification (MLPA) was performed using the SALSA P133-C1 probe mix (MRC-Holland) for detection of large deletions or duplications.

Results:

Sequence analysis of the FGFR1 gene revealed a heterozygous duplication of two nucleotides (GG) in exon 8 (c.1011_1012dupGG) in the patient, which is also called p.Glu338fs. The duplication leads to a premature termination codon at position 357 and therefore to a highly truncated and misfunctional protein. Deletions of the FGFR1 gene were excluded by MLPA.

Conclusion:

Our results strongly indicate that the novel duplication of two base pairs in exon 8 of the FGFR1 gene is the genetic cause of the Kallmann syndrome in our patient.

Genetic counselling and genetic testing of the gene should be considered in young patients to confirm the diagnosis and to enable in time therapeutic decisions.

P-ClinG-061

Frequency of CIZ1 mutations in a cohort of sporadic dystonia patients

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Primary torsion dystonia (PTD) is a disabling movement disorder disorder characterized by involuntary muscle contractions, causing twisting and repetitive movements or abnormal postures. PTD has a wide clinical spectrum and its severity is largely determined by the age of onset. Whereas most cases of early-onset generalized dystonia are caused by a 3 bp-deletion in the DYT1 gene (Torsin1A), the underlying gene defect for the almost 10fold more prevalent adult-onset and localized subtypes of dystonia (focal, segmental) is still elusive.

Recently, mutations in CIZ1 have been reported to cause primary cervical dystonia in a large Caucasian pedigree. CIZ1 codes for Cip1-interacting zinc finger protein 1 which was shown to cooperate with cyclin-A-CDK2 to activate DNA replication in late G1-phase nuclei.

To investigate the role of CIZ1 mutation as underlying genetic basis of adult onset primary torsion dystonia, we evaluated the contribution of mutations in the CIZ1 gene for its pathogenic relevance in a series of 93 Patients with sporadic and familiar forms of predominantly cervical dystonia of different movement disorder outpatient clinics in Germany. Mutational analysis of the coding region of the DYT1 gene and in the DYT6 gene has been performed previously and mutation carriers have been excluded. Detection of mutations in the CIZ1 gene was performed by means of next generation sequencing on an Illumina MiSeq Sequencer. Sequencing was carried out after massive parallel amplification of the coding exons (n=17) and adjacent intronic bases and simultaneous barcoding of each sample. For this purpose the Fluidigm Access Array System, following the 4-Primer Amplicon tagging protocol, was used.

Apart from 7 annotated SNPs (dBSNP 135) we found 2 two rare polymorphisms with unknown significance in exon 8 (c.G1250T, p.R417M and c.T1199C, p.L400P) in our cohort of 93 patients suffering from predominatly cervical dystonia thus indicating that mutations in CIZ1 are most likely a rare cause of adult onset cervical dystonia.

P-ClinG-062

The European Network for Human Congenital Imprinting Disorders

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Imprinting disorders (IDs) are a group of rare congenital diseases affecting growth, development and metabolism with a lifelong impact on patients´ quality of life. Despite their common underlying (epi)genetic aetiologies, IDs are usually studied separately by small research groups working in isolation, and the basic

pathogenesis and long term clinical consequences of IDs remain largely unknown. Efforts to elucidate the aetiology of IDs are currently fragmented across Europe and standardisation of diagnostic and clinical management is lacking. This COST Action will, for the first time, draw together researchers of all eight known human IDs in an interdisciplinary pan-European Network for Human Congenital IDs, working to advance understanding of the pathophysiology with the major aim of translating this knowledge to improvement of diagnostic and clinical management for the benefit of the patients and their families. The Action will harmonise a common ID classification system, develop guidelines for treatment through consensus, create standard operation procedures (SOPs) for diagnosis based on best current practice, coordinate databases held in different countries to make them compatible and useful as a springboard for collective research initiatives, identify new imprinting disorders and causative mechanisms through collaborative effort, educate researchers and stimulate translational exchange. The ID network currently consists of 25 groups from 11 countries. It will join forces and complement studies to reduce health care costs and increase the life quality of patients. The Action will start in spring 2013.

P-ClinG-063

The 17q21.31 microdeletion syndrome and the reciprocal microduplication partially encompassing KANSL1 - molecular characterization and phenotypic comparison in 7 unrelated patients

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The 17q21.31 microdeletion syndrome phenotype consisting of facial dysmorphism, moderate-to-severe intellectual disability, hypotonia and friendly behavior has been shown to be caused by haploinsufficiency due to deletion or loss-of-function mutations in the KANSL1 (KIAA1267) gene. The reciprocal 17q21.31 duplication has been described in association with mental retardation and behavior problems in six patients in the literature so far.

We present four patients with overlapping microdeletions in 17q21.31 and three patients with the reciprocal microduplication partially encompassing KANSL1. All seven patients presented with developmental delay, usually with a more severe speech delay, mild to moderate abnormalities of the fingers and/or toes and some degree of facial dysmorphism which was rather unspecific, variable and mild. One patient with a microdeletion presented with macrocephaly, tall stature and adipositas, while one of the patients with a microduplication was microcephalic. Neurodevelopmental and growth abnormalities were present in some patients. All deletions and duplications at least partially encompass the KANSL1 gene.

Referring to our patients we report in detail clinical and molecular genetic aspects of the 17q21.31 microdeletion syndrome and the reciprocal microduplication syndrome.

P-ClinG-064

Results from massively parallel next-generation sequencing of known deafness genes challenge MYO1A haploinsufficiency as pathomechanism for autosomal dominant deafness type DFNA48

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Hearing loss is the most common sensory deficit and genetically extremely heterogeneous, with more than 60 genes known to be involved. We have conducted massively parallel sequencing of 67 deafness genes in a 4-year-old girl with profound congenital hearing impairment and detected a heterozygous nonsense mutation, c.2220T>G (p.Y740X), in myosin-1A (MYO1A), the gene known to be mutated in autosomal dominant hearing loss, type DFNA48. However, neither the severity of the patient's hearing loss nor the segregation analysis with several healthy individuals carrying p.Y740XMYO1A was compatible with a

causative role of this mutation. Of note, the patient also carries a homozygous missense mutation, c.3719G>A (p.R1240Q), in the myosin-7A gene (MYO7A). Homozygosity for p.R1240QMYO7A is a known cause of Usher syndrome type 1, an autosomal recessive condition with congenital deafness and retinitis pigmentosa with onset in the first decade of life. While the MYO7A mutation can be regarded causative and predicts visual impairment in the patient in the near future, the MYO1A nonsense mutation does not seem to interfere with normal hearing. All DFNA48 mutations reported to date are in-frame MYO1A mutations, except a heterozygous nonsense mutation in one patient whose mother carried the nonsense mutation, too, but was said to have normal hearing. We propose that dominant deafness of the DFNA48 type rather results from dominant-negative in-frame alterations than from haploinsufficieny, and that truncating MYO1A mutations may either act recessively or may not be pathogenic at all. Our study demonstrates that accidental and potentially misleading findings are not necessarily confined to large-scale approaches such as exome or genome sequencing, but also represent a challenge in the analysis of (large) gene panels for genetically heterogeneous phenotypes. The pathogenicity of apparently deleterious mutations has to be assessed carefully in the context of the overall variant load in all analyzed genes, the patient's phenotype and the segregation pattern. Moreover, the study illustrates the potential of NGS for the early unmasking of deafness syndromes before the onset of second-system manifestations, thereby allowing for early and targeted supportive management.

P-ClinG-065

HOXA10 and HOXA13 sequence variations in human female genital malformations including congenital absence of the Uterus and Vagina

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Background: Congenital genital malformations occurring in the female population are estimated to be 5 per 1,000 and associate with infertility, abortion, stillbirth, preterm delivery and other organ abnormalities. Complete aplasia of the uterus, cervix and upper vagina [Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome] has an incidence of 1 per 4,000 female live births. The genes responsible for the etiology of congenital genital malformations including MRKH are unknown. The homeobox (HOX) genes HOXA10 and HOXA13 located on chromosome 7 are involved in the development of human genitalia.

Methods: HOXA10 and HOXA13 genes of 27 patients with the MRKH syndrome, 10 non-MRKH patients with genital malformations and 53 control women were sequenced. A novel exonic nucleotide deletion discovered in HOXA10 was analysed by real time PCR in a total of 103 patients with MRKH and 109 non-MRKH patients with genital malformations.

Results: A total of 15 DNA sequence variations (11 novel and 4 known) within exonic and untranslated regions (UTRs) were detected in HOXA10 and HOXA13 among our cohorts. Five HOXA10 and two HOXA13 DNA sequence variations were found solely in patients with genital malformations. In addition to mutations resulting in synonymous amino acid substitutions, missense mutations in the HOXA10 gene were found in one MRKH patient and one non-MRKH patient with a bicornate uterus, with the latter predicted by computer analysis as probably damaging to protein function. An exonic HOXA10 cytosine deletion was identified in a non-MRKH patient with a septate uterus and renal malformations resulting in a premature stop codon and loss of the homeodomain helix 3/4. This cytosine deletion in HOXA10 demonstrated an incidence of one in a total of 212 MRKH and non-MRKH patients with genital malformations. Lastly, in the 5'UTR of HOXA10 in a non-MRKH patient with genital malformations, a cytosine to an adenosine base change was found.

Conclusions: Rare DNA sequence variations in the HOXA10 gene were detected in MRKH patients but especially in non-MRKH patients with genital malformations. Consequences of non-synonymous mutations, a cytosine deletion or a 5'UTR mutation, could result in altered protein function or abnormal gene expression of HOXA10.

P-ClinG-066

Phenotypic variability of X-linked megalocornea in a large pedigree with an inherited CHRDL1 frameshift mutation.

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X-linked megalocornea is an inborn error of the anterior eye segment with a corneal diameter greater than 13 mm. In combination with an enlarged ciliary ring and a deep anterior eye chamber it is termed anterior megalophthalmos. Usually patients have mild to moderate myopia and astigmatism and some complain of iridodonesis. Additional ocular findings can be congenital cataract and a pigment dispersion along the inner corneal surface (Krukenberg-Spindel). Although the intraocular pressure is primarily normal, megalocornea predisposes to secondary glaucoma due to a widened ciliary body and a potential pigment deposition in the trabecular meshwork. There is a higher susceptibility for corneal dystrophy, vitreous degeneration and retinal detachment. As megalocornea is an important differential diagnosis for congenital glaucoma (buphthalmos) which needs a prompt treatment a fast diagnostic approach for megalocornea is necessary. Furthermore, megalocornea can be observed in a syndromic context e.g. in patients with Marfan syndrome or with Megalocornea-Mental-retardation syndrome. Recently, different mutations in CHRDL1 have been identified as the disease causing genetic alterations for X-linked megalocornea. CHRDL1 encodes for the protein ventroptin, which is a bone morphogenetic protein 4 antagonist and is involved in cell fate determination as well as cell differentiation. The now identified etiology of X-linked megalocornea provides the opportunity to explore the underlying pathogenesis and to refine the management of megalocornea patient care by analyzing further genotype-phenotype correlations. We report here on a four generation family with inheritance of a CHRDL1 frameshift mutation most probably causing a loss of function of the ventroptin in affected males with X-linked megalocornea and discuss the intrafamilial phenotypic variability.

P-ClinG-067

Moyamoya disease and limb malformation in a girl with RASopathy: new insights into the origin of vasculopathies?

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Introduction:

The term “RASopathies” defines a group of developmental syndromes caused by dysregulation of the Ras/mitogen activated protein kinase (MAPK) signaling pathway. Activating mutations in genes encoding components of this pathway are the cause of Noonan-, CFC-, Costello- and LEOPARD-syndrome. Somatic mutations of the same genes are found in different types of leukemia and other neoplasias. About 15% of patients with juvenile myelomonocytic leukemia (JMML) carry somatic mutations in the CBL gene which is a negative regulator of the RAS-MAPK-pathway. Recently, germline mutations of this gene have been described in patients with a Noonan-like phenotype.

Clinical Report and Genetic Findings:

We describe a girl with developmental delay, short stature, pulmonary stenosis (PS), facial dysmorphism including downslanting palpebral fissures, deep set, posteriorly rotated and dysplastic ears, hyperpigmented skin patches, transverse reduction defect of the left upper extremity, moyamoya disease and JMML. Molecular genetic analysis of bone marrow cells showed the splicing mutation c.1096-85_1096-8delinsT in the CBL gene. This mutation was also present in DNA derived from cultured fibroblasts of the patient but not in DNA of both parents, indicating that it is a de novo germline mutation.

Discussion:

The girl has phenotypic features in common with other patients carrying a CBL germline mutation, e.g. facial dysmorphism, PS and pigmentary skin anomalies. Moyamoya disease and dysmelia, however, have not been described as a feature of RASopathies so far. Interestingly, a few previously reported patients with

CBL germline mutations show signs of vasculopathy. We suggest that the moyamoya disease and dysmelia in our patient may be due to a defect in the embryonic vasculogenesis caused by the CBL mutation.

P-ClinG-068

Is a microdeletion in KANSL1 causing the 17q21.31 microdeletion syndrome phenotype?

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The chromosome 17q21.31 microdeletion syndrome was one of the first previously unidentified genomic disorders discovered by microarrays in the year 2006. A 424-kb critical region encompasses at least six genes, C17orf69, CRHR1, IMP5, MAPT, STH and KANSL1 (or KIAA1267). Haploinsufficiency of one or more of these genes may underlie the phenotype seen in individuals with the 17q21.31 deletion syndrome. MAPT was of particular interest, as the gene is highly expressed in brain and is involved in several neurodegenerative diseases. Recently, several atypical microdeletions were identified encompassing only parts of the MAPT and /or KANSL1 genes as well as heterozygous point mutations just only in the KANSL1 gene causing intellectual disability and typical features of the 17q21.31 microdeletion syndrome.

We report a case of a several months old baby girl with upward slanting palpebral fissures, neonatal hypotonia and poor sucking which required naso-gastric feeding for a short period of time. Blood was sent to our lab with the suspected diagnosis of a trisomy 21. Fluorescence in situ hybdridization (FISH) with locus-specific probes detected no trisomic pattern in 40 metaphases and 500 interphases. As cytogenetic karyotyping revealed a marker chromosome of unknown origin in 20% of the metaphases, we performed arrayCGH analysis.

ArrayCGH analysis did not detect any additional chromosomal material but revealed a new atypical microdeletion in 17q21.31 which spans over 478 kb of size, disrupts the KANSL1 gene (breakpoint: intron 2-3) and encompasses the genes LRRC37A, ARL17B, LRRC37A2, ARL17A, C17orf88, NSF. Until now, the parents refused any further analysis to determine if the detected microdeletion results from a familial or a “de novo” structural aberration and especially, if they harbor the common 900-kb inversion polymorphism for this region.

Our data show that a heterozygous microdeletion of this region, disrupting the KANSL1 gene, might cause the classical 17q21.31 microdeletion syndrome phenotype.

P-ClinG-069

Exome sequencing reveals a diagnosis dismissed on clinical grounds: SLC2A10 mutations in individuals with cutis laxa, but without arterial tortuosity.

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Differential diagnosis of autosomal recessive cutis laxa (ARCL) syndromes relies on clinical hallmarks that allow sorting of the cases into increasingly narrow categories. We investigated an extended consanguineous family from Oman with a form of ARCL most closely overlapping with gerodermia osteodysplastica (GO; OMIM 231070). Affected individuals displayed a typical long face with sagging cheeks, periorbital fullness and mild ptosis. They were relatively tall with a slender built, had joint hypermobility and thin, translucent skin with reduced elasticity. In cardiological investigations two siblings showed signs of hypertension and mild non-progressive aortic root dilatation. One individual had urinary bladder diverticula. After excluding relevant candidate genes (GORAB, PYCR1, ATP60A2) we performed whole exome sequencing in three affected individuals. After filtering for homozygosity runs using the tool Gene-Talk, we found a novel 1bp deletion in exon 2 of the SLC2A10 gene. Such loss-of-function mutations in this transporter usually cause arterial tortuosity syndrome (OMIM 208050), which can entail life-threatening vascular complications. This shows the relevance of next-generation sequencing in differential diagnosis in rare hereditary disorders.

P-ClinG-070

Multiple methylation errors at imprinting control regions in patients with S-adenosylhomocysteine hydrolase (AHCY) deficiency

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S-adenosylhomocysteine hydrolase (AHCY) deficiency is a novel human disease, which was first discovered in Croatia in 2004. Main characteristics are psychomotor delay and severe myopathy (hypotonia, absent tendon reflexes and delayed myelination) from birth, associated with hypermethioninaemia, elevated serum creatine kinase levels and increased genome-wide DNA methylation. The prime function of AHCY is the efficient removal of S-adenosylhomocysteine (SAH), the by-product of transmethylation reactions. As SAH is one of the most potent methyltransferase inhibitors, its rapid removal is crucial to avoid product inhibition of methyltransferases. Thus, AHCY plays a critical role in regulation of biological methylation processes. We set out to more specifically characterize DNA methylation changes in blood DNA samples of five AHCY-deficient patients as well as HepG2 and HEK293 cell lines after shRNA-mediated knockdown of the AHCY gene by determining the quantitative DNA methylation patterns of different imprinting control regions (ICRs). Two of the analyzed patients showed aberrant hypermethylation at all up to now analyzed ICRs (MEST, NESPAS, SNRPN and PEG3) whereas the remaining three patients displayed normal differential methylation patterns. The knockdown cell lines also exhibited methylation changes to different degrees at the ICRs. Microarray-based experiments to analyze the complete DNA methylome of AHCY-deficient patients in comparison to normal individuals are planned. Our preliminary data indicate that AHCY deficiency may represent a good model disease for studying the biological consequences of multiple methylation errors in epigenetic research. Thus, findings from this study may make an important contribution to develop standard and high-throughput tools for the diagnosis of AHCY deficiency and other diseases associated with aberrant epigenetic modifications.

P-ClinG-071

Experiences on next-generation sequencing (NGS) in more than 300 patients with different (poly)cystic kidney diseases and other ciliopathies

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Cilia-related disorders (ciliopathies) are characterized by extensive clinical and genetic heterogeneity. Literally all organs can be affected, frequent cilia-related manifestations are cystic and polycystic kidney disease, retinal degeneration, cardiac defects, situs inversus, polydactyly, other skeletal features, and defects of the central and peripheral nervous system. These can occur isolated or as part of syndromes, such as Bardet-Biedl, Joubert, Meckel, Jeune, and Ellis-van-Crefeld syndrome. Variable expressivity and overlaps between different entities often make it difficult to give a clear clinical diagnosis. Genotype-phenotype correlations are usually not convincing and mutations in the same gene can cause very different phenotypes. Overall, it is more the rule than the exception that multiple, often dozens of genes are to be considered to be disease-relevant in a patient with suspected ciliopathy. Second-site modifiers are expected to exert an aggravating effect in an epistatic way. In this scenario, altered dosage of disease proteins may disturb cell homeostasis and network integrity contributing to early and more severe disease expression. We designed an NGS (next-generation sequencing) based panel for all ciliopathies. All exons and adjacent intronic boundaries of currently 258 genes known or hypothesized to cause ciliopathies were targeted by a custom SeqCap EZ choice sequence capture library and subsequently sequenced on a Roche 454 GS FLX or an Illumina MiSeq platform. Patients were analyzed with an average coverage of currently more than 100x. We present our experiences from the analysis of more than 300 unrelated families from a broad spectrum of cilia-related disease phenotypes in which we have used our NGS-panel. In the great majority of cases, underlying mutations could be clearly identified. Notably, we were also able to detect mutations in novel genes for cystic kidney diseases and other ciliopathies as well as disease-causing CNVs (copy number variations). Overall, we demonstrate that NGS-based testing approaches can considerably improve genetic diagnostics from a quantitative and qualitative point of view.

P-ClinG-072

Disruption of EXOC6B in a Patient with Developmental Delay, Epilepsy, and a de novo Balanced t(2;8) Translocation

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Most balanced chromosomal aberrations are not associated with a clinical phenotype, however in some patients they may disrupt gene structure. With the development of various next-generation sequencing techniques fast analyses of the breakpoint regions of chromosomal rearrangements are possible.

Here, we report on a 19-year-old woman with a de novo balanced translocation t(2;8)(p13.2;q22.1) and a severe clinical phenotype. The patient is the first child of a 27-year-old mother and her unrelated 37-year-old husband. Three younger siblings are healthy. Family history and pregnancy were unremarkable. Spontaneous birth was at 38 weeks of gestation. Length (51 cm), weight (3590 g), and occipitofrontal head circumference (OFC) (36 cm) were above the 90th centile. Parents described developmental delay starting at the age of 8 months, severe behavioural disorder with autistic and aggressive features, epilepsy, and stereotypical movement disorder. Walking was possible at the age of 22 months. Menarche was at the age of 16 years. At the age of 19 years height was 162 cm (25th centile), weight was 58 kg (50th-75th centile), and OFC was 54 cm (25th–50th centile). Speech was reduced to a few words and features resembling autism were observed. Dysmorphic features included strabismus, a thin upper lip, and slightly posteriorly rotated ears.

By next-generation sequencing we defined the breakpoints within two Alu elements with a homology of 81 % leading to the disruption of the EXOC6B gene in intron 1 on chromosome 2p13.2. No gene was found at the breakpoint on chromosome 8. Expression analysis of the EXOC6B in blood lymphocytes and buccal smear revealed reduced expression in the patient in comparison to the control. Our findings in combination with one recently published case (Borsani et al., 2008) and one patient listed in DECIPHER v5.1 (#249203) indicate that EXOC6B may be relevant for intellectual development and electrophysiological stability.

P-ClinG-073

Widening the phenotypic spectrum of 15q25.2 microdeletion: description of another patient with developmental delay, stenosis of the aortic isthmus, and facial dysmorphism

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Background: To date, nine patients with confirmed interstitial microdeletion of chromosome 15q25.2 have been described in the literature. The smallest common region overlaps 1.67 Mb. The clinical data show that individuals with interstitial microdeletion 15q25.2 have an increased risk for cognitive deficits, behavioral problems, congenital diaphragmatic hernia, and Diamond-Blackfan anaemia.

We here report on a boy with interstitial deletion 15q25.1-q25.2, widening the phenotypic spectrum.

Patient: The 16 months old boy presented with psychomotor developmental delay. His body weight and body length was between the 3rd and 10th centile. During pregnancy, a stenosis of the aortic isthmus was diagnosed; therefore surgery at the second day of life was performed. During the first months of life an iron supplementation was necessary. The boy showed facial dysmorphism including rotated ears, flat midface and prominent lips.

Methods: We performed chromosomal analysis (with a resolution of about 500 bands), array CGH analysis (Agilent® Sure Print G3 Human CGH 180K Microarray) and FISH (using a specific probe for 15q25.2).

Results: Classical chromosomal analysis was normal. Molecular karyotyping revealed a 3.7 Mb interstitial deletion of 15q25.1-q25.2 containing 55 genes including CPEB1, HOMER2 and RPS17. Since the deletion included the RPS17 gene, hematologic investigations were initiated due to the known risk of Diamond-Blackfan anaemia.

P-ClinG-074

Li-Fraumeni Syndrome and Hereditary Diffuse Gastric Cancer in one patient identified by next generation sequencing

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Li-Fraumeni syndrome (LFS) caused by germline mutations of TP53, is a cancer predisposition syndrome associated with sarcomas, breast cancer, brain tumors, adrenocortical carcinoma (ACC), and a variety of other neoplasms. The lifetime risk of cancer is estimated at 90%, individuals with LFS are at increased risk of developing multiple primary cancers.

Hereditary diffuse gastric cancer (HDGC) is a different cancer syndrome caused by germline mutations of the gene CDH1 coding for E-cadherin. The syndrome is dominated by highly penetrant diffuse-type gastric cancer and an elevated risk of lobular breast cancer. Both syndromes are inherited in an autosomal-dominant manner and require intensive clinical management. In particular, prophylactic operations are recommended in HDGC.

Here, we present a 46-year old female patient who developed bilateral breast cancer at the age of 38 and 43 years, respectively. Recently, she was diagnosed with an adenocarcinoma of the uterus. Her son died of a brain tumor when he was 17 years of age, the sister of our index patient developed breast cancer and died at the age of 28. Family history revealed further tumor affected relatives in the paternal line, mainly presenting with gastric and breast cancer.

As a consequence, the patient was referred to our genetic service and molecular genetic diagnosis was initiated. Sanger sequencing and MLPA analysis of the genes BRCA1 and BRCA2 did not reveal a disease associated mutation. Due to the high familial tumor burden, we extended our diagnostic work-up and enriched 150 genes (890kb) highly associated with breast cancer according to the COSMIC database. Sequencing was performed on the Roche 454FLX® platform. The analysis identified a heterozygous germline mutation in two high-penetrance genes, TP53 (c.673-1 G>A) and CDH1 (c.892 G>A), respectively. Both mutations were confirmed by Sanger sequencing, the damaging effect in mRNA splicing (TP53) was proven on the basis of cDNA analysis.

New sequencing techniques now allow improved strategies in the work-up of hereditary cancer, especially the identification of causative germline variants. This significantly extends our genetic counseling options and may improve the treatment and prevention of manifestations and secondary complications in familial cancer.

P-ClinG-075

Congential LMNA-related muscular dystrophy due to a homozygous mutation in the LMNA gene (c.1444c>T, p.Arg482Trp) that is recurrently associated with partial lipodystrophy

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We report on a 15 year old girl with symptoms of a congenital muscular dystrophy who was born to consanguineous Turkish parents. She shows a generalized proximal muscle weakness of all limbs suggesting a limb girdle myopathy. Further symptoms are a rigid spine, thorax rigidity, severe scoliosis and contractures of the lower extremities. Sleep associated hypoventilation requires assisted ventilation. Supraventricular extrasystoles are treated with a betablocker. A main feature in the girl is a severe dystrophy/kachexia. Her weight is 21,9 kg (20 kg < P3), head circumference 51,5 cm (P 3-10). Height is not measureable because of scoliosis and contractures. Her intellectual ability is normal and she shows no dysmorphic features.

Rigid spine, heart conduction defects and contractures are landmarks of a laminopathy. Mutations in the LMNA gene have been linked to several disorders called laminopathies, which display heterogeneous phenotypes including diseases affecting muscles and axonal neurons, progeroid syndromes and lipodystrophies. In more detail the muscular phenotype splits into: 1) Emery-Dreifuss muscular dystrophy (EDMD2), 2) autosomal dominant limb-girdle muscular dystrophy (LGMD1B) and 3) congenital muscular dystrophy (MDCL), characterized by progressive generalized weakness, a dropped head and early contractures. Furthermore LMNA mutations were identified in subtypes of lipodystrophy, being mainly a

metabolic syndrome. Many forms of laminopathies are of de novo occurrence and dominant inheritance, but autosomal recessive inheritance is possible.

The MDCL phenotype covers most of our patient's symptoms. Analysis of the LMNA gene revealed a homozygous mutation in exon 8 (c.1444c>T, p.Arg482Trp). Both parents and the brother of the index patient are mutation carriers. The above mutation is causal for partial lipodystrophy type Dunnnigan. The father and brother of our patient were not aware of any clinical symptoms till now. The mother showed an unusual muscular build, characteristic for a partial lipodystrophy. Metabolic examinations are on the way.

This family broadens our knowledge on laminopathies: the first reported case carrying the "lipodystrophy mutation pArg482Trp" in a homozygous state shows symptoms of a MDCL.

P-ClinG-076

Clinical outcome in an IL-10 receptor–deficient patient with haematopoietic stem cell transplantation

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IL-10 receptor (IL-10R) deficiencies are rare primary immunodeficiencies and present with severe life-threatening early-onset inflammatory bowel disease (IBD) and various inconsistent immunological abnormalities.

Here we present a 3 month old boy with severe IBD and perianal disease that was refractory to any immunosuppressive therapy, including steroids and anti–TNF-α antibodies. Genetic analysis showed that the patient is compound heterozygous for a novel mutation in exon 2 of IL10RA (c.170A>G) and a 5.5-kb deletion including exons 2- 4 of IL10RA, the gene encoding the alpha-unit of the IL-10R. In contrast to healthy controls, peripheral blood mononuclear cells of the patient did not show any IL-10–mediated inhibition of LPS-mediated TNF-α release, giving evidence that the observed mutation resulted in a loss-of-function.

identification of a matched donor made a haematopoietic stem cell transplantation (HSCT) possible which resulted in significant clinical improvement.

Since the defect in patients with IL-10R deficiency resides in hematopoietic lineage cells and their colitis is resistant to standard immunosuppressive therapy, HSCT should be considered early as a potentially curative therapeutic option.

P-ClinG-077

WRD72 deletion causes Amelogenesis imperfecta in a consanguineous family

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Introduction: Amelogenesis imperfecta (AI) is a rare genetically inherited teeth development disorder. Do date, mutations in nine genes (AMELX, ENAM, C4orf26, DLX3, FAM20A, FAM83H KLK4, MMP20, SCLA4, and WDR72) are known to cause AI resulting in clinical heterogeneous forms of AI and several others are discussed as candidate genes. They are classified in the major phenotypes "hypoplastic", "hypomineralized", and "hypomaturated" AI. Autosomal recessive and dominant as well as X-chromosomal traits are known. Until now, a clear genotype-phenotype correlation is missing.

Methods: We analyzed genomic DNA of three affected siblings of healthy consanguineous parents. The siblings suffer from hypomineralized AI with rough, soft and discoloured enamel. First, mutational analysis of Enamelin was done using Sanger Sequencing. Microsatellite (MS) analysis of chromosome 11 (MMP20), 15 (WDR72) and 19 (KLK4) was done to identify recessive candidate genes for further investigation due to regions of homozygosity. Subsequently, the DNA of four subjects was subjected to Exome Sequencing (Illumina HiSeq2000 platform (2x100-base pair paired end reads) after undergoing exome enrichment (Illumina's TruSeq Exome enrichment Kit). The reads in FastQ files were aligned on hg19 reference with the Novoalign software. Then the read counts per exon were calculated using BedTools. We evaluated NGS results using PCR.

Results: We found no alterations within the ENAM gene. Whole exome sequencing of the affected siblings revealed a homozygous 103 kB deletion spanning exons 13-18 of the WDR72 gene, which is an

important gene within the enamel maturation pathway. The parents were found to be heterozygous in this genomic region. The deletion was confirmed using conventional PCR, qPCR and long-range PCR.

Discussion: In the WDR72 gene (15q21.3) so far only one missense mutation and two nonsense mutations were reported as causative for AI. Our data for the first time report a WDR72 deletion causing autosomal recessive AI.

Currently, we are working on an NGS panel for AI diagnostics to improve genotype-phenotype correlations in the AI-spectrum disorders.

P-ClinG-078

Mutations in the PAX8 promoter region cause thyroid dysgenesis

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Thyroid dysgenesis (TD) is the cause of approximately 80% of patients diagnosed to have congenital hypothyroidism. So far, mutations in five candidate genes (PAX8, TSHR, TTF1, TTF2 and NKX2.5, respectively) have been identified to cause TD. We screened 190 patients with TD for mutations in these genes. Besides previously described and characterized mutations, we detected new so far unreported mutations in the coding regions of PAX8, TSHR, TTF1, TTF2 and NKX2.5. 100 normal individuals did not harbour those mutations. Interestingly, in addition to mutations in the coding region of the known candidate genes we also detected four different base pair exchanges in the PAX8 promoter region. Very recently, we have found one of these base pair changes in a patient diagnosed with thyroid dysgenesis who was heterozygous for a mutation in the NKX2.5 gene and heterozygous for a base pair change in the PAX8 promoter. In vitro studies were performed to unravel the mechanisms by which these newly identified promoter base pair exchanges might be causative. Electromobility shift assay (EMSA) studies suggest no specific protein or protein complex binding to the altered promoter elements. Transient transfection studies in different cell lines showed that at least one of these base pair changes leads to a significantly decreased promoter activity and thus to an impaired PAX8 gene expression. In summary, we identified a new group of PAX8 promoter sequence alterations that might cause TD. Further studies are needed to prove this hypothesis.

P-ClinG-079

Milder presentation of Angelman syndrome patients due to somatic mosaicism

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Angelman syndrome (AS; OMIM 105830) is a neurogenetic disease caused by loss of functional maternal UBE3A (ubiquitin E3 ligase) gene on chromosome 15. In most cases the patients do not have a maternal allele as a result of a deletion of chromosomal region 15q11–q13 (70%). Uniparental disomy (1-2%) and UBE3A mutations (15%) are less frequently found. Approximately 2–4% of patients have a paternal imprint on their maternal chromosome 15. The clinical presentation in AS is characterised by severe intellectual disability (ID) with gait ataxia, seizures, sleep disorder, absence of or severely limited speech and unique behaviour with paroxysms of laughter. The patients also show mild facial abnormalities, postnatal microcephaly and characteristic EEG signs.

Here, we present two patients with milder manifestations of AS due to somatic mosaicism in copy number or methylation pattern, respectively.

Patient one is a girl of four years who presented with ID, delayed speech and motor development. Brain MRI and routine chromosome analysis gave normal results. She had a normal length and a low normal OFC (10th centile). The girl never had seizures and no ataxia. Microarray analysis using the Affymetrix® CytoScan HD array demonstrated a loss in copy number of 1,5 Mb in the chromosomal band 15q11.2 encompassing SNRPN and UBE3A. The size of the deletion is smaller than class 1 and class 2 deletions commonly found in AS and Prader-Willi-Syndrome patients. Analysis showed an incomplete heterozygous deletion indicating a possible mosaicism of this deletion. The partial absence of the maternal methylated allele was verified by methylation-sensitive multiplex-ligation-dependent probe amplification (MS-MLPA, MRC-Holland) procedure. Additional interphase fluorescence in situ hybridisation (FISH) studies confirmed mosaicism for the deletion of the SNRPN probe (Metasystems) with 68% of peripheral lymphocytes carrying the deletion.

The second patient showed a normal growth and development during his first two years of life, including normal milestones of motor development. At about 30 months of age, a speech development disorder became obvious and he showed a sleep disturbance. EEG was normal at that age but showed paroxysmal activity during sleep a year later when he suffered from nocturnal terror fits. He never had obvious epileptic seizures and showed no ataxia nor other movement disorder. Length, weight, OFC (60th centile), and motor development were normal. By MS-MLPA we could identify an approx. 50% reduction of methylation signal in the SNRPN region indicating a mosaicism in methylation status. A microsatellite analysis of chromosome 15 revealed biparental inheritance, suggesting an imprinting defect.

These patients will be compared to cases of milder AS due to somatic mosaicism in methylation and deletion that have been previously published. They demonstrate the wide spectrum of molecular genetic causes of AS, emphasising the need of robust molecular genetic diagnostic tools and precise clinical investigation of patients with milder presentations of AS.

P-ClinG-080

Expanding the Mutation Spectrum for Fraser Syndrome: Identification of a Novel Heterozygous Deletion in FRAS1

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Fraser syndrome (FS) is a rare autosomal recessive inherited disorder characterized by cryptophthalmos, laryngeal defects and oral clefting, mental retardation, syndactyly, and urogenital defects. The incidence has been found to be 0.43 per 100,000 live births. Mutations in the FRAS1, FREM2, and GRIP1 gene have been identified causing FS. Mutations in FRAS1 on chromosome 4q21.21 are responsible for the classical phenotype of FS. Almost 50% of the patients exhibit mutations in either of the genes. So far, 26 mutations have been identified in FRAS1, most of them are truncating mutations. The mutational spectrum includes nucleotide substitutions, splicing defects, a gross insertion, and small deletions/insertions. Moreover, single heterozygous missense mutations in FRAS1 seem to be responsible for non-syndromic unilateral renal agenesis.

A family presented to our department in 2012 for genetic counseling. The medical history revealed a pregnancy in 2005. Prenatal ultrasound of the female fetus at a gestational age of 28 weeks showed renal agenesis on the left side and a multicystic dysplastic kidney on the right side. The fetus was stillborn at 29 weeks of gestation. Post mortem investigations additionally showed bilateral anophthalmia, bilateral cleft lip and palate, tracheal stenosis, and an anal atresia type II. Retrospectively, we made the clinical diagnosis of a FS in 2012 and initiated genetic analysis of the fetus and the parents. While molecular examination of the fetus was ongoing, the mother became pregnant again. Ultrasonography of the second female fetus at eleventh weeks of gestation showed bilateral cleft lip and palate. Post mortem investigations at a gestational age of thirteen weeks (induced abortion) showed a syndactyly of the second to fourth fingers on the right hand and between the second to fifth toes on both feet. Additionally, an atresia of the epiglottis resulting in an occlusion of the trachea could be found.

Because of poor DNA quality of the first fetus, direct sequencing was first performed in the parents for all 74 FRAS1 exons. The DNA sequence analysis of both fetuses was done by target diagnostic. In exon 66 of FRAS1 the novel heterozygous mutation c.10346delA resulting in a premature stop (p.Glu3449GlyfsX2) was identified in the father. Target diagnostic revealed that the fetuses were carrier of the paternal mutation. The mother did not show any causative mutation in FRAS1, but presented with multiple homozygous polymorphisms, which were located between exons 6-9 and 25-71. Because of the unexpected accumulation of homozygous polymorphisms in the mother, array-CGH was additionally performed and revealed a heterozygous 63.87 kb interstitial deletion on chromosome 4q21.21. The minimal deleted region extends from 79,125,989 to 79,189,854 and contains the exons 3 to 9 of FRAS1. The maximum deleted region extends from rs79,108,116 to 79,209,252 (101,14 kb) including the exons 3-14. Array-CGH of both fetuses showed the deletion of the FRAS1 gene as well.

Here we report the first case of a family with two patients affected by Fraser syndrome due to a deletion of 64 kb (deletion 4q21.21) and an additional novel frameshift mutation in exon 66 of the FRAS1 gene. To date, large deletions of the FRAS1 gene have not yet been described. Large deletions seem to be a rare cause for Fraser syndrome, but should be considered in patients with a single heterozygous mutation. Patients with a single mutation in FRAS1 should be examined by array-CGH as of now. A commercial MLPA probe set for the detection of large deletions or duplications in FRAS1 is not available so far. Our findings expand the spectrum of causative mutations in FS and the possibility for diagnostic testing, prenatal diagnosis for FS patients and their families.

P-ClinG-081

First trimester use of allopurinol - outcome of 31 prospectively ascertained pregnancies and a phenotype possibly indicative for teratogenicity

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Allopurinol, a purine analogue, that inhibits xanthine oxidase, is mainly used for the treatment of hyperuricemia in patients with gout or tumor lysis syndrome. As these conditions are rare in women of childbearing age, there are no prospective data on first trimester exposure of allopurinol. In 2011, Kozenko et al. reported on a child with multiple malformations after maternal treatment with allopurinol throughout pregnancy. Possible teratogenicity of allopurinol was proposed due to the similarity of the pattern of malformations in this child and those described in mycophenolate embryopathy. In addition, a possible common mechanism of both drugs, i.e. disruption of purine synthesis, was discussed.

We report on pregnancy outcome of 31 prospectively ascertained pregnancies with at least first trimester allopurinol exposure. Pregnancy outcomes were 2 spontaneous abortions, 2 elective terminations of pregnancy and 27 live born children. The overall rate of major malformations (3.7%) and of spontaneous abortions (cumulative incidence 9%, 95%-CI 2-32) are both within the normal rage. However, there was one child with severe malformations including microphthalmia, cleft lip and palate, renal hypoplasia, low-set ears, hearing deficit, bilateral cryptorchidism, and micropenis. The striking similarity of the pattern of anomalies in this child and the case described by Kozenko et al. might be considered as further signal for possible teratogenicity. Thus, we would recommend caution with allopurinol treatment in the first trimester, unless further prospective data are available.

P-ClinG-082

Novel mutation in the CLDN1 gene in a Turkish family with neonatal ichthyosis sclerosing cholangitis syndrome

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Neonatal ichthyosis sclerosing cholangitis (NISCH) syndrome (MIM #607626) is a rare autosomal recessive disorder characterized by scalp hypotrichosis, scarring alopecia, ichthyosis and sclerosing cholangitis. It is caused by mutations in the CLDN1 gene (MIM *603718) encoding the tight–junction protein claudin-1. A lack of claudin-1 leads to increased paracellular permeability between epithelial cells. Until now, twelve patients in five families with NISCH syndrome and two homozygous mutations in the CLDN1 gene have been reported. Here we describe two patients from a consanguineous family of Turkish origin with the clinical characteristics of NISCH syndrome carrying a novel homozygous nonsense mutation in the CLDN1 gene (c.181C>T, p.Q61X, exon 1). Patient 1 is a two year old boy with ichthyosis, alopecia/hypotrichosis and cholestasis which regressed at age of 6 months. Patient 2 is a 35 year old woman and a cousin of the father of patient 1. She shows a similar skin and hair phenotype than patient 1. Two of her brothers suffered from ichthyosis and cholestasis and died in the neonatal period. A light microscopic investigation of a skin biopsy from the upper arm in patient 2 revealed an unremarkable non-acanthotic epidermis and minor abnormalities in electron microscopy. The parents of both patients are heterozygous carriers of this mutation and phenotypically normal. Our report confirms the causal association of the CLDN1 gene with NISCH syndrome, the phenotypic heterogeneity, and the lack of pathognomonic histological features in skin biopsies.

P-ClinG-083

A small de novo copy number loss within the 16p12.2-p12.1 deletion region in a girl with mild unspecific intellectual disability

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De novo microduplications and microdeletions are a frequent cause of developmental delay.

We report on a 12 years old girl of German origin with mild intellectual disability (IQ of 60±6), who speaks in whole sentences but has problems even with basic arithmetic.

The girl has a narrow palate, strabismus and sandal gaps of toes but no major dysmorphisms.

Body measurements were within the normal range.

After normal chromosomal analysis and exclusion of Fragile-X syndrome we performed molecular karyotyping on a high density microarray. A microdeletion of 821 kb on chromosome 16p12.2-p12.1 encompassing 700 array markers was identified. Validation and segregation testing by FISH-analyses confirmed the copy number loss in the patient and excluded the deletion in both healthy parents, thus suggesting de novo occurrence. This aberration lies within the recurrent 8.7 Mb microdeletion syndrome of 16p11.2-p12.2 but is not contained in the database of Genomic variants and was not found among 820 in house healthy controls. It contains six genes without known human phenotype, two being highly expressed in brain suggesting them as candidate genes for unspecific intellectual disability. Mutational screening of these genes in patients with unexplained mental retardation is ongoing.

P-ClinG-084

Mutations in IL36RN in patients with generalized pustular psoriasis

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Generalized pustular psoriasis (GPP) is a rare disorder affecting 2 out of 1 million individuals in Europe. This severe disease is characterized by repeating flare-ups of pustular psoriasis and potentially life-threatening accompanying multisystemic inflammation. Recently, missense mutations in IL36RN have been identified in GPP patients from Tunisia and Europe. Therapy with a recombinant interleukin-1 receptor antagonist (anakinra) is effective in some GPP patients, which supports the role of IL36RN as a disease-causing gene for GPP.

We screened a group of 17 GPP patients recruited at five German university hospitals and one rehabilitation hospital for IL36RN mutations by Sanger sequencing. We identified mutations in 6 patients: three patients were homozygous for p.H32R, p.P76L or p.S113L, and three further individuals were compound heterozygous for p.S113L and either p.R48W, p.E94X or p.P76L. The stop mutations as well as the two missense mutations p.H32R and p.P76L are novel and all affect highly conserved residues. The stop mutation disrupted the C-terminal part of a binding domain of its protein IL36RA. Molecular modeling of p.P76L revealed a reduced stability of the protein as well as local structural rearrangements, indicating its disease-causing nature. The wildtype residue of p.H32R was predicted to interact tightly with the IL-36 receptor, suggesting a pathogenic role of this mutation as well. The newly identified mutations were not present in 190 control individuals. All available unaffected parents were tested as heterozygous mutations carriers. Eleven patients did not carry IL36RN mutations.

Phenotype genotype correlation including published cases was difficult due to different mutations in few carriers. In general, we did not observe a significant difference in age of onset between carriers and non-carriers of mutations ($p= 0.09$). The widely varying age of onset (1, 5 and 51 years of age) in three homozygous patients for the most common European mutation p.S113L (two of a previous publication) can currently not be explained. An additional coding variant (p.S200N) in another gene (CARD14), more recently identified to be relevant for the pathogenesis of psoriasis vulgaris and GPP, was identified in our homozygous carrier of p.S113L with the earliest age of onset (1 year). This CARD14 variant has been shown to be functionally relevant and might be a modifier for disease onset/ severity.

In the patient compound heterozygous for p.R48W and p.S113L, a further flare-up of GPP occurred very recently. As many usual therapeutics were not effective anymore or could not be applied due to additional medical problems, her doctors' decision to give the recombinant interleukin-1 receptor antagonist was considerably alleviated by her positive mutation screening. This therapeutic intervention proved to be very successful.

The rate of 35.2% IL36RN mutation carriers in our study provides further evidence that GPP is a heterogenous disease. As therapy with a recombinant interleukin-1 receptor antagonist in some GPP patients is effective, especially in those carrying IL36RN mutations, revealing the molecular basis of GPP in single patients is important for therapeutic decision-making.

P-ClinG-085

Girl with developmental delay, nearly absent speech and oligodontia: First case of an intragenic SATB2 gene duplication

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Background:

Recurrent chromosomal deletions of 2q32-q33 with a minimal deleted region of about 8 Mb have recently been described as a new microdeletion syndrome characterized by intellectual disability (ID), growth retardation, dysmorphic features, thin and sparse hair, feeding difficulties and cleft or high palate. The common deletion contains at least seven genes, including SATB2, a DNA binding protein that plays an important role in craniofacial patterning and brain development. Haploinsufficiency of SATB2 has been discussed to be causative for most of the clinical features. Hence, about 10 patients with smaller deletions in 2q33.1, including an intragenic deletion in SATB2, have been reported with a similar phenotype (severe developmental delay, challenging behavior, tooth abnormalities, cleft or high arched palate) supporting SATB2 as the candidate gene.

Clinical report and genetic findings:

We report on an 11 year old female patient with ID, nearly absent speech, oligodontia and dysmorphism (short and broad hands, broad thumbs and big toes, broad nasal root and short palpebral fissures). Microarray analysis (Affymetrix® CytoScan HD) showed a 84 kb duplication within the 2q33.1 region encompassing part of the SATB2 gene. Subsequent MLPA (multiplex ligation dependent probe amplification) analysis of the girl and her parents confirmed a de novo duplication of exon 3 of the SATB2 gene.

Discussion:

To our knowledge, this is the first case of an intragenic SATB2 duplication leading to a similar phenotype as described for patients with a microdeletion 2q33.1. Due to the striking similarities between our patient and the patients with 2q33.1 deletions we suggest that the intragenic SATB2 duplication leads to a functionally impaired or non-functional protein and may be disease causing. This provides further evidence for SATB2 as the candidate gene for most of the clinical features of 2q32-q33 microdeletion syndrome. Additional investigations are in progress.

This study is part of a project, which is winner of the Trinational Metropolitan Region's "Offensive Sciences" and cofinanced by the European Union's European Fund for Regional Development (EFRE) in the framework of the “INTERREG IV Upper Rhine Program".

P-ClinG-086

Floating-Harbor Syndrome caused by a truncating mutation in Exon 33 of the SRCAP gene

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Floating-Harbor syndrome (FHS) is a rare disorder characterized by short stature, delayed bone age, speech delay, and dysmorphic facial features. Recently, truncating mutations within a small region of the final exon (Exon 34) of the SRCAP gene (encoding SNF2-related CREBBP) have been identified as the molecular basis of FHS. We report on an 8-year-old patient with intellectual disability and typical clinical features of FHS. Sequencing of the SRCAP gene revealed a de novo truncating mutation in Exon 33. We conclude that – in case of clinical suspicion of FHS – sequence analysis of the SRCAP gene should not be restricted to the final exon (where all other mutations reported so far were located).

P-ClinG-087

Application of targeted next-generation sequencing for molecular diagnostics of Malignant Hyperthermia and Central Core Disease

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Malignant Hyperthermia (MH) is a pharmacogenetic disorder of the calcium metabolism in skeletal muscles. A hypermetabolic response is triggered by exposure to anesthetic gases such as halothane, desflurane, isoflurane but also by muscle relaxants like succinylcholine. The classic signs of a MH crisis are

muscle rigidity, rhabdomyolysis, tachycardia, acidosis and high fever. Central Core Disease (CCD) is an inherited myopathy characterized by muscle weakness and wasting. The clinical presentation is highly variable and ranges from nearly unnoticed to more severe congenital forms. Most patients with CCD show also a susceptibility to MH.

Both diseases are inherited in an autosomal dominant pattern and in most cases caused by mutations in the ryanodine receptor gene (RYR1). More than 180 mutations in the RYR1 gene have been identified so far and linked to MH, CCD and other neuromuscular disorders. Mutations are believed to be concentrated in two hotspot regions in the middle and at the C-terminal end of the gene. With routine molecular diagnostics, sequence analysis of the hotspots of the RYR1 gene of MH and CCD patients identifies mutations in only ~ 25 % of cases. Thereby in a large proportion of cases the causative mutations appear to be missed.

Therefore we decided to analyze all 106 exons of the RYR1 gene in more than 30 MH/CCD patients by next generation sequencing (NGS). For target enrichment the Access Array System™ of Fluidigm was used which allows the amplification of 48 target regions for 48 samples in parallel. Sequencing was performed with the 454 GS Junior System of Roche. For more than one third of those patients mutations could be found outside the hotspot regions. The results show that obviously several additional mutations exist outside the known MH and CCD hotspots. For an economic diagnostic protocol, it will therefore be useful to screen the hotspot regions first, but in case of negative results to analyze also the whole RYR1 gene by a cost-effective method like targeted NGS.

P-ClinG-088

Mutational analysis of the PRKAR1A and PDE4D genes in patients with acrodysostosis and brachydactyly type E

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Acrodysostosis is characterized by a peripheral dysostosis that is accompanied by short stature, midface hypoplasia, accelerated bone age, and developmental delay.

Recently, it has been discovered that mutations in two genes, PRKAR1A and PDE4D, which both play a role in the cAMP pathway, cause acrodysostosis with or without hormone resistance.

By mutational analysis of PRKAR1A and PDE4D we detected five different PRKAR1A mutations (p.Arg369Stop, p.Ala213Thr, p.Tyr373Cys, p.Gly289Glu, p.Arg335Cys) and one PDE4D mutation (p.Tyr667Cys) in 6/8 affected patients with acrodysostosis. The combination of clinical results, endocrinological parameters and in silico mutation analysis gave evidence to suppose a pathological effect of each mutation. This assumption is supported by the de novo origin of these mutations.

A review of the existing clinical and laboratory findings showed that apart from typical radiological abnormalities of the hand bones, elevated TSH and PTH values are the most common findings in patients with PRKAR1A mutations. Less frequent features in these patients are characteristic facial dysmorphisms, sensorineural hearing loss, and mild intellectual disability. These results lead to the conclusion that mutations of PKRAR1A are the major molecular cause for acrodysostosis with endocrinological abnormalities.

In addition, in our cohort of 44 patients affected with brachydactyly type E (BDE) we detected only one sequence variant of PRKAR1A (p.Asp227Asn) which is probably benign and no PDE4D mutations. Thus, we conclude that PRKAR1A and PDE4D mutations may play no major role in the pathogenesis of BDE.

P-ClinG-089

Noonan Syndrome With Multiple Giant Cell Lesions

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A small group of patients with Noonan syndrome develop multiple giant cell lesion (MGCL) of the jaw or even more rarely of other bones or soft tissues, which are classified as pigmented villonodular synovitis (PVNS). Originally, these patients were described as a distinct entity. However, this association is now considered as a part of the phenotypic spectrum of Noonan syndrome.

We here describe a female patient with short stature, relative macrocephaly, ocular hypertelorism, ptosis, downslantig palpabral fissures, low-set, posteriorly rotated ears, short webbed neck, cubitus valgus, hypertrophic cardiomyopathy, pulmonic stenosis and mild developmental delay. At the age of 6 years, she first showed a mild swelling of the right maxilla and CT confirmed a cystic enlargement located in the anterior wall of the right maxillary sinus.Two years later, this tumor had progressed and in addition she had a swelling of the left knee and the left lower leg. Histological examination of the maxillary cyst showed multinuclear giant cells, the biopsy of the knee showed a multinuclear giant cell synovitis. CT of the knee was consistent with PVNS. Within 1 ½ years tumors of both maxillae developed, resulting in a completely disfiguring face with obstruction of the nasal airway, orbital deformation and misalignment of the teeth. Intralesional injections of corticosteroids had no effect. Surgical treatment is planned. Chromosome analysis revealed a normal 46,XX karyotype. However, until now, no mutation in the RAS/MAPK pathway could be detected in our patient.

24 published patients with molecularly confirmed Noonan/CFC/LEOPARD syndrome and MGCL had very different, but known classic mutations of the RAS/MAPK pathway. Hence, it seems that no specific mutation leads to MGCL, but that general disturbances of this pathway can predispose to MGCL.

P-ClinG-090

Cystic Fibrosis caused by compound heterozygosity for a de novo novel frameshift mutation in exon 20 and the p.Phe508del mutation

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Cystic fibrosis (CF, MIM: 219700) is one of the most common life-shortening autosomal recessive disorders in the Caucasian population, affecting approximately 1 in 2500 newborns. The disease is caused by dysfunction or complete deficiency of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel (MIM: 602421) in the apical epithelial cell membrane. Since the identification of the CFTR gene in 1989, >1860 different molecular defects were recognized and submitted to Cystic Fibrosis Mutation Database

Here we report on a 3 months old boy with classical phenotype of Cystic Fibrosis. In the first instance, 36 common mutations were analyzed in him. By this the most common mutation p.Phe508del was identified on one CFTR allele but no second disease causing mutation. Subsequently, by sequencing the whole coding region of the CFTR gene we could confirm the p.Phe508del and additionally, identified a small heterozygous deletion (c. 3294delG) in exon 20 (legacy region name: exon 17b) resulting in a frameshift and a premature stop codon (p.Trp1098Cysfx*4). This mutation has not been reported in the Cystic Fibrosis Mutation Database or the Human Gene Mutation Database so far, but due to its nature it can be considered as disease causing.

Usually, each parent of a child with Cystic Fibrosis has been considered to carry one copy of the abnormal allele giving a 25% risk for having further affected children. To confirm carrier status in the parents, CFTR exons 11 and 20 were analyzed by sequencing. While we could confirm the mutation p.Phe508del in the mother, the mutation c.3294delG was neither identified in the father nor in the mother. Therefore, we confirmed paternity with a probability of higher than 99,99999% by testing 15 microsatellite markers from 13 different chromosomes. The absence of this mutation in the father and the mother of the CF patient indicates de novo occurrence of the c.3294delG mutation in this family. Although this mutation was not found in the blood lymphocytes of the father, germline mosaicism, as demonstrated for for other disorders, cannot be excluded.

While de novo mutations are common in dominantly inherited disorders, such as neurofibromatosis and tuberous sclerosis, the appearance of new mutations is very uncommon in recessive diseases. From the genetic counseling point of view, the identification of de novo mutations in CF families highlights the importance of segregation studies to test carrier status in a given family. In order to give accurate genetic counseling, particularly regarding possible prenatal testing, molecular analysis of each of the parents of a child affected with an autosomal recessive disease must be routinely performed.

P-ClinG-091

A novel deletion comprising 5'UTR and exon 1 of MPZ is associated with late-onset demyelinating Charcot-Marie-Tooth disease

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Introduction: Charcot-Marie-Tooth disease (CMT), also known as hereditary motor sensory neuropathy, is the most common inherited disorder of the peripheral nervous system with significant clinical and genetic heterogeneity. The main clinical manifestations of CMT include progressive distal muscular atrophy and weakness, impaired distal sensation and diminishing or loss of tendon reflexes. According to electrophysiological and histopathological characteristics CMT can be classified into demyelinating and axonal types.

The demyelinating phenotype may be caused by defects in the myelin protein zero (MPZ) gene which varies from early onset and severe forms to late onset and milder forms. The MPZ gene encodes the major structural protein component of myelin in the peripheral nervous system which mediates adhesion in the spiral wraps of the Schwann cell's myelin sheath. More than 120 mutations have been detected in this gene so far.

Case report: A 54-year-old man presented with decreased sensation in both feet starting 6 years before, increasing over time with decreased muscle strength in the right leg. Additionally, he showed atrophied small foot muscles and pes cavus bilaterally. Neurography revealed severe demyelinating polyneuropathy with extensively increased distal motor latencies and diminished nerve conduction velocities. Ultrasound indicated thickening of the median nerve. His 19-year-old son showed similar neurographic and ultrasonographic results.

Methods: For mutation analysis the MPZ gene was analyzed by MLPA (multiplex ligation-dependent probe amplification) analysis and direct sequencing. Polymorphisms located in the binding sites of the MLPA probes were excluded in the exon in question.

Results and conclusion: MLPA analysis of MPZ revealed 50%-diminished gene dosage of two probes for exon 1 and the 5' untranslated region (5'UTR), respectively. To our knowledge, this is the first report of CMT caused by a gross deletion in MPZ. The identified deletion in heterozygous state comprised part of 5'UTR and at least a major part of exon 1 including the start codon. As the start codon is deleted, we suggest impaired transcription. The next possible start codon is located 48 bp downstream in exon 2 of MPZ, but it would result in out-of-frame translation of only 7 amino acids.

The novel MPZ mutation is responsible for the severe late-onset form of demyelinating CMT in the investigated patient with high probability.

P-ClinG-092

Targeted Next-generation Sequencing identifies two novel Mutations in the IGHMBP2 gene

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an autosomal recessive disorder characterized by an early life-threatening respiratory failure ascribed to diaphragmatic dysfunction and a subsequent generalized atrophy of body muscles. SMARD1 results from mutations in the gene encoding the immunoglobulin micro-binding protein 2 (IGHMBP2). Mutations in this gene are also known to be causative for patients with distal spinal muscular atrophy type 1 (DSMA1) and hereditary motor neuropathy type 6 (HMN6).

We report on a 3 years old female patient who initially presented with muscular hypotonia and congenital polyneuropathy. The nerve conduction velocity was not inducible. Karyotyping, array-CGH and MLPA in the SMN1 gene revealed normal results. We performed targeted Next-generation Sequencing with a panel including 50 genes that are associated with hereditary motor and sensory neuropathies (HMSN) and related disorders. We identified two heterozygous novel nonsense mutations in the IGHMBP2 gene [c.676G>T (p.Glu226X) and c.2083A>T (p.Lys695X)] leading most probably to a truncated protein. One of these mutations could also be detected in paternal and maternal DNA, respectively. This demonstrates the compound heterozygosity of the two detected genetic alterations.

Gene-Panel diagnostic with Next-generation Sequencing is an appropriate method to detect causative mutations in case of diseases with clinical and genetic heterogeneity.

P-ClinG-093

Microdeletion of 831 kb in a young woman leads to haploinsufficiency of more than 30 genes at 17q including a copy of the BRCA1-gene. Consequences for genetic and clinical management.

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Using array-CGH analysis by application of a 60k Agilent oligonucleotide array we identified a de novo microdeletion, that leads to a loss of the important tumour suppressor gene BRCA1. These unintentional findings in the patient were obtained because cytogenetic results did not provide any clue for presence of her congenital moderate mental impairment and dysmorphic facial features which on the other hand suggested that a small genomic imbalance might be responsible which finally could be confirmed. Besides this dn microdeletion (chr17:40869151-41700815) also a microduplication of about 332 kb at 1q25.1 (chr1:172199642-172532314) inherited from her phenotypically normal mother was found. Because of that latter fact and since the microduplication only affects 4 genes without any known important function, we assume that the microduplication is presumably without any additional clinical relevance. The observations made in this particular case certainly demonstrate again that accidental or coincidental findings in specific subsets of patients should seriously be considered and councelling has to be performed accordingly. The list of well defined inherited cancer predisposition syndromes, which can be attributed to a hereditary susceptibility and having far reaching implications for all family members, is steadily growing. De novo mutations or even microdeletions of such tumour suppressor genes are rather rare events, which for different gene loci obviously will show a remarkable variable probability. We recently identified the first patient with a contiguous gene syndrome due to microdeletion of 17p that also caused a loss of the entire TP53 gene as well as several flanking genes in a mentally retarded person. The current patient is now 28 years of age. Her length is 153.5 cm and weight is 38 kg. A microcephaly with an OFC of 51 cm was noticed and distinct dysmorphic facial features like ear anomalies and bilateral ptosis were recognized. No comparable microdeletions also causing a loss of the entire BRCA1-gene as well as up- and downstream flanking genes is reported thus far or included in databases like ISCA or Decipher. Because of the achieved diagnosis the patient and her family were already subjected to an additional extented genetic counceling with information about results and recommendations and specialists in breast cancer screening and prevention are already included in continued medical and psychological assistance and support.

P-ClinG-094

Third case of somatic mosaicism and familial transmission of Rubinstein-Taybi syndrome

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Rubinstein-Taybi syndrome (RTS) is a rare genetic disorder (~1 in 125,000 newborns) of broad thumbs and great toes, facial anomalies, growth delay and developmental delay. Autosomal dominant mutations of the CREBBP or EP300 genes have been reported in, respectively, ~50-70% and 3% of patients clinically diagnosed with RTS. Somatic mosaicism in RTS patients is extremely rare. Only 6 individuals with RTS and somatic mosaicism have been reported in the literature, all with CREBBP gene mutations and mild or absent clinical features. Two of these individuals had offspring with classical RTS. We present here a third case of inherited RTS due to somatic mosaicism in one parent. Both the mother and daughter had a clinical diagnosis of RTS and demonstrated, by Sanger sequencing of the CREBBP gene, a heterozygous c.2168C>A mutation (predicting p.Ser723*). Inspection of sequence chromatograms and quantification of C and A peak areas showed that in the mother, the mutant A allele peak was smaller than the normal C allele peak. For confirmation and quantification of this rare result, we performed a quantitative pyrosequencing assay and identified abnormal allele frequencies (30% mutant allele, 70% normal allele) in the mother's DNA, confirming a somatic mosaicism. The daughter's DNA showed normal allele peaks in the sequencing and normal allele frequencies (50% : 50%) in the pyrosequencing assay. We hypothesize that somatic mosaicism in RTS may be more frequent than estimated in the past. We have initiated a systematic re-analysis of molecularly confirmed RTS cases using the methods described here.

P-ClinG-095

Phenotypic Spectrum in a large Cohort of Patients with SHOC2 Mutations

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Noonan-like syndrome with loose anagen hair (Mazzanti syndrome) (MIM 607721) is caused by a specific mutation in the SHOC2 gene. The disorder was first described by Mazzanti and colleagues in 2003 and is characterized by Noonan-like features, including heart defects, short stature, developmental delay and typical facies, but also by a specific hair phenotype. This includes easily pluckable and slow growing hair. Additionally trichograms typically show the characteristic pattern of loose anagen hair. Noonan syndrome is considered a RASopathy, along with Costello syndrome, Cardio-facio-cutaneous syndrome, Neurofibromatosis type 1 and others. All these disorders are caused by mutations in genes encoding components of the RAS/MAPK pathway.

In 2009 Cordeddu and colleagues found the causative mutation for Noonan-like syndrome with loose anagen hair in the SHOC2 gene (c.4A>G, p.S2G). The reported consequence of this mutation has been described as an aberrantly acquired N-myristoylation of the protein, which results in increased RAS/MAPK signalling. The same effect is seen for mutations in all other genes associated with Noonan syndrome. SHOC2 mutations account for about 1% of the RASopathy genes (excluding NF1).

We report on a large cohort of patients from an international research collaboration with the same common SHOC2 mutation. This cohort includes the patients from Cordeddu et al (2009) as well as 53 novel cases. Clinical data from a total of 78 patients was available. The ages ranged from 0.08 – 39 years with a median at 9.0 years. Short stature and an intellectual disability were present in most of the patients with a SHOC2 mutation and more common than in a cohort of 166 patients with PTPN11 mutations. Proven growth hormone deficiency was diagnosed in 42% of patients with known test results. These patients also have a recognizable facial phenotype that is similar to other RASopathies. Heart defects were common, but differently distributed than in patients with mutations in other known Noonan syndrome genes. 35% had a single heart defect and 51% had multiple ones. Pulmonary valve stenosis was present in 42% of the patients with a SHOC2 mutation. Other heart defects included ASD and VSD (40%), HCM (29%) and others (29%).

Noonan-like syndrome with loose anagen hair represents a recognizable condition within the spectrum of RASopathies and a single recurrent mutation, p.S2G, accounts for this syndrome.

P-ClinG-096

De novo deletion of NUFIP1 in a female patient with mild developmental delay, intellectual deficits and piebaldism

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The NUFIP1 gene encodes the nuclear FMRP interacting protein 1. It is expressed in neurons of cortex, hippocampus and cerebellum (Bardoni B et al., 1999). Deficiency of its interaction partner FMRP, the Fragile X Mental Retardation Protein, encoded by FMR1 on Xq27.3, causes the fragile X mental retardation syndrome. NUFIP1 is localized on 13q14.12, approximately 3.5 Mb proximal to RB1. All patients with retinoblastoma and an interstitial 13q deletion that includes the NUFIP1 gene exhibit intellectual disability (ID). It has been suggested that haploinsufficiency of NUFIP1 may be the cause of ID in these patients (Caselli R et al., 2007 & Mitter D et al., 2011).

Here, we describe a 15-year-old girl with primary microcephaly and ID. She was born after 39.5 weeks of gestation by Caesarian section due to breech presentation (birth weight 2860 g (-1.5 SD), length 49 cm (-1.2 SD), OFC 31 cm (-3 SD)) to healthy, non-consanguineous parents. The mother's head circumference (54 cm) is also in the lower normal range. The patient has a healthy elder sister. A cecum perforation on the second day after birth required surgical intervention. Developmental delay was first noted at the age of one year. At the age of 15 years she attends a regular school, but needs special support. Her body measurements are normal except for mild microcephaly (length 1.69 m (0 SD), weight 46.5 kg (-1.1 SD), OFC 52 cm (-2 SD)). Additionally, she presents with hypopigmented areas on both eyelids, her right armpit and her lower legs. A syndromic form of intellectual disability was suspected.

A conventional chromosome analysis had revealed a normal female karyotype, 46,XX. In a search for a suspected microdeletion, we performed an array analysis using the CytoScan HD array by Affymetrix. Analysis of raw data was done with the Affymetrix Chromosome Analysis Suite v.1.2. Even at a resolution of

5 kb for losses or gains of genetic material, we found only one bona fide pathogenic deletion. The 28.66 kb-deletion, arr[hg19] 13q14.12(45.531.275-45.559.939)x1, spans exons 2 through 7 of the ten exons the NUFIP1 gene, and must be regarded as inactivating mutation. Presence of the deletion was confirmed by a specific quantitative PCR, and determined to be de novo.

There is no obvious association between the partial NUFIP1-deletion and the suspected piebaldism. The known loci for piebaldism, KIT on 4q12 and SNAI2 on 8q11.22, did not show an altered dosage. Thus, we assume that the ID and the hypopigmentation occurred independently in the patient, and that the small head circumference may also be an independent trait in the maternal line.

In conclusion, our findings support the hypothesis that haploinsufficiency of NUFIP1 impairs cognitive functions. However, examination of a larger cohort of patients with ID and identification of further inactivating mutations in NUFIP1 are necessary to prove this hypothesis.

P-ClinG-097

Emerging phenotype of microdeletion 1p34.3

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We report on a young boy seen for evaluation of his developmental delay. He was born at term small for gestational age with hypospadias, suffered from perinatal depression, neonatal infection and transient renal failure. He showed failure to thrive, length and OFC dropped below the 3rd centile and his development was delayed. On examination at the age of one year, he presented in particular with motor delay, axial hypotonia and mild hypertonia of the limbs, length and OFC below the 3rd centile, and facial features partly also present in his older healthy brother (prominent forehead, deep set eyes, short palpebral fissure, depressed nasal bridge, and small chin). SNP Array analysis (Affymetrix CytoScan HD) revealed a 3 Mb deletion in chromosomal region 1p34.3 encompassing 43 RefSeq genes, among which several are involved in transcriptional regulation, cell cycle control and synaptic transmission. Subsequent metaphase FISH analysis of the patient and his parents confirmed the deletion and showed de novo occurrence. To our knowledge, no comparable deletions of 1p34.3 have been reported in the literature, so far. However, several patients listed in databases (Decipher, ECARUCA) show comparable or overlapping small deletions and similar clinical features like developmental delay, hypotonia, and feeding problems, thus suggesting pathogenicity of the deletion.

P-ClinG-098

Pronounced instability of the amyotrophic lateral sclerosis causing C9orf72 hexanucleotide repeat in immortalized cell lines

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Amyotrophic lateral sclerosis (ALS) is a progressive, adult-onset neurodegenerative disorder caused by degeneration of upper and lower motor neurons. The clinical prognosis of ALS patients is poor, and death due to respiratory failure occurs after 3-5 years of disease duration on average. To secure a long term access to patient cells and nucleic acids for biological studies as well as for genetic testing and counseling of family members, immortalized cell lines as e.g. lymphoblastoid cell lines (LCLs) have been established in many laboratories assuming a similar behaviour and cellular phenotype of the immortalized cells as compared to primary patient cells. An intronic GGGGCC hexanucleotide repeat expansion in C9ORF72 has recently been found to be the most common genetic cause of familial ALS and frontotemporal dementia (FTD) as well as in a substantial fraction of sporadic ALS patients. Molecular testing of C9ORF72 relies on a repeat-primed polymerase chain reaction method and Southern blotting. However, Southern blotting is the only way to determine the exact repeat size and thus necessary to learn more about a possible genotype-phenotype correlation. We now wanted to test whether molecular diagnosis for C9ORF72 is possible with good sensitivity and specificity on genomic DNA isolated from LCLs by Southern blotting. We therefore studied three different patients with C9ORF72-caused ALS, from each of whom we simultaneously analyzed DNA from whole blood in comparison to DNA derived from at least three independent LCL cultures. Southern blotting indeed revealed different patterns depending on the source of DNA. Whereas DNA derived from whole blood shows a smear rather than distinct bands for the expanded allele (showing somatic instability), DNA from LCLs revealed clonal effects represented by a number of distinct bands in the expanded allele range. These bands were in general smaller than the main expansion in the blood sample. Even more, DNA samples of independent LCLs of the same patient showed different banding patterns, arguing for a more stochastic mechanism of clonal selection in the LCL cultures. Our data show that in

principle, DNA from LCLs can be used for confirming the diagnosis of a C9ORF72 repeat expansion in ALS, as all LCL cultures showed the existence of expanded alleles. However, the determination of the exact C9ORF72 repeat length with DNA derived from LCLs is problematic as there is only a poor – if any - correlation between repeat lengths observed in primary cells from whole blood and immortalized cells from the same patient. Our study thus broadens the knowledge of the diagnostic and methodological setting for molecular testing in the most common genetic cause of ALS and FTD and faces us with diagnostic problems in repeat-diseases possibly occurring with immortalized cell lines.

P-ClinG-099

Is it Down Syndrome? Challenging differential diagnosis in newborns and early infants

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Down syndrome is among the most common syndromal diseases seen in newborns. For neonatologists even in specialized centers Down syndrome is the major known syndromal disease.

Thus, in newborns with dysmorphic signs - atypical or typical for Down syndrome - this differential diagnosis is frequently considered resulting in a request for human genetic consultation. Individual characteristic Down syndrome signs however may not be specific. As well individual phenotype variants as other diagnoses might be reflected in part of these early captured cases.

Here we present four cases of newborns or early infants, in which possible diagnosis of Down syndrome lead to human genetic investigation. Diagnosis of Down syndrome could be established by cytogenetic finding of trisomy 21 in only one of these babies. Instant chromosome analysis is recommended especially in dysmorphic newborns to early confirm or exclude chromosomal disease.

P-ClinG-100

Isodisomic LMNA mutation p.R435C leading to a unique progeroid syndrome with Restrictive Dermopathy-like features

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Restrictive dermopathy (RD) is an extremely severe congenital genodermatosis characterized by tight rigid skin with erosions at flexure sites, multiple joint contractures, general low bone density and pulmonary insufficiency generally leading to death in the perinatal period. We report a female patient affected by a progeroid syndrome with RD-like features. Besides missing hairiness, stagnating weight and growth, RD-like features including skin swelling and solidification, acrocontractures, osteolysis and muscular hypotension were continuously progressive until the patient died at the age of 11 months. For mutational analysis, the complete coding regions including intron/exon boundaries of the LMNA and FACE1 genes were amplified and used for direct Sanger sequencing. As a result, the homozygous LMNA mutation c.1303C>T (p.R435C) was found. The heterozygous mutation was present only in the mother while the father was wild type. Sequencing of polymorphic markers on chromosome 1 revealed a partial uniparental disomy of chromosome 1 (1q21.3 to 1q23.1) including the LMNA gene. Interestingly, LMNA p.R435C is not located at the cleavage site necessary for processing of prelamin A by ZMPSTE24 as reported in other RD patients with LMNA mutations. This might explain the atypical phenotype compared to other published cases of RD. In the course of the disease, immunoreactivity for lamin A was decreasing and double strand breaks were observed in different tissues by γH2AX staining at the age of 11 months. These results suggest a correlation between decreasing amounts of LMNA and increasing DNA damage.

P-ClinG-101

A missense mutation in the dystrophin gene causes BMD in the original family of Becker and Kiener

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In their first description of Becker muscle dystrophy in 1955 Becker and Kiener wrote:

„Die neue X-chromosomale Muskeldystrophie ist eine genetisch selbständige Art, die sich von der bisher bekannten [DMD] durch das spätere Erkrankungsalter, den mehr gutartigen Verlauf und die weniger schwere Ausprägung unterscheidet. Die Mutationen der beiden recessiven X-chromosomalen Arten [DMD und BMD] stehen vermutlich im Verhältnis der Allelie zueinander." („The new X-chromosomal muscle dystrophy is a genetic independent entity differing from the already known form [DMD] by the later onset, a more benign course and a less severe phenotype. The mutations of both X-chromosomal recessive forms [DMD and BMD] are probably allelic.") He was right in all his predictions.

The original BMD family originating from Upper Palatinate (Oberpfalz) consisted in the first description of 14 BMD patients. Seeing the family again, T. Grimm met 1989 another patient, a cousin of no.14 in the original pedigree. From a stored DNA sample of patient no.13 a deletion or duplication in the dystrophin gene could be excluded by MLPA. In 2007 the whole dystrophin gene was sequenced using the same DNA sample, and a hemizygous missense mutation was found in exon 3: c.136G>T (p.Asp46Tyr). It was not already described, and at that time it could not be decided whether it was pathogenic.

In 2012 we found another BMD patient in our diagnostic lab harbouring the same missense mutation. A detailed family story revealed that his grandfather was also affected and identical with the cousin of family member no.14 in the original Becker-Kiener family. Now we could track the family down again.

Meanwhile there was a new entry in the Leiden mutation data base (www.dmd.nl) describing an index patient from Italy suffering from BMD, habouring the same missense mutation and showing reduced dystrophin expression in his muscle biopsy by immuno-histological analysis. Gene mutation interpretation programs classified it as probably pathogenic.

The underlying mutation in the original BMD family described by Becker and Kiener could be identified as a missense mutation in exon 3 of the dystrophin gene. It is one of the rare causal missense mutations in this gene.

Lit.: Becker, P. E., Kiener, F. ; Eine neue X-chromosomale Muskeldystrophie. Arch Psychiat Zeitschr Neurol 1955; 193:427–428.

P-ClinG-102

VACTERL-H association – a previously undescribed family with a FANCB mutation

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The X-chromosomal VACTERL-H-association is a rare condition with vertebral defects, anal atresia, cardiac malformation, tracheoesophageal fistula, esophageal atresia, radial or renal dysplasia, limb abnormalities and hydrocephalus. Mutations in the FANCB gene (Fanconi anemia complementation group B) have been described to be causative for this distinct entity. Until now, there are just a few patients with VACTERL-H caused by mutations in FANCB reported (McCauley et al. 2011, Holden et al. 2006, Meetei et al. 2004).

Here, we present the first child of a non-consanguineous couple. Pregnancy was complicated by an anhydramnios, which was diagnosed at 14 weeks of gestation. Prenatal sonographic evaluation showed bilateral absence of kidneys, malformations of the upper limbs, hydrocephalus and hypertrophy of the myocardium. Delivery took place after a preterm rupture of membranes at 25+6 weeks of gestation. The boy died shortly after birth. The tentative diagnosis of VACTERL-association plus hydrocephalus, which was established prenatally was confirmed at postmortal clinical examination. It is interesting to note that the mother gave life to three children, all of them girls. The remaining eight pregnancies ended in spontaneous abortions during the first trimester of pregnancy. The maternal grandmother had a stillborn boy.

We sequenced the FANCB gene in the mother and her son and identified a heterozygous nonsense mutation in the mother (p.Q278*) and the same homozygous mutation in the boy. In addition, we performed an X inactivation analysis at the AR locus in the mother and found a complete skewing at the AR locus.

We would like to present the clinical and molecular data of this family and give a review on this rare condition belonging to the clinically and genetically heterogenous Fanconi anemias .

P-ClinG-103

A splice-mutation in intron 4 of FLCN leads to loss of exon 5 and causes a Birt-Hogg-Dubé Syndrome like phenotype with pneumothorax and cutaneous manifestation, but no renal tumors

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Birt-Hogg-Dubé Syndrome (BHD, OMIM #135150) is a rare autosomal dominantly inherited disorder characterized by variable combinations of pulmonary cysts and spontaneous pneumothorax, different cutaneous manifestations as well as renal tumors. The only gene, in which mutations are known to cause BHD is FOLLICULIN (FLCN, OMIM*607273), located on chromosome 17p11.2.

Here we describe a three generation family with 14 affected members, who did not present the full classical clinical phenotype of BHD. Five family members had pneumothorax, four members had cutaneous manifestations, mainly angiofibromas, and five members suffer from pneumothorax and cutaneous manifestations. Remarkably no renal tumors have been diagnosed so far.

A splice mutation, c.250-2A>G in FLCN was detected in the affected family members. To examine whether the mutation in the splice acceptor site of exon 5 causes aberrant mRNA splicing, we performed cDNA analysis in the patient. One prominent PCR band, corresponding to an isoform containing exon 5 was detected in the healthy control. Two prominent bands were detected in the patient, corresponding in size to the full-length transcript and a novel transcript lacking exon 5, respectively. A small amount of exon 5-skipped transcript was also detected in the control, suggesting that the splice acceptor site of exon 5 is a weaker one. Sequencing of each of the two PCR products confirmed the aberrant mRNA splicing and the presence of the two isoforms.

Here we present the largest family so far with the mutation c.250-2A>G leading to aberrant mRNA splicing, resulting in a Birt-Hogg-Dubé Syndrome like phenotype with no renal tumors.

P-ClinG-104

Phenotypic variability of the same unbalanced translocation in two brothers

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We report on two brothers who presented at the age of 5 and 12 years with a phenotypically different picture of a syndromic retardation.

The younger boy was profoundly retarded and did not have any active speech. Furthermore he had a short stature (<3rd perc.) and microcephaly (<3rd perc.). Craniofacial dysmorphism included midface hypoplasia, hypotelorism, epicanthal folds, broad nose and high arched palate.

Pregnancy was complicated by preeclampsia of the mother and delivery per caesarean section was necessary after 28 weeks of pregnancy because of CTG-deterioration. The birth-weight was 1190g, length 40 cm and head circumference 25 cm.

The older brother (length: 25th perc., weight: 97th perc.) possessed a very mild speech delay. Apart from microcephaly he had only mild dysmorphic features (broad nasal bridge, thin upper and lower lip). Pregnancy and delivery after 41 weeks of pregnancy were uneventful, birth-weight 4340g, head circumference 34.5 cm, length 53 cm.

Conventional cytogenetic analyses revealed apparently normal karyotypes (46,XY). We then performed array CGH analysis in the more severely affected younger boy and uncovered a loss (4,5 Mb) in 2q37.3 and a gain (2,1 Mb) in 12p13.33. These imbalances were validated by telomere specific FISH probes (D2S2142-,D12S158+) and revealed an unbalanced translocation with a partial monosomy 2q37.3 and a partial trisomy 12p13.33. The father carried the balanced translocation (46,XY.ish t(2;12)(q37.3;p13.3)).

Unexpectedly the array findings of the phenotypically different older brother showed the same unbalanced translocation.

The loss in 2q37.3 encompassed the region of the 2q37 deletion syndrome including mental retardation, autism spectrum disorders, facial dysmorphism, skeletal anomalies, epilepsy, cardiac defects and overweight.

The fact that both brothers carry the same unbalanced chromosomal rearrangement but presented with largely different phenotypes can not be explained by the results of our analysis. Recently, differential expression of HDAC4, a gene localized in the deleted region 2q37.3, was supposed to influence the phenotypic differences in 2q37 deletions (Morris et al. 2012). Dosage effects of HADAC4 or other genes in this region or in 12p13.33 or epigenetic mechanisms could influence phenotypic variability.

P-ClinG-105

A balanced 1/22 translocation leading to the diagnosis of Phelan-McDermid syndrome in an adult with non-specific intellectual disability

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Our patient is the third child of a German non-consanguineous couple. He was born after an uneventful pregnancy with normal birth weight, length and OFC. His two older sisters are healthy and gave birth to a healthy son each. Among the paternal relatives of the patient some additional males with learning difficulties or attention deficit disorders were reported.

Clinical examination at the age of 51 years revealed a patient with moderate intellectual disability and particular impairment of speech development. His receptive and nonverbal communication skills were higher than his active speech. Facial characteristics included a long face with slightly deep-set eyes, a high nasal bridge, long eyelids, mild down-slanting palpebral fissures, and a pointed chin. The face was looking older than his age. Neurological examination revealed mild muscular hypotonia and normal reflexes. His behaviour was characterized by impaired communication and irritability. He was reported to have a decreased perception of pain. Physical health appeared otherwise remarkably good. No hearing or visual impairment or gastrointestinal problem was known. A cardiac examination two years ago revealed nothing exceptional. He worked as an unskilled worker since he was 14 years old. Independent living is not possible and therefore he lives in a sheltered accommodation.

Molecular testing regarding Fragile X syndrome and molecular karyotyping (array-CGH) were unremarkable. By conventional chromosome analysis (GTG-banding), however, a derivative chromosome 22 was suspected. This impression was confirmed by fluorescent in-situ hybridization (FISH) with the DiGeorge "Tuple" (22q11) / 22q13 (SHANK3) probe mix (Kreatech) containing a probe for SHANK3 as internal control. A third signal for SHANK3 was observed at the telomeric region of the short arm of one chromosome 1. Application of subtelomeric 1p and 22q probes showed a reciprocal translocation between 1pter and 22qter with one breakpoint being situated within the 22qter probe (split signal). Only two genes, SHANK3 and ACR, are included within the 40 kb overlapping region of the Kreatech SHANK3 probe and the 22q subtelomeric probe. In accordance with the patient's phenotype, these results strongly suggest that a disruption of the SHANK3 gene is the cause of his disease. Since molecular karyotyping revealed no remarkable copy-number changes at the translocation-breakpoints, this reciprocal translocation between 1pter and 22qter is assumed to be balanced. Chromosome analyses in the patient's parents showed normal karyotypes, therefore the karyotype of the patient is: 46,XY,t(1;22)(p36.3;q13.33)dn.

To the best of our knowledge this is the second patient with a balanced translocation disrupting SHANK3 resulting in the Phelan-McDermid syndrome. Furthermore, this is one of the oldest patients known to have this syndrome. Further investigations for revealing the exact deletion breakpoints are in progress.

P-ClinG-106

Autosomal-dominant thrombocytopenia-2 (THC2) caused by a novel point mutation in the promotor region of the ANKRD26 gene

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We report the case of a 62-year-old woman with severe isolated thrombocytopenia. Ankyrin repeat domain 26 (ANKRD26)-related thrombocytopenia-2 (THC2) was suspected due to autosomal dominant pattern of inheritance and normal thrombocyte morphology.

The patient presented in good general condition (Eastern Cooperative Oncology Group (ECOG) status 0) and normal nutritional status (Height: 156cm, Weight: 60kg, Body Mass Index (BMI) 25 kg/m). The blood pressure was 150/90mmHg, heart rate at 100 beats per minute. Bleeding tendency was grade 0 according to the World Health Organization bleeding scale. The peripheral lymph node status, the neurological examination and the heart, lung and abdominal status remained inconspicuous. We could confirm the initial clinical diagnosis of THC2 by DNA sequencing. A heterozygous single nucleotide substitution c.-126T>C in the 5'-untranslated region of the ANKRD26 gene was identified in the patient and all affected family members. A specific therapy was not necessary but would consist of thrombocyte supplementation.

This is the first case of THC2 reported in Germany. We are currently preparing to screen a larger cohort of patients because we anticipate that THC2 might be a frequent form of hereditary thrombocytopenia and an underestimated differential diagnosis of isolated thrombocytopenias. The correct diagnosis is important to avoid unnecessary treatment of a patient with thrombocytopenia and to provide sufficient information with respect to prognosis and genetic counseling.

P-ClinG-107

Novel STRA6 null mutations in the original patient with Matthew-Wood syndrome

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Microphthalmia/anophthalmia can be associated with craniofacial dysmorphic features, heart and vascular malformation, skeletal and limb anomalies, skin or gut defects, mental retardation and hydrocephalus, or combinations thereof. The combination of anophthalmia with pulmonary hyploplasia, diaphragmatic hernia and a cardiac malformation was originally referred as Matthew-Wood syndrome (MWS) by Seller in 1996, while in 2007 Chitayat devised the acronym of PDAC (Pulmonary hyploplasia,/agenesis, Diaphragmatic hernia/eventration, Anophthalmia/microphthalmia and Cardiac defect). Mutations in STRA6 gene (Pasutto 2007) have been reported in patients with an extremely variable clinical phenotype resembling MWS and PDAC syndrome, however considering them as different diseases. In fact, to date STRA6 mutations in the original patient with MWS (Seller 1996) had not been detected. To show that the original MWS phenotype is also caused by mutations in STRA6, we could obtain and sequence the DNA of the first described MWS patient. Indeed, we identified two novel sequence alterations in STRA6 in exons 6 and 15: a heterozygous deletion of two nucleotides, c.343-344delTG, predicting a frameshift with a premature stop codon at position 138 of the protein, p.Cys115PhefsX24; and a heterozygous transition, c.1177C>T, resulting in the generation of a stop codon at position 393 of the protein, Arg393X. The fetus's parents were also tested for both STRA6 mutations and we could confirm that the mutations were occurring in trans, consistent with autosomal recessive inheritance. We excluded the presence of both alleles in a further 270 healthy subjects by sequence analysis of exons 6 and 15. We conclude that mutations in STRA6 are indeed responsible for Matthew-Wood syndrome which shares common clinical features both with PDAC syndrome and with the "syndromic microphthalmia 9" (MCOPS9, OMIM601186), a term later adopted by the Mendelian Inheritance in Man Database. On the basis of their common clinical features we would suggest a detailed reclassification of this phenotype under the original name of Matthew-Wood syndrome.

P-ClinG-108

A new interesting case of mosaic trisomy 9

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Usually complete trisomy 9 results in first trimester spontaneous abortion and is rarely seen in live-born infants. The few known patients showed multiple malformations that resulted in early death. Mosaicism is the only way for long term survival but seems to be a rare occurrence. Here we present a female patient, born from the first pregnancy of a healthy couple in the 37th week. Birth weight was 2670 g. Neonatal examination showed: dolichocephaly, microphthalmia, broad-based nose with bulbous tip, micrognathia, low-set malformed ears, abnormal hands and feet, no other malformations. The initial analysis showed a normal karyotype (46,XX). At 8 months of age the patient showed severe psychomotor retardation and seizures and Array comparative genomic hybridization analysis (ArrayCGH) was requested. The analysis revealed a genomic imbalance of chromosome 9. Fluorescence in situ hybridization (FISH) analysis showed that twelve (8,6%) of 139 cells were trisomic for chromosome 9. Compared to other known patients this level of mosaicism is rather low. This case demonstrates the advantages of ArrayCGH and FISH for the diagnosis of chromosomal mosaicism for trisomy 9.

P-ClinG-109

A girl with X/XX mosaicism, Osteopathia striata with cranial sclerosis and severe intellectual disability caused by a contiguous gene deletion in Xq11.2

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Osteopathia striata with cranial sclerosis (OSCS) is an X-linked bone dysplasia caused by truncating point mutations or deletions of the WTX (FAM123B) gene. Heterozygous female patients with OSCS typically present with short stature, macrocephaly and longitudinal striations of long bones visible on X-rays. Hemizygous males exhibit a much more severe phenotype consisting of craniofacial anomalies, skeletal

defects and heart, gastrointestinal or kidney malformations. Usually theses defects lead to early fetal loss or a poor prognosis in males.

Intellectual disability is not a common sign of OSCS. A disease causing deletion of the WTX gene is found in about 20% of affected females. As has been reported recently, some of these patients show intellectual disability, suggesting an effect of neighboring genes on cognitive development as part of a contiguous gene deletion. We report on a 7-year-old girl with X/XX mosaicism, mild facial features of Turner syndrome, OSCS and profound intellectual disability caused by a de novo contiguous gene deletion of the X-linked genes WTX, ARHGEF9 and MTMR8 detected by routine array CGH.

P-ClinG-110

Heterozygous partial HDAC4 deletion in a family with brachydactyly type E and short stature but normal intelligence

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Deletions of the chromosomal region 2q37 cause the brachydactyly-mental retardation syndrome (BDMR) also known as Albright hereditary osteodystrophy-like syndrome. The condition is characterized by facial dysmorphism and brachydactyly type E (BDE) in association with intellectual disability, behavioural problems, autism or autism spectrum disorders of varying severity, and overweight or obesity. Recently, haploinsufficiency of HDAC4 has been suggested as a critical genetic mechanism, and monogenic mutations were found in two individuals with BDE and intellectual disability. Here we report on a two generation family in which a mother and her two children show BDE in combination with particular facial features (round face, frontal bossing, midface hypoplasia) and short, sturdy stature. Intelligence in all affected individuals is normal. Quantitative PCR analysis in the mother revealed a partial deletion of HDAC4 extending at least from intron 4 to intron 26. Sequences from HDAC4 intron 2 showed normal amplification but the centromeric limit of the deletion remains to be determined. This observation indicates that heterozygous loss of HDAC4 function may be considered as a cause of typical skeletal abnormalities in the absence of intellectual disability.

P-ClinG-111

Syndromal mental retardation in a boy with a maternally inherited interstitial duplication Xp21.3p22.12

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We report on a 12-year-old obese boy presented to us with hypogenitalism, hypothyroidism, mild dysmorphic features, mental retardation, delayed speech development, and early infantile epileptic seizures. He is the second child of healthy unrelated parents and has a phenotypically and normal sister.

In 2000, the at that time 28-year-old mother underwent amniocentesis at the 19th gestational week because of an elevated AFP-MoM at the second trimester biochemical screening. Prenatal ultrasound was normal. The cytogenetic analysis of fetal cells revealed a normal male karyotype 46,XY (GTG-banding, 400 bands per haploid set). The boy was spontaneously born at 39 weeks of gestation. From the early milestones, a global developmental delay was observed and he received special advancement therapies and education.

In 2012, after exclusion of other frequent causes of mental retardation in the proband (Prader-Willi- and Fragile-X syndrome), array-CGH analysis using a CytoChip Oligo 2x105K v1.1 (BlueGnome, Camridge, UK) disclosed an interstitial duplication within the short arm of the X chromosome of about 7,3 Mb (arr Xp22.12p21.3 [20,597,612-27,931,253x2]; ISCN 2009;hg18).

The duplicated region contains 42 HGNC genes, 19 of them listed in the OMIM database. Five of the latter are well-known disease causing genes: SMPX (*300226, #300066), MBTPS2 (*300294, #308205/#308800), SMS (*300105), which causes X-linked mental retardation Snyder-Robinson type (#309583), PHEX (*300550, #307800) and ARX (*300382). Mutations affecting the ARX gene are known to be associated with X-linked Lissencephaly (#300215), agenesis of corpus callosum with or without abnormal genitalia (#300004), early infantile epileptic encephalopathy (#308350), X-linked mental retardation with or without seizures, and Partington X-linked mental retardation syndrome (#309510).

First of all, the duplication within Xp21.3p22.12 was fortified by the results of the Multiplex Ligation-dependent Probe Amplification analysis (MLPA) using the SALSA-P189-B1/2- and -P015-Kit (MRC Holland), which contain ARX gene sequences.

Secondly, repeated conventional karyotyping on a 550-bands-level in the boy (and his mother) confirmed the maternally inherited interstitial Xp duplication: karyotype 46,XY,dup(X)(p21.3p22.12)mat.

In two further steps, we performed MCB-FISH to determine the direction of the duplication and X-inactivation studies to provide more evidence for the disease causing effects of the interstitial Xp-duplication in our proband, but not in his phenotypically normal mother.

Finally, we review the small number of reported cases with interstitial duplication of distal Xp and present the clinical and molecular data of our patient in comparison to the literature.

P-ClinG-112

Deep intronic OCRL mutation analysis in suspected Lowe syndrome patients

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Background: OCRL gene analysis usually comprises all exons with their corresponding splice sites. With the application of this strategy, the causative mutation remains undetected in around 25% of the patients with suspected Lowe syndrome (LS).

Methods: Routine PCR analysis was carried out in 15 patients with suspected LS and RNA analysis with RT-PCR was performed in patients negative for OCRL defects.

Results: Classic mutation analysis revealed exonic or splice site mutations in 11 patients. Three of these defects have not been reported yet and the de novo occurrence could be established in four cases. In the remaining four patients, RT-PCR yielded normal products of exons 1-14. However, amplification of the remaining exons using an OCRL 3'-UTR reverse primer failed in all cases. These findings indicate absence of the normal distal part of the transcript and suggest that LS might here be caused by intronic events leading to an abnormal OCRL mRNA, currently under investigation.

Conclusions: In line with other panels of patients investigated yet, we initially identified the causative OCRL mutation in ~75% of patients with suspected LS. Studies are underway to elucidate the molecular mechanism in the four cases with yet unknown defects.

P-ClinG-113

Allan-Herndon-Dudley syndrome: increased serum triiodothyronine (T3) is a key diagnostic marker

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Allan-Herndon-Dudley syndrome (AHDS) is an X-linked intellectual disability syndrome caused by mutations in SLC16A2 which encodes the monocarboxylate thyroid hormone transporter 8 (MCT8). Clinically, the affected male patients are characterized by severe intellectual disability, initial hypotonia with subsequent spastic paraplegia, dystonic posturing and superimposed paroxysmal dyskinesia. The patients typically have elongated faces, but other dysmorphic features are less consistent. Leukodystrophy and white matter changes are present in early childhood and improve with age. The most specific laboratory finding in AHDS patients is an increase of serum triiodothyronine (T3), while serum thyroxine (T4) is decreased and thyroid-stimulating hormone (TSH) is either normal or mildly increased.

We report on a 17-year old patient with severe intellectual disability and neurological problems in whom increased serum T3 led us to suspect AHDS which was confirmed by the detection of a SLC16A2 mutation (c.812G>A, p.R271H).

P-ClinG-114

Intrauterine dystrophy in a discordant monozygotic twin affected with Beckwith-Wiedemann syndrome due to hypomethylation of KCNQ10T1

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Background. Beckwith-Wiedemann syndrome (BWS) is commonly classified as an overgrowth condition characterized by abdominal wall defect macroglossia and increased prenatal growth, as indicated by the former acronymic term EMG syndrome. While most of BWS subtypes are related to an abnormal epigenetic regulation in a gene cluster region in chromosome 11p15.3, signs and symptoms differ depending on the particular imprint disorder. Frequent findings are changes in the methylation pattern of the KCNQ1OT1 gene. We report on the clinical course of a discordant female monozygotic (MZ) who was born in an even dystrophic state, and discuss the variable phenotype in comparison to two other children with the same genotype.

Case report. Diana G. is the product of the first pregnancy of her mother who was treated for infertility by ART. She was the second born of female twins considered monozygous due to biamnial-monozygotic membranes. On preterm delivery (30th week of gestation) she was dystrophic (birth weight 910g, length 34 cm), with marked macroglossia, frontal nevus flammeus and thickened umbilical cord with a small hernia. Her neonatal period was difficult with impeded respiration due to the large tongue. Hypoglycemia was not observed. At 3 months L-thyroxin support was started for mild hypothyroidism. So far her neurological development has been normal (16 months). As we have the possibility to compare the patient's development to two other children's with the same genetic background we are observing the difference with respect to growth and other clinical features.

Lab investigation. Molecular studies (kindly performed by F.Laccone, Vienna) revealed an abnormal methylation state of the promotor region of the KCNQ1OT1 gene (maternal allele) in 11p15.3 in a mosaic pattern by means of methylation-sensitive MLPA indicating an abnormal function in the imprinting center 2. Monozygosity of the twins was also confirmed by VNTR studies. Analysis of the methylation pattern of the unaffected twin is still pending.

Discussion. BWS is the most common genetic cause of macroglossia which is a nearly constant feature in an otherwise variable clinical picture. Prenatal growth depends on cooperation of several genetic and epigenetic influences. Our case is interesting for the patient's unusual dystrophic newborn state lacking any period of hypoglycemia. However, gaining weight is influenced by epigenetic factors only partially as we have observed normal and even increased growth velocity in two other children with the same genetic background who have shown early dental loss as a very unusual clinical feature. Another interesting point is the variable tumour risk for this particular genotype sparing Wilms tumor in most cases but including other tumours like RMS. Lastly this reports adds to the known phenomenon of discordant female MZ twins with BWS explained by incorrect methylation in early blastogenesis.

P-ClinG-115

Case report: A new point mutation in the androgen receptor (AR) gene causing complete androgen insensitivity syndrome (CAIS) in a 44 year old woman

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Introduction: The Complete Androgen Insensitivity Syndrome (CAIS, alias testicular feminization syndrome) is characterised by a female phenotype in a person with an XY karyotype. CAIS is caused by hormone resistance and results from mutations in the androgen receptor (AR) gene (Xq12). The AR gene mutation database (last updated March 2012) lists 79 different point mutations in exon 1 causing CAIS, mostly due to premature stop codons.

Clinical background: A 44-year-old woman with primary amenorrhoea and muscular dystrophy sought genetic counselling after surgery of a large gonadal tumor (bilateral sertoli cell adenome). Since childhood she had been extremely slender and engaged in sports, so that nobody had wondered why she never had menstrual bleedings. Her pubertal growth and breast development had been appropriate. Aged 35 years, a bilateral inguinal hernia was operated and the surgeon repositioned "macroscopically female gonads" into her abdomen. Aged 36 years, genetic evaluation of her muscular dystrophy revealed 300-500 CTG repeats in the DMPK gene, indicating a diagnosis of adult-onset myotonic dystrophy (DM1).

Genetic studies and findings: Chromosomal analysis revealed a normal male 46,XY karyotype and molecular genetic analysis of the AR gene disclosed in exon 1 a heterozygous c.827delC mutation (predicting p.P276*). The single nucleotide deletion predicted a frame shift followed by 19 incorrect amino acid residues and a premature stop codon.

Conclusion: We report on another case of complete AIS caused by a stop mutation in the AR gene. Studies are pending if the mutation occurred de novo; the patient has two apparently healthy sisters who could possibly be healthy carriers of the mutation. The diagnosis of AIS and a mutation in the AR gene was grossly delayed until age 44 years due to the coincidence of a severe neurogenetic disorder, DM1, in the patient and her personality combining extreme shyness and a secluded lifestyle.

P-ClinG-116

Expanding the spectrum of PTH1R gene mutations in patients with primary failure of tooth eruption

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Primary failure of tooth eruption (PFE) is a rare autosomal-dominant disease characterized by severe lateral open bite as a consequence of incomplete eruption of posterior teeth. Heterozygous mutations in the parathyroid hormone 1 receptor (PTH1R) gene cause PFE likely due to protein haploinsufficiency. So far eight mutations in the PTH1R gene leading to PFR have been described. To further expand the mutational spectrum of PFE-associated mutations, we have analyzed the 14 coding exons of the PTH1R gene and their immediate exon/intron boundaries in 71 index PFE cases. We identified a total of 30 unique sequence variants and classified them into three categories, namely pathogenic, uncertain and benign variants based on their allele frequency, their pathogenicity prediction in several prediction programs and additional evidence of pathogenicity such as familial segregation of variant with disease. Accordingly, twenty of the sequence variants were categorized as pathogenic, of these four were nonsense, five frame-shift, three splice site and eight missense mutations. In three additional missense cases, the prediction of pathogenicity was inconclusive leading to a classification as uncertain variants. The remaining seven variants likely represent very rare, rare or common polymorphisms and should be benign in their effect on disease pathology. Our data increase the number of known unique PTH1R mutations with probably pathogenic effects resulting in PFE from previously 8 to now 26 and provide a basis to further explore the molecular pathomechanisms of these variants.

P-ClinG-117

Database for hereditary neuropathies: experiences of genetic diagnostics based on 1515 index patients

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Since 2001 we offer genetic analyses for patients with hereditary neuropathies, mainly hereditary motor and sensory neuropathy (HMSN), hereditary neuropathy with liability to pressure palsies (HNPP) and a smaller number of patients with hereditary motor neuropathies (HMN) or distal spinal muscular atrophy (dSMA). Over time diagnostic algorithms changed with the identification of new genes. Facing the considerable genetic heterogeneity (2012: more than 40 genes) it is necessary to develop new labor and cost efficient genetic tools.

At the starting point of genetic testing for HMSN electrophysiological characterization to either demyelinating HMSN type I or axonal HMSN type II (or mixed) is important. Reference cut-offs are 38-40 m/s for motor conduction in the median nerve. Patients with HNPP show fluctuating signs of mechanically irritated nerves but also can be indistinguishable from HMSN. In HMN/dSMA there is distal weakness without sensory deficits, electroneurography shows normal results or reduced amplitudes of motor nerve action potentials. We also ask for information about the main clinical features (age at onset, distribution of weakness, additional signs), histology findings, and the family history (further affected relatives, parental consanguinity). For many patients, we received full medical reports mainly from departments who had sent in larger patient numbers.

Following the frequency of gene defects in the different patient populations, we currently offer a stepwise analysis for HMSN I genes (PMP22 duplication, mutations in Cx32, MPZ/P0, PMP22, only rarely in NEFL, LITAF/SIMPLE, EGR2, FGD4 und NDRG1) and for HMSN II genes (mutations in MFN2, Cx32, MPZ/P0, only rarely in NEFL, GDAP1, BSCL2 and GARS). If autosomal recessive HMSN is suspected, the genes GDAP1 und SH3TC2 are analysed, while further candidate genes are screened following linkage analysis in suitable

families. HNPP patients are tested primarily for PMP22 deletion and more rarely for point mutations of PMP22. In HMN we offer an analysis of the only known BSCL2 mutation.

The database includes all patients for whom a genetic report was written over a period of 11 years (2001-2011). Of 1515 index patients, 656 (43%) were classified as HMSN I and 270 (18%) as HMSN II, 108 (7%) showed a mixed HMSN, and no designation to a specific HMSN type was available in 234 (15%) patients. 202 patients (13%) were analysed because of suspicious HNPP, 22 (2%) under the clinical picture of HMN, 23 patients (2%) had various diagnoses.

The genetic detection rate was 52% for HMSN I, 13% for HMSN II, 19% for mixed HMSN, 9% for unknown HMSN, 38% for HNPP and 18% for HMN. Of a total of 417 HMSN families with known gene defects, 266 (64%) were PMP22 duplications, 61(15%) Cx32, 30 (7%) MPZ/P0, 18 (4%) SH3TC2, and 16 (4%) MFN2 mutations, while each of the other genes was responsible for less than 2% of all index patients.

Aims of our study are i) to describe the clinical picture of hereditary neuropathies observed in different gene defects, ii) to define diagnostic algorithms on a large number of patients, and iii) to evaluate our database in respect to a possible extension of data documentation in the national and international context.

P-ClinG-118

Congenital reticular ichthyosiform erythroderma (CRIE) – clinical and genetic characteristics of a 4-year-old girl

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Congenital reticular ichthyosiform erythroderma (CRIE) is an extremely rare autosomal dominant skin disease caused by mutations in the KRT10 gene. Clinically, CRIE is presented by erythroderma, leading to defective skin barrier function, prominent scaling and palmoplantar keratoderma. Because of the development of hundreds to thousands of pale, normal-appearing confetti-like spots starting from childhood the condition is also known as ichthyosis en confetti. Recently, it was shown that the confetti-like spots arise due to independent reversion events in the KRT10 gene.

We present a 4-year-old girl, with congenital erythema, and marked lamellar desquamation on the trunk, extremities and the head. Furthermore, extensive exfoliation on the hands and feet were observed. Otherwise, the patient was considered a healthy child. Clinical examination at the age of 4 years showed severe erythroderma with isolated confetti-like spots of normal-appearing skin on the trunk and face.

Due to the typical phenotypic features of CRIE, we performed the mutation analysis of the KRT10 gene (chromosome 17q21.2, OMIM *148080) and identified a heterozygous intron 6 splice donor site mutation (c.1373+2T>C). To date, all identified mutations in CRIE patients resulted in cDNAs encoding frameshifts that entered the same alternative C-terminal reading frame. Therefore it is supposed, that the splice site mutation c.1373+2T>C of this report leads to the same pathological mechanism.

Our report confirms that CRIE is caused by dominant mutations in the KRT10 gene. Further, this case supports the revertant mosaicism mechanism in patients with CRIE. Revertant mosaicism has also been described for other skin diseases such as epidermolysis bullosa and Kindler syndrome.

P-ClinG-119

Renpenning-like syndrome in two brothers with no mutation in PQBP1 provides evidence for heterogeneity

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Renpenning syndrome is an X-linked recessive disorder characterized by intellectual disability, short stature and leanness relative to other family members. PQBP1 is the only gene known to cause Renpenning syndrome. Recently, it has been recognized that there are several allelic disorders caused by mutations in PQBP1 (including cases of Sutherland-Haan, Golabi-Ito-Hall and Hamel cerebropalatocardiac syndrome). Here we present a Turkish family with two affected sons and a history of three pregnancy losses. The index patients presented with microcephaly, short stature, intellectual disability, microorchidism and a history of psychiatric problems (social phobia, schizophrenia). We noted considerable intrafamilial variability concerning facial dysmorphism, which was more pronounced in the older brother and comprised brachycephaly, a long narrow face, large ears, malar hypoplasia, short philtrum and prognathism. Moreover, he also presented with bilateral hearing loss and congenital heart disease. With the exception of leanness – which was not overt in our patients – the phenotypical presentation fit well to the diagnosis of Renpenning

syndrome, but we did not detect a causative mutation in Sanger sequencing of the PQBP1-gene. We performed whole-exome sequencing in one of the patients and, using stringent filter strategies, we have now identified several highly interesting variants in candidate genes, which are currently under further investigation. The presented family provides evidence for further genetic heterogeneity in Renpenning syndrome and identification of the causative gene in the presented family will give novel insights into the molecular pathogenesis of short stature syndromes associated with intellectual disability.

P-ClinG-120

Familial cerebral cavernous malformations with cutaneous manifestations caused by a novel CCM3 mutation

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Cerebral cavernous vascular malformations occur with a frequency of 1:200 and can cause recurrent headaches, seizures, and hemorrhagic stroke. Cavernous malformations have also been described in other organs such as skin, liver, kidney, and retina. Cutaneous lesions were originally described in 1928 by Kufs et al. and are found in 9% of individuals affected with CCM. Thus far, cutaneous lesions are reported to be phenotypically heterogeneous and have only been described in CCM1 germline mutation carriers. We here report the first CCM3 germline mutation carrier with a positive family history for multiple cerebral and cutaneous cavernous malformations.

The 53 year-old index case presented with painful bluish nodules on his lower legs which he had noticed since his adolescence. Similar lesions were seen all over his body, except the mucosa, and histologically classified as angiokeratoma. In addition, he reported headaches since fifteen years. Subsequently, MRT showed multiple infra- and supratentorial cavernous malformations, one with subacute bleeding. Direct sequencing of the CCM1, CCM2 and CCM3 genes revealed a novel mutation in exon 5 of the CCM3 gene, c.113delT, resulting in a frameshift (p.Leu38Argfs*7). Thus far, therapy consisted of surgical excisions of cutaneous lesions. Cryotherapy appeared to be as effective as lasertherapy.

P-ClinG-121

UPD is rare in patients with intellectual disability: analysis of 350 parent-patient-trios using UPDtool

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Uniparental disomy (UPD) describes the inheritance of a pair of chromosomes from only one parent. It may occur as isodisomy (two copies of one parental homolog), heterodisomy (both homologs of one parent) or a combination of both. UPD may involve a complete chromosome or only chromosome segments. Some parts of chromosomes are subject to „parent-of-origin imprinting“ and the phenotypic effect in UPD syndromes is mainly due to the functional imbalance of imprinted genes. Furthermore, isodisomy can be the cause of mutation homozygosity in autosomal recessive inherited diseases.

UPD is the basis of a small number of well-defined clinical conditions associated with intellectual disability (ID). However, there is little knowledge on the occurrence of UPD in the group of patients with unexplained syndromic or non-syndromic ID mainly due to the lack of qualified screening techniques.

We have applied our newly developed UPDtool - a computational tool for detection and classification of UPD in trio SNP-microarray experiments – onto a cohort of 350 parent-patient-trios with unexplained ID. All patients were initially analysed for pathogenic copy number variants using Mapping 6.0 SNP arrays (Affymetrix). Applying standard analysis parameters (stretches with 300 mendelian errors (ME), ME density > 1 % / kb) no evidence for non-mosaic UPD, including both iso- and heterodisomic segmental UPDs, could be found. Therefore we suspect UPD to be a rare cause of intellectual disability. The project is on-going with altered algorithms for an in-depth-analysis of segmental UPDs and expansion of the patient cohort.

P-ClinG-122

Mutations in the TSPYL1 gene are not associated with sudden infant death syndrome in a Swiss cohort of deceased infants

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Background: Sudden infant death syndrome (SIDS) is currently the major cause of an unexpected and unexplained death of infants in the first year of lifetime in industrialized countries. Besides environmental factors also genetic factors, such as mutations in mitochondrial genes or mutations in the long QT syndrome genes SCN5A, KCNQ1 and CAV3 may play a role as a risk factor for SIDS. Notably, a frameshift mutation (c.457dupG, p.Glu153Glyfs*17) in the TSPYL1 gene has been reported to be causative for an autosomal recessive sudden infant death with dysgenesis of the testes syndrome (SIDDT) in an Old Order Amish community in Pennsylvania. Because the Amish community was originally founded in the German speaking part of Switzerland, including people from Alsace and Palatinate, a mutation analysis of the entire TSPYL1 gene was performed in a cohort of 145 SIDS cases originating from the Swiss population around Zurich. The TSPYL1 gene of all DNA samples was amplified and the PCR products sequenced using an ABI PRISM® 3130xl Genetic Analyzer.

Eight known SNP variants (rs61746509, rs3828743, rs3749895, rs61746508, rs56100880, rs3749894, rs45490498 and rs9400897) were detected in the analyzed SIDS cohort, none of which was significantly associated with SIDS. All detected SNPs in the SIDS group were in Hardy-Weinberg equilibrium. In addition, one affected infant was heterozygous for the rare variant rs150144081 (c.718C>T, p.Leu240Leu), and two children were heterozygous for the missense mutation rs140756663 (c.1098C>A, p.Phe366 Leu) in the TSPYL1 gene. In silico analyses predicted a non-pathogenic effect for p.Leu240Leu and pPhe388Leu, respectively, although protein features might be affected by p.Phe366Leu. This heterozygous exchange was previously identified in one out of 126 selected German SIDS cases and in one out of 104 Caucasian infertile men with OAT syndrome. The founder nonsense mutation c.457dupG (p.Glu153Glyfs*17) was not detected in our analyzed SIDS cohort.

Conclusions: Mutations in the TSPYL1 gene are not associated with SIDS in Swiss infants.

P-ClinG-123

Expanded mutational spectrum of SRCAP in patients affected with Floating-Harbor syndrome

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Floating-Harbor syndrome (FHS) is a rare genetic disorder characterized by short stature, delayed bone age, retarded speech and intellectual development as well as dysmorphic facial features. Recently mutations strictly located in exon 34 of the Snf2-related CREBBP activator protein (SRCAP) gene, encoding the core catalytic component of the multiprotein chromatin-remodeling SRCAP complex, were found to cause FHS. SRCAP locates to chromosome 16p11.2, comprises 34 exons and encodes 3230 amino acids.

Here we report 5 patients fulfilling the diagnostic criteria of FHS. All of them presented with short stature, speech delay as well as psychomotor delay and typical facial dysmorphism including broad nasal bridge, prominent columella and short philtrum. Similar to previous findings, two patients demonstrate heterozygous de novo frameshift mutations in exon 34 (c.7395delC and c.7218dupT) leading to premature stop mutations in SRCAP (Pro2465GlnfsX10 and Gln2407SerfsX36, respectively). Interestingly, in one patient, Sanger sequencing identified a stop mutation in exon 33 (c.6985C>T, Arg2329X) clearly demonstrating that not all FHS cases are caused by mutations in exon 34 of SRCAP. Our data confirm a mutational hot spot in the final exons of SRCAP in the majority of FHS patients.

Although no major clinical differences were observed in this series supporting clinical homogeneity, absence of SRCAP mutations in exons 33 and 34 in two further patients could support genetic heterogeneity. Further DNA analysis will clarify whether FHS could also be caused by mutations in other parts of SRCAP or in other genes.

P-ClinG-124

Compound heterozygosity for a paternally inherited deletion of the TUSC3 gene and a stop mutation in the maternal allele is associated with autosomal recessive mental retardation.

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We report on an 4 1/2 year old boy who presented with a general psychomotor retardation including a severe deficiency of active speech. He is the first child of healthy, non-consanguineous parents. The parents orginate from Kasachstan and the father has asian roots. Besides mild unspecific craniofacial dysmorphism (epicanthic folds, low set ears) and a borderline microcephaly (3rd centile), no major malformations were present. Ultrasound and MRI of the brain did not show any structural defects.

Conventional karyotyping revealed no numeric abnormalities (46,XY) but the resolution of 450 bands (GTG banding) did not allow a sufficient structural analysis. Array-CGH analysis uncovered a small heterozygous deletion of 51.5 kb on chromosome 8p22 comprising the TUSC3 gene. Quantitative PCR analysis of both parents also showed a deletion in the father. Homozygous deletions of the TUSC3 gene have already been described as a possible cause of autosomal recessive mental retardation (Garshasbi et al 2008). A homozygous nonsense mutation in this gene has also been described in a consanguinoeus family. We therefore hypothesized, that the retarded boy might have a point mutation in the second allele of the TUSC3 gene. Indeed sequence analysis showed the mutation c.992C>A in the boy and his mother. This mutation leads to a premature stop in codon 331 (p.Ser331X). The mutation is localized in the region of chromosome 8 which is deleted on the paternal allele, so that the boy is hemizygous for the mutation. Since inactivating mutations in both alleles of the TUSC3 gene have already been described in patients with autosomal recessive non-syndromic mental retardation, we conclude that the alteration is causative for the mental retardation in our propositus.

This case illustrates, that the inheritance of a small deletion from a healthy parent does not exclude pathogenicity. It is indispensable to look for candidate genes in this region and to search for mutations which may lead to an inactivation of the second allele.

P-ClinG-125

ARSACS: expanding the genetic, clinical and imaging spectrum

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Mutations in SACS, leading to autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), have been identified as a frequent cause of recessive early-onset ataxia around the world. Here we aimed to expand the spectrum of ARSACS mutations outside Quebec, establish the pathogenicity of novel variants, and further explore the clinical and imaging phenotype.

We identified 10 index patients from across Europe, harbouring 18 novel ARSACS variants. 9/10 patients harboured two variants of at least probable pathogenicity (≥UV4) which were located in highly conserved domains and not observed in 1750 European control chromosomes. These 9 patients accounted for 11% (9/82) in our series of unexplained early onset ataxia subjects. The presenting phenotype will be described showing that each feature of the classical ARSACS triad (ataxia, spasticity and peripheral neuropathy) might in fact be missing in ARSACS. On the other hand, our imaging findings extend the range of diagnostic features – which also extend to supratentorial regions and involve the cerebral cortex – and will help to establish the diagnosis in most cases.

P-ClinG-126

IGF1R mutation analysis in short children with Silver-Russell syndrome features

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The insulin-like growth factor 1 receptor (IGF1R) is a key factor in intrauterine and postnatal growth by mediating the biological function of IGF-I. Mutations of the IGF1R gene are usually associated with growth retardation but the clinical picture of IGF1R mutation carriers is heterogeneous. Indeed, these patients show clinical signs compatible with Silver-Russell syndrome (SRS), and some IGF1R mutation carriers have been identified in SRS cohorts. We therefore investigated DNA samples of 19 growth retarded patients with SRS features. Apart from 8 apathogenic variants, we detected heterozygosity for the unknown duplication, c.1056_1057dup, leading to a premature termination in one patient and his growth retarded sister. Due to its nature, we assumed that this variant is probably pathogenic. However, the patient and his sister exhibit a spontaneous catch-up growth in later life. We therefore delineate that the c.1056_1057dup does not result in a disruption of the GH-IGFI axis. Thus, this IGF1R mutation without obvious clinical consequences for growth challenges the actual concept of IGF1R haploinsufficiency as a general cause for disturbed growth in IGF1R mutation carriers. Mutation analysis in IGF1R should only be considered in growth retarded patients with minor SRS features, but not in probands with the characteristic SRS phenotype.

P-ClinG-127

Application of the next generation sequencing technique 454 (GS Junior, Roche) to diagnostic sequence analysis of the VWF gene. Evaluation and development of protocols.

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Objectives. We designed and validated a 454 sequencing (NGS) assay for mutation detection in patients with suspected Von Wilebrand diesease (VWD) to improve diagnosis and classification.

Methods. A multiplex- based NGS- and a conventional amplicon- sequencing approach for the 51 coding exons, exon 1 and approximately 1500 base pairs of the promoter region of the VWF gene was established. MLPA (MRC Holland) was used to check for gross deletions of the VWF gene. The blood group O allele c.261delG was analysed by a pyrosequencing assay and laboratory specific reference ranges for routine VWD assays were determined for G/- and -/- individuals.

Results. 17 biochemically pre-characterized patients were subjected to molecular genetic testing. NGS results were in agreement with conventional sequencing results for all detected variants (241 heterozygous single base exchanges, 1 heterozygous small deletion, 50 homozygous variants). In patients 7 VWD causing mutations were detected (2 novel, 1 type 1, 1 2B, 2 2N and 1 “unclassified” mutation). Moderately decreased VWF levels in 5 patients were sufficiently explained by blood group O. No mutation was detected in 3 patients with borderline and in 2 patients with significantly pathologic functional VWD analyses.

Conclusion. NGS is a reliable, convenient and cost efficient alternative to conventional sequencing of VWF in routine laboratories. c.261delG genotyping and the application of adapted reference ranges to functional assays prior to genetic VWF testing reduces the sequencing load significantly.

P-ClinG-128

Familial 12q12 microdeletion suggests ARID2 as a novel gene for Coffin-Siris syndrome

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Coffin-Siris syndrome is a rare disorder characterized by intellectual disability, short stature, course facial features with bushy eyebrows, full lips, hypertrichosis, sparse scalp hair and hypoplastic 5th finger and toe nails. Very recently genetic heterogeneity was shown with dominant de novo mutations in one of six SWI/SNF subunit genes, including SMARCB1, SMARCA4, SMARCA2, SMARCE1, ARID1A and ARID1B accounting for 87% of patients. We now identified a novel 990 kb microdeletion including another SWI/SNF

subunit gene, ARID2, in a boy and his mother with some features of Coffin-Siris syndrome. Our proband is the first child of non-consanguineous parents aged 23-years (mother) and 22 years (father) at delivery. He was born at term (40+5) by caesarean section with a weight of 2420g (<P3), a length of 47 cm (<P3) and an OFC of 34 cm (P10) indicating intrauterine growth retardation. Apgar scores were 7/9/10. At birth he displayed micrognathia, wide sagittal sutures, convergent strabismus and bilateral inguinal hernias. Fingernails were hypoplastic and hyperconvex and the fifth toenails were nearly absent bilaterally. He walked independently at 18 months. At age 3 9/12 years he showed global developmental delay and his language was limited to a few simple words. He showed short stature (P<3; -4.15 SDS) with a relatively large head circumference (P10-25) and weight (<P3; BMI 16) and distinctive facial features such as sparse hair, high forehead, hypoplasia of the supraorbital ridge, deep-set eyes, short nose with anteverted nostrils, wide mouth with a full cupid bowed upper lip and a slightly everted lower lip, short philtrum, and low-set posteriorly rotated ears. At the age of 6 years facial gestalt appeared more course with fair but thick eyebrows and bulbous nasal tip with broad columella. While the father appeared normal, the mother also showed mild short stature and microcephaly, mild intellectual disability, and similar minor anomalies as seen in her son: low-set posteriorly rotated ears, short philtrum, full upper and lower lips and nearly absent fifth toenails bilaterally.

Our findings therefore suggest that ARID2 haploinsufficiency is a further cause of the Coffin-Siris syndrome clinical spectrum.

P-ClinG-129

Clinical Characterisation of a novel Mutation in FBN1

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Mutations in the FBN1 gene (OMIM *134797) are known to result in the clinically well recognisable Marfan syndrome spectrum (OMIM #154700). We report on the novel FBN1 mutation c.1838A>G (p.Asp613Gly) which results in a substitution of the highly conserved amino acid aspartic acid to glycine at position 613. Based on the calculated functional predictions (SIFT: not tolerated, AGVGD: C65, Polyphen-2: 0.98) we expect that this mutation has a deleterious effect on the fibrillin 1 protein. We detected this mutation in a 10-year-old boy. He was first evaluated at the age of 2 years presenting with statomotor and psychomotor retardation. Subsequently he developed mental and language impairment, in addition to severe behavioural abnormalities. We saw him at the age of 10 years with a slender build, long extremities, aortic dilatation and distinct facial features (downslanting palpebral fissures, retrognathia, high palate) suggesting Marfan syndrome. The same mutation was found in the boy's 38-year-old mother. Aside from similar facial features she did not present any skeletal, cardiologic or ocular signs of Marfan syndrome or developmental abnormalities. We conclude that the FBN1 mutation c.1838A>G (p.Asp613Gly) does not always result in a typical Marfan phenotype according to the Ghent nosology. This observation emphasizes the role of genetic counselling and testing of all first degree family members, as even seemingly unaffected family members might be mutation carriers and at risk to develop life-threatening aortic dissections.

P-ClinG-130

Autosomal Recessive Adams-Oliver Syndrome (AOS2): Novel DOCK6 Mutations and Delineation of the Phenotype

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Adams-Oliver syndrome is a hereditary disorder characterized by aplasia cutis congenita (ACC) and terminal transverse limb defects (TTLD). To date, two genes have been identified for the autosomal dominant form of AOS, namely ARHGAP31 (AOS1 #100300) and RBPJ (AOS3 #614814). The autosomal recessive type can be caused by mutations in DOCK6 (AOS2 #614912). Nevertheless, many cases of AOS still remain unsolved.

Two homozygous truncating mutations of the DOCK6 gene (*614194) have previously been described in two unrelated consanguineous families with autosomal recessive AOS (p.Asp416* and p.Thr455Serfs*24). We analysed this gene by direct sequencing in a cohort of 25 patients including 12 index cases from families with evidence of autosomal recessive inheritance as well as 13 sporadic cases. We identified a homozygous

frameshift mutation in a patient born to consanguineous parents and compound heterozygosity for a frameshift and splice site mutation in another sporadic case, thus confirming AOS2 in these patients. Additionally one patient was found to harbour a homozygous missense variant. In a family with two children affected by AOS a heterozygous nonsense mutation was found, while a mutation on the second allele remained undetected. The phenotype of DOCK6-mutated patients in or cohort was strikingly similar to the one of affected individuals published by Shaheen et al. (2011). All patients had TTLD and, except for one patient, they all presented with ACC of the scalp, the clinical hallmarks of AOS. Notably and in contrast to patients with AOS1 and AOS3, individuals carrying mutations in the DOCK6 gene consistently exhibited additional abnormalities affecting the central nervous system (developmental delay, seizures/convulsions, speech delay) and the eyes (poor vision, optic atrophy, microphthalmia, retinal detachment).

We conclude that DOCK6 mutations account for a considerable proportion of cases with AOS and a family history suggesting autosomal recessive inheritance but can also be found occasionally in sporadic cases. Central nervous system and ocular involvement appears to be a frequent sign of AOS2 and a possible clinical differentiator.

P-ClinG-131

The Phenotypic Spectrum Of Duplication 12p11.21-12p13.31

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Background: Pure trisomy 12p is a rare chromosome aberration. The phenotype of complete trisomy 12p consists of severe intellectual disability (ID), muscular hypotonia, increased birth weight, and unusual facial features including a round, flat face with prominent cheeks, epicanthal folds, flat wide nasal bridge, and upturned tip of the (short) nose. Frequent findings are also a high forehead, macrocephaly, and a broad everted lower lip. Pure partial trisomy 12p has been reported in 13 patients so far. The facial features correspond to those of complete trisomy 12p but ID was moderate in most cases.

Clinical report: Here we present a girl with a de novo duplication of the chromosomal region 12p11.21-p13.31. The girl was born at term with normal birth measurements after an uneventful pregnancy. She presented with developmental delay and muscular hypotonia from the beginning. At the age of 14 months, she had short stature, microcephaly, marked muscular hypotonia and was not able to sit without support. Facial dysmorphism consisted of a high forehead, short palpebral fissures, bilateral epicanthal folds, a short nose with anteverted nostrils, full cheeks, micrognathia, and full, prominent lips. She had no major malformations. Chromosome analysis in the girl and her parents revealed a de novo duplication of chromosome region 12p11.21-p13. This duplication could be confirmed and narrowed down to 22 Mb in 12p11.21-p13.31 by microarray analysis using Affymetrix® CytoScan HD array.

Discussion: The facial features of this girl are consistent with the clinical presentation of 12p duplication cases described in the literature. Most of the reported partial duplications affected the terminal region of 12p with the exception of one familial case with a more proximal duplication and a patient presented by De Gregori et al. (2005) with a similar duplication as the present case. Both patients show facial features of 12p duplication but microcephaly, which is in contrast to the macrocephaly of 12p duplication syndrome. Genes responsible for macrocephaly may therefore possibly be localized proximal to 12p11.21. Reviewing all partial and complete duplications 12p so far, the SRO for the facial phenotype can be reduced to 12p13.2-p13.31.

P-ClinG-132

17p13.1 microduplications are associated with intellectual disability, obesity and mild dysmorphic features

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Only few cases of patients with a 17p13.1 microduplication have been reported so far. Here we describe a further de novo 484 kb microduplication of chromosomal band 17p13.1 encompassing 38 genes in an 8-year-old boy with intellectual disability, attention deficit, pathologic electroencephalogram, mild dysmorphic features, reduced feeling of satiety and overweight. Conventional cytogenetic analysis revealed an apparently normal male karyotype. FMR1 gene analysis and methylation test of the SNRPN locus were normal.

Our case resembles a 15-year-old boy with a 790-830 kb microduplication in 17p13.1 who has been reported to show metabolic and endocrine anomalies leading to mild obesity, metabolic syndrome and hypothyroidism. Further clinical symptoms included moderate mental retardation and recurrent afebrile seizures with aspecific anomalies on EEG.

Obesity and neonatal seizures are features that have previously been described in the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) in another case with a 260 kb duplication in 17p13.1, so possible common genetic mechanisms for these pathologies could be of interest. In contrast to these cases, several patients with a 17p13.1 microdeletion have been reported with poor growth.

P-ClinG-133

A new mutation in the PTEN gene in a three years old boy with Bannayan-Riley-Ruvalcaba syndrome boy with macrocephaly and increased birth length

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The preceding pregnancy was achieved by in-vitro-fertilization and was uneventful. The delivery was by vacuum-assisted extraction. The birth weight was 4005 g (+1SD), birth length 57 cm (+3.5 SD) and head circumference 39 cm (+3SD). The boy was breastfed for six months. The milestones such as head control, sitting and crawling were achieved timely. At the age of 18 months, he began to speak first words and was able to follow small orders. Dental development was normal. He had no epilepsy nor EEG abnormalities. He learned to walk at the age 21 months but a global developmental delay with a regression of language was observed (he lost skills like to follow orders or use words already learned) and he developed autism.

The parents were not consanguineous. His father and paternal grandfather also present with macrocephaly. Cytogenetic analysis, Array-CGH, testing for Sotos- and Beckwith-Wiedemann syndrome revealed unremarkable findings. In clinical examination the child showed a macrocephalus (head circumference 54.5 cm (+3SD)) with normal height 89 cm (0SD) and weight 15 kg (+1SD). Further findings are a cafe-au-lait spot and a hemangioma in the neck area, facial dysmorphisms like prominent forehead, sloping palpebral fissures, deep-set eyes and a muscular hypotonia. These symptoms lead us to the clinical diagnosis of Bannayan-Riley-Ruvalcaba syndrome (BRRS). The moleculargenetic analysis of the PTEN gene revealed a novel mutation c.228T>A (p.Tyr76X) in exon 4, creating a premature stop codon. 65% of individuals with a clinical diagnosis of BRRS have a detectable PTEN mutation. Patients with BRRS are in risk for developing multiple lipomas and hamartomatous polyps. A tumor prevention is indicated in these cases.

P-ClinG-134

Epigenetic changes in different lung diseases: towards molecular diagnosis

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Diagnosis of severe lung disease like chronic obstructive pulmonary disease (COPD), Idiopathic pulmonary disease (IIP) and lung cancer remain difficult and mostly invasive. High resolution computed tomography (HRCT), which is capable of detecting IIP and lung cancers, is mostly followed by biopsy for final diagnosis, but differential diagnosis remains challenging. The situation may be impoved by the introduction of molecular markers for differential diagnosis. For this purpose we assed the methylation pattern of patients with various lung diseases using Illumina´s Infinium HD assay for Methylation 450k. Snap frozen tissue material from IIP patients of different grade (n=38), COPD patients of different GOLD stages (n=40) and lung cancer patients consisting of patients with adenocarcinomas or Squamous-cell carcinoma (n=18) and concordant normal controls for each patient group were assessed for their methylation changes on genome wide scale. A clear differentiation between the patient groups and normal controls could be achieved applying various uni- and multivariate analysis and classification algorithms for feature-selection. Further pathway and network analysis for the methylation changes in every subgroup will be presented. Subsequently validation of promising markers were performed on Fluidigm´s Biomark, a high throughput qPCR device, allowing 9216 parallel qPCR reactions per run. qPCR analysis allowed a correct classification of more than 90% of all tested samples to their specific lung disease or the un-diseased status of their lung.

P-ClinG-135

An unusual severe case of Sotos syndrome

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Sotos syndrome is an autosomal-dominant disorder, first described in 1964 by Juan F. Sotos. It is caused by haploinsufficiency of the NSD1-gene and characterized by a distinct facial gestalt, macrocephaly (> 2 SDS), overgrowth and mostly by mild to moderate, but variable intellectual disability. It is associated with several further anomalies like cardiac defects, brain malformations, neonatal jaundice, renal and skeletal anomalies, and many more.

We present a 3 years and 9 months old girl with severe global retardation and multiple anomalies. She was born prematurely at 36 weeks after an uneventful pregnancy. The girl developed severe hypoglycemic episodes, seizures, jaundice and required neonatal intensive care medicine. Furthermore the cranial ultrasound showed a small corpus callosum, later on a central discoordination was diagnosed.

She developed a gastroesophageal reflux and required a gastric tube because of feeding problems. Extensive metabolic screening tests and a muscle biopsy were normal.

At presentation at the age of 3 years and 9 months she showed a most severe delay in her motoric skills without any head control and unability to sit or stand. She had no speech, either. Her head circumference was above the 97th centile, her length between the 50th and 75th centile, and her weight on the 50th centile. She showed some facial features of Sotos syndrome like a high bossed forehead, a long, triangular shaped face with a pointed chin, a hypertelorism, downslanting palpebral fissures, and hypoplastic teeth. Prompted by this, sequencing of the NSD1-gene was performed and revealed a heterozygous de novo truncating mutation in Exon 5 (c.2530_2537delGAGAAAACinsTGTT; pGlu844Cysfs*8). This mutation has not been described before but leads to a frameshift, which is one of the most common mutation types in NSD1-gene.

The clinical symptoms leading to the diagnosis of Sotos syndrome in this patient were the large head circumference and the distinct facial gestalt. Other clinical aspects of our patient such as the hypoplastic corpus callosum and seizures are in accordance with Sotos syndrome as well. However, though severe intellectual disability in Sotos syndrome is reported in a small fraction of patients, the severe motor disability in our patient with poor head control at the age of 3 years and 9 months as well as lacking further motor and speech development at that age is rather unusual, as most of the patients are reported to have delayed motor milestones and mild to moderate ID.

This case therefore illustrates impressively the wide phenotypic spectrum of Sotos syndrome.

P-ClinG-136

Mutations in C12orf65 cause autosomal recessive intellectual disability and show a distinct genotype phenotype correlation

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We undertook autozygosity mapping in a large family with autosomal recessive syndromic intellectual disability and multiple consanguineous marriages. A sibling pair (a 29 years old female and a 25 years old male) and their 36 years old maternal aunt presented with mild intellectual disability, arthrogryposis of small joints and malposition and weakness of feet with suspected paraplegia, recognizable facial anomalies (low set eyebrows, mild hypertelorism, broad nasal bridge, thin upper lip, and round face) and a pleasant and satisfied character. Growth parameters were unremarkable and head circumferences were in the lower norm. We undertook a genome-wide autozygosity mapping and identified eight candidate loci with a total length of 91Mb. After exome enrichment with Agilent SureSelect Kit 50 Mb we performed paired-end large scale sequencing of one of the index patients on a SOLiD 5500xl platform. Stringent filtering including in silico analysis and segregation tests, led to the identification of only one novel variant in C12orf65; p.Q139X, which probably leads to a prematurely truncated protein within the Pfam domain. C12orf65 encodes a protein that is critical for the release of newly synthesized proteins from mitochondrial ribosomes.

Antonicka et al. (AJHG, 2010) already reported two homozygous mutations in C12orf65, c.210delA and c.248delT, in two independent families. Both mutations lead to the same prematurely truncated protein of 84 amino acids (AA) instead of the full length protein of 166 AA. This stop mutation lies within the important Pfam domain that extends between AA positions 54 to 146. Reported patients have severe encephalomyopathy with mitochondrial translation defect, regression of cognition and mobility in early childhood, nystagmus, optic atrophy in childhood or adolescence, ophtalmoplegia, respiratory insufficiency

and early death. The symptoms of our patients are significantly milder in comparison to the reported patients. We suggest that mutations in C12orf65 may cause a syndromic form of intellectual disability and postulate a variable degree of severity in dependence on the position of the mutation. In the severely affected patients the mutations lead to an early truncation of the protein in the middle of the Pfam domain. In mildly affected patients the truncation is at the end of the Pfam domain. This hypothesis has been very recently substantiated by the study of Shimazaki et al. (J Med Genet, Dec 2012) who reported a truncating mutation at AA 132 in a patient with also a milder phenotype.

In summary, we confirmed C12orf65 as a further intellectual disability gene in the oxidative phosphorylation system and found evidence for a distinct phenotype genotype correlation. These results also show the efficiency of next generation sequencing to overcome the difficulties originating from variable spectrums of phenotypes.

P-COMPLEX GENETICS / COMPLEX DISEASES

P-Compl-137

Association study and differential gene expression of Dupuytren's disease

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Dupuytren's disease (DD) is a multifactorial fibromatosis that causes progressive and permanent contracture of the palmar fascia with subsequent flexion contracture of the fingers. A strong genetic predisposition exists but little is known about the epidemiology and the molecular aetiology and pathogenesis of the disease. It is the most frequent genetic disorder of connective tissue with a prevalence of about 3% in Germany and up to 40% in parts of Scandinavia.

We have collected clinical data and samples from over 800 DD patients and evaluated the importance of different risk factors. We found that a genetic predisposition had the strongest influence on the age at the first surgical treatment compared to environmental factors. Patients with a positive family history were on average 5.2 years younger than patients without known family history (P 2.2x10-08). The percentage of familial cases decreased with age from 55% in the group of 40-49 years old patients to 17% in patients aged 80 years or older. Manifestation in patients with and without family history differed in regard to other risk factors, namely diabetes, smoking and occupational exposure.

We have performed a genome wide association study (GWAS) with 565 unrelated DD patients and 1,219 controls. Data for 5,204,451 single-nucleotide polymorphisms (SNPs; 186 cases genotyped with Affymetrix Genome-Wide Human SNP Array 6.0 and 379 cases, 1,219 controls genotyped with Axiom CEU 1 Array; data imputed with HapMap CEU reference panel) were analyzed for association with DD. SNP rs2290221 on chromosome 7p14 showed the strongest association signal with a P-value of 2.2x10-10 and odds ratio of 2.13. It is located intronic of the genes for secreted frizzled-related protein 4 (SFRP4) and ependymin related protein 1 (zebrafish) (EPDR1). Moreover, we identified SNPs in four further regions with significant association with DD.

Consistent with these and other GWAS findings, a whole genome expression analysis with primary disease tissue samples from from 12 DD patients and 12 normal fascia controls revealed upregulation of the Wnt/β-catenin signalling pathway and also changes in proteins important in mitochondrial function and oxidative stress response in the disease tissue. The Wnt signalling pathway is therefore likely to be a key player in the susceptibility to fibromatosis as observed in DD. Primary disease tissue derived fibroblasts retained their disease associated characteristics at least in part in vitro, they exhibited higher proliferation rates and generated strong contraction forces in 3-D collagen gels, and thus present an excellent model for investigating the mechanisms of DD in the context of aging and aging associated diseases.

P-Compl-138

Replication of genome-wide significant susceptibility factors for nonsyndromic cleft lip with or without cleft palate in a European population: Support for 1p36, 1p22.1 and 20q12

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Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is one of the most frequent congenital defects. The prevalence rate in the European population is estimated to be about 1 in 1000. The etiology is complex, with both environmental and genetic factors involved in the development of this malformation. Research into NSCL/P has seen major advances by the application of genome-wide association studies, given that there are six genome-wide significant loci with comparably large effect sizes which were identified by GWAS using relatively small sample sizes. Recently, meta-analyses of the two largest NSCL/P datasets identified six additional genome-wide significant susceptibility loci. Based on these data, the number of conclusively identified risk loci for NSCL/P has now increased to twelve. Six of these twelve risk loci have previously been confirmed by successful replication in an independent large European NSCL/P triad sample (Mangold et al. 2010).

The present study aimed at replicating the hitherto unreplicated risk loci (1p22.1, 1p36, 3p11.1, 8q21.3, 15q22.2 and 20q12) in the same, family-based sample of European descent (715 NSCL/P nuclear triads). The top associated SNPs at each locus were genotyped by either use of multiplex mass spectrometry or a single marker genotyping assay. We successfully replicated the NSCL/P association for three of the six analyzed risk loci and thus confirmed the strong association of 1p22.1, 1p36 and 20q12. The strongest association was observed for rs742071 at the 1p36 locus (Pcorrected=3.67x10-5). The rs742071 SNP is located in an intron in the PAX7 gene which encodes paired box 7, a member of the paired box (PAX) family of transcription factors. Interestingly, PAX7 has already been functionally implicated in craniofacial development (Mansouri et al. 1996).

Non-replication of the three susceptibility loci 3p11.1, 8q21.3 and 15q22.2 might be explained by insufficient sample power, technical issues or sample characteristics respectively.

P-Compl-139

Allelespecific expressionanalysis of putative members of a network of imprinted genes in human

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Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are rare developmental disorders presenting with marked intrauterine and postnatal overgrowth (BWS) or growth retardation (SRS). The heterogeneous associated molecular defects are mainly due to epigenetic changes in imprinting control regions (ICR). ICR1 regulates the monoallelic expression of IGF2 and H19 in cis. A subgroup of SRS and BWS patients present with epigenetic alterations of other chromosomal regions, arguing for a functional dependence of the affected imprinted genes. To date the molecular mechanisms of the ICR1 effects are incompletely defined and their analysis often has to be performed using genetically engineered model organisms. The description of a network of imprinted genes (IGN) in mice gives a new starting point to decode multilocus imprinting defects in humans and depicts candidate genes possibly involved in associated clinical symptoms.

We analyzed unique fibroblasts with paternal (BWS) or maternal (SRS) uniparental disomy 11p15 which provided us with an unusual mechanistic insight into ICR1-driven regulation of gene expression in human cells. We focused on imprinted genes on different chromosomes. In keeping with findings in murine orthologues these genes may be part of an IGN involved in regulating developmental growth in human.

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P-Compl-140

First genome-wide analysis of copy number variants in alopecia areata

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Alopecia areata (AA; MIM 104000) is a hair loss disorder with a lifetime risk of 1-2% and a risk of recurrence for first degree relatives of 6-7%. It is one of the most frequently occurring autoimmune disorders and the second most common reason for hair loss. Both sexes are affected equally and it can occur at any age, with an increased prevalence in the 2nd and 3rd decade. The trait is characterized by sudden onset and an unpredictable, often recurrent course. Total remission as well as chronic ongoing conditions can be observed. Overall, the psychological strain for patients is high, especially because treatment options to date are still unsatisfactory.

There is general agreement that the genetic basis of AA is multifactorial. The underlying pathomechanism is a T-Cell mediated autoimmune response. The genetics of AA is not yet fully understood, however about ten susceptibility loci are known which are mostly involved in autoimmune processes.

Over the last ten years we have collected the largest sample of AA patients worldwide in collaboration with several medical centers in Germany and Belgium. In the current project we want to determine the effect of copy number variants (CNVs) on AA. Although there are no existing CNV studies for this trait so far, previous findings underline the influence of CNVs for other autoimmune disorders.

We are currently analyzing a sample based on SNP-array data from 750 cases and 1300 matching controls genotyped on the Illumina OmniExpress-Chip. Cases and controls are all of Central European descent. For the project, we employ a well established CNV analysis pipeline in our lab. CNV calls are calculated on the Hidden-Markov-based programs QuantiSNP and PennCNV. We currently utilize three approaches to analysis: a gene-specific analysis for a set of candidate genes, a genome-wide burden analysis and a genome-wide association analysis. Verification will be done by qPCR. Although the analysis is still ongoing, early results for the CCL3L1 gene, which is involved in immunoregulation, were promising. Further results will be presented at the conference.

P-Compl-141

Expression analysis of miRNA 185, located in 22q11.2 microdeletion region, and mutational analysis in schizophrenia

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Schizophrenia is a complex neuropsychiatric disorder with a lifetime prevalence of about 1% and a high heritability of up to 80%. The disease is characterized by positive symptoms such as hallucinations or delusions, negative symptoms as reduced emotions or interest and cognitive deficits. The 22q11.2 deletion

syndrome (22q11.2DS), also known as velocardiofacial/DiGeorge syndrome is caused by a hemizygous deletion at chromosome 22q11.2. It occurs approximately 1 in 2,000 - 4,000 births and about 30% of carriers develop schizophrenia, making this syndrome one of the strongest known genetic risk factors for schizophrenia (Odds ratio=21.6-∞). Symptoms of 22q11.2DS-related schizophrenia are largely indistinguishable from those of the idiopathic disease suggesting that the investigation of deletion-related forms may reveal further insight into the genetic mechanisms of schizophrenia in general. Most of the deletions at 22q11.2 are either 1.5 or 3 megabases in size spanning 35 and 60 known genes, respectively. So far, none of the affected genes has conclusively been pinpointed as a schizophrenia risk gene.

There is growing evidence regarding the involvement of micro RNAs (miRNAs) in the pathogenesis of schizophrenia as well as in brain development and plasticity. The minimally deleted 1.5 megabases region removes miRNA 185 which has two validated targets (RhoA, Cdc42) previously associated with schizophrenia. The aim of this study was to identify rare small-sized susceptibility variants at the miRNA 185 gene by resequencing of 1,000 schizophrenia patients and 500 controls. Furthermore we investigated the expression pattern of miRNA 185 in mice brain with relevance to schizophrenia and other neurological manifestations of 22q11.2DS.

Resequencing detected two rare patient-specific novel variants directly flanking the miRNA 185 sequence. Both variants are currently being followed up by genotyping in a large independent sample of 3,530 patients and 4,018 controls. No variants were detected in the pre-mature miRNA sequence itself suggesting that this gene is highly conserved. At this point, however, we cannot exclude that rare schizophrenia susceptibility variants exist in miRNA 185 which would require larger samples to detect them. In situ hybridization in developing and adult mice brain revealed overlapping expression patterns of miRNA 185 with brain regions reported to be involved in schizophrenia and other neurological manifestations of 22q11.2DS.

As a conclusion, we found no direct genetic evidence for the involvement of miRNA 185 in schizophrenia susceptibility. Our expression results suggest that miRNA 185 plays a role in the control of messenger RNAs involved in brain development.

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

P-Compl-142

Identification of DMBT1 single nucleotide polymorphisms and their haplotypes as novel susceptibility variants for Crohn's disease and ulcerative colitis

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DMBT acts as a pattern recognition and scavenger receptor with antibacterial properties, suggesting a protective role for DMBT1 in inflammatory bowel disease (IBD). Aim of this study was to analyze novel DMBT1 gene variants regarding disease susceptibility in Crohn's disease (CD) and ulcerative colitis (UC). Seven single nucleotide polymorphisms (SNPs) in the DMBT1 gene region (rs2981745, rs2981778, rs11523871, rs3013236, rs2981804, rs2277244, rs1052715) were analyzed in 2073 individuals of Caucasian origin, including 818 CD patients, 283 UC patients and 972 healthy unrelated controls in two independent case-control panels. Comprehensive genotype-phenotype and haplotype analyses were performed and DMBT1 variants were tested for epistasis with allele variants in the known CD susceptibility genes NOD2, IL23R and IL27.

Several gene variants in DMBT1 were associated with the susceptibility to CD and UC. SNP rs2981804 was most strongly associated with CD in the combined discovery and replication panels (p=1.5x10-7, OR 0.70) while SNP rs2981745 was most strongly associated with UC (p=1.8x10-4, OR 1.46). In haplotype analyses, 100% of all haplotype groups tested showed highly significant associations with CD and 85% of all haplotype groups were associated with UC, respectively, including omnibus P-values of less than 10-10 in 45% of the cases. Genotype-phenotype analysis revealed an association of the minor allele of rs2981745 with increased colonic involvement in CD and UC patients. We identified novel variants in DMBT1 that are associated with the susceptibility to CD and UC suggesting an important function of this molecule in the pathogenesis of IBD.

P-Compl-143

IRGM Variants and Susceptibility to Inflammatory Bowel Disease in the German Population

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Genome-wide association studies identified the autophagy gene IRGM to be strongly associated with Crohn's disease (CD) but its impact in ulcerative colitis (UC), its phenotypic effects and potential epistatic interactions with other IBD susceptibility genes are less clear which we therefore analyzed in this study. Genomic DNA from 2060 individuals including 817 CD patients, 282 UC patients, and 961 healthy, unrelated controls (all of Caucasian origin) was analyzed for six IRGM single nucleotide polymorphisms (SNPs) (rs13371189, rs10065172=p.Leu105Leu, rs4958847, rs1000113, rs11747270, rs931058). In all patients, a detailed genotype-phenotype analysis and testing for epistasis with the three major CD susceptibility genes NOD2, IL23R and ATG16L1 were performed.

Our analysis revealed an association of the IRGM SNPs rs13371189 (p=0.02, OR 1.31 [95 % CI 1.05-1.65]), rs10065172=p.Leu105Leu (p=0.016, OR 1.33 [95 % CI 1.06-1.66]) and rs1000113 (p=0.047, OR 1.27 [95 % CI 1.01-1.61]) with CD susceptibility. There was linkage disequilibrium between these three IRGM SNPs. In UC, several IRGM haplotypes were weakly associated with UC susceptibility (p<0.05). Genotype-phenotype analysis revealed no significant associations with a specific IBD phenotype or ileal CD involvement. There was evidence for weak gene-gene-interaction between several SNPs of the autophagy genes IRGM and ATG16L1 (p<0.05), which, however, did not remain significant after Bonferroni correction. Our results confirm IRGM as susceptibility gene for CD in the German population, supporting a role for the autophagy genes IRGM and ATG16L1 in the pathogenesis of CD.

P-Compl-144

PTGER4 expression-modulating polymorphisms in the 5p13.1 region predispose to Crohn's disease and affect NF-κB and XBP1 binding sites

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Genome-wide association studies identified a PTGER4 expression-modulating region on chromosome 5p13.1 as Crohn's disease (CD) susceptibility region. The study aim was to test this association in a large cohort of patients with inflammatory bowel disease (IBD) and to elucidate genotypic and phenotypic interactions with other IBD genes. A total of 7073 patients and controls were genotyped: 844 CD and 471 patients with ulcerative colitis and 1488 controls were analyzed for the single nucleotide polymorphisms (SNPs) rs4495224 and rs7720838 on chromosome 5p13.1. The study included two replication cohorts of North American (CD: n=684; controls: n=1440) and of German origin (CD: n=1098; controls: n=1048). Genotype-phenotype, epistasis and transcription factor binding analyses were performed.

In the discovery cohort, an association of rs4495224 (p=4.10 x 10-5; 0.76 [0.67-0.87]) and of rs7720838 (p=6.91 x 10-4; 0.81 [0.71-0.91]) with susceptibility to CD was demonstrated. These associations were confirmed in both replication cohorts. In silico analysis predicted rs4495224 and rs7720838 as essential parts of binding sites for the transcription factors NF-□B and XBP1 with higher binding scores for carriers of

the CD risk alleles, providing an explanation of how these SNPs might contribute to increased PTGER4 expression. There was no association of the PTGER4 SNPs with IBD phenotypes. Epistasis detected between 5p13.1 and ATG16L1 for CD susceptibility in the discovery cohort (p=5.99 x 10-7 for rs7720838 and rs2241880) could not be replicated in both replication cohorts arguing against a major role of this gene-gene interaction in the susceptibility to CD. We confirmed 5p13.1 as a major CD susceptibility locus and demonstrate by in silico analysis rs4495224 and rs7720838 as part of binding sites for NF-kB and XBP1. Further functional studies are necessary to confirm the results of our in silico analysis and to analyze if changes in PTGER4 expression modulate CD susceptibility.

P-Compl-145

Haplotypes containing the susceptibility genes SLC22A4 and SLC22A5 within the IBD5 locus are associated with inflammatory bowel disease (IBD)

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Variants within the SLC22A4 and SLC22A5 in the IBD5 region encoding the organic cation/carnitine transporters OCTN1 and OCTN2 are associated with Crohn's disease susceptibility. However, due to strong linkage disequilibrium in this region comprising a cytokine gene cluster, the causal variant has not yet been identified. Moreover, the SNP rs12521868 within the IBD5 region was associated with susceptibility in a recent genome-wide meta-analysis including more than 20,000 cases with Crohn's disease. In a very recent meta-analysis investigating more than 75,000 IDB cases and controls the IBD5 region was also identified as a susceptibility locus for ulcerative colitis. Therefore, three additional variants in the IBD5 region were investigated. Genomic DNA from 2865 individuals of Caucasian origin including 844 patients with Crohn's disease (CD), 467 patients with ulcerative colitis (UC), and 1544 healthy, unrelated controls was analyzed for three SNPs within the IBD5 region (rs17622208=IGR2230, rs12521868=IGR2096, rs7705189=IGR3096). A detailed haplotype analysis and genotype-phenotype analysis were performed.

All three SNPs within the IBD5 region were strongly associated with CD. The variants IGR2230 (p=8.52 x 10-3; OR 1.17 [1.04-1.32]), IGR2096 (p=4.40 x 10-2; OR 1.13 [1.01-1.27]) and IGR3096 (p=9.81 x 10-3; OR 1.17 [1.04-1.32]) were identified as risk factors for developing CD. Moreover, several haplotypes containing these three SNPs and additional IBD5 risk variants investigated in a previous study were strongly associated with CD of as well as with UC susceptibility (minimal p values 1.96x10-11 for CD and 7.08x10-07 for UC, respectively). In conclusion, IBD5 haplotypes are strongly associated with CD as well as with UC susceptibility in the German population. Given the accumulating evidence for an important role of variants within the cytokine gene cluster of the IBD5 region in intestinal inflammation, further analyses are required to investigate the functional implications of the IBD5 susceptibility locus in IBD.

P-Compl-146

Analysis of the association of LPL–Hind III polymorphism on the risk of coronary artery disease

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Background: Coronary artery disease (CAD) is one of the leading causes of death and disability around the world. Interaction between genetic and environmental factors determines susceptibility of an individual to develop coronary artery disease lipoprotein lipase (LPL) play an important role in the metabolism of HDL-C, LDL-C and triglycerides. LPL dysfunction is associated with increased risk of CAD . Genetic variants of lipoprotein lipase gene is associated with coronary heart disease . The aim of present study was to investigate the relationship between the risk of coronary artery disease and LDL-C, HDL-C and TG levels by lipoprotein lipase gene Hind III polymorphism.

Materials and Methods : A total of 202 subjects including 114 patients with coronary artery disease and 88 control participated in this study. The Hind III polymorphism of the lipoprotein lipase gene was determined by PCR and RFLP .

Results : In this survey, a highly significant association between the frequent H+/+ genotype and unfavorable TG levels was observed in our population . For the Hind III genotypes, within the healthy subjects (n=88), the H+/+ genotype was found in 67 individuals (58.8%), H-/+ genotype in 38 individuals (33.3%) , and 9 individuals (7.8%) carried the H-/- genotype. Within the CAD group (n=114), 47 individuals (53.4%) with H+/+ genotype, 36 (41%) with H-/+ genotype, and 5 (5.6%) carried the H-/- genotype.

Conclusion: There was a difference in the distribution of LPL–Hind III genotypes between the healthy subjects and the patients with CAD. Lipoprotein lipase–Hind III polymorphisms were not detected as independent risk factors for CAD in this study group, but had significant associations with TG levels ($P<0.05$).

P-Compl-147

Identification of genes involved in altered pain perception in patients with Crohn´s disease (CD) using expression profiling of small intestine biopsies

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Crohn's disease (CD) is a painful inflammatory bowel disease with complex polygenic inheritance. It has been shown that a number of CD patients require significantly higher post-operative opioid doses than patients undergoing comparable abdominal surgery. We recently demonstrated that this is not due to the most common variants in components of opioid metabolism. CD, therefore, may be a suitable model for the identification of novel pain susceptibility genes. In order to further investigate the molecular and genetic basis of this difference in pain perception within CD patients, we focused our attention on the affected tissue. RNA was extracted from sections of inflamed and non-inflamed small intestine tissue of 3 CD patients with high and 3 patients with low postsurgical opioid requirement. Expression profiling of all 12 tissues was performed using Affymetrix U133 Plus2.0 microarrays. Comparison of the expression profiles revealed 18 transcripts with significantly altered expression in high versus low consumers (fold change of >2; p-value <0.05), independent of the inflammation status of the tissue.

Using quantitative real-time PCR we were able to confirm the expression changes for 5 out of the 18 genes in the original RNA set as well as in a second set of RNAs extracted from different regions of the original biopsies. These genes include factors already known to be involved in pain perception and/or inflammation as well as genes with yet unknown function. Immunohistochemical analysis of murine intestine revealed a highly interesting expression pattern for two of these genes (SPIB and TCF21). We identified strong expression in yet not further characterized cells of the lumen as well as in enteric nerve cells of the plexus myentericus, respectively. This turns these two genes into highly interesting candidates for the altered pain perception and post-surgical opioid requirement in CD patients.

P-Compl-148

Analysis of the association of APOE4 polymorphism on the risk of coronary artery disease

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Background: Coronary artery disease responsible for more than half of all deaths occurred in middle age and One-third of all deaths in old age in the most advanced countries. One of the most important risk factors, genetic risk factors, and apolipoprotein E genotype among them has the greatest impact. The aim of present study was to determine the genotypes of polymorphism APOE4 and study their association whit coronary artery disease in population Iran.

Material and Methods: In this case-control study, the ApoE gene polymorphism was analysed in 100 Iranian patients with coronary artery disease and 48 control subjects. The Apoe4 polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphism.

Results: The frequency of the different Apo E genotypes in our population was in Hardy-Weinberg equilibrium ($\chi^2= 1.511$, p-value= 0.53, although not significant). Subjects with the ε4 allele were less than two times less likely to develop low-density lipoprotein (LDL), but subjects with the ε2 allele were more than two times more likely to develop low-density lipoprotein.

Conclusion: Apo E polymorphism is not significantly associated with the risk of low-density lipoprotein (LDL), high-density lipoprotein (HDL), VLDL, triglyceride and cholesterol. Allele frequencies reported in the present study were similar to those of Japanese, and Mexican, Korean population. The frequency of ε4 appears to be 2 or 3 folds lower in Iranian subjects, compared to Finland, Nigerian , African-Americans , Norwegian , German 9) and Swiss .

P-Compl-149

Whole Exome Sequencing reveals uncommon mutations in the recently identified Fanconi anemia gene SLX4/FANCP

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Known as a rare genetic disorder, Fanconi anemia (FA) is characterized by congenital malformations including short stature, skin hyper- or hypopigmentations and radial ray defects. Patients suffer often from progressive bone marrow failure (BMF) and susceptibility to hematological and non-hematolocigal malignancies with an increased risk of the myelodysplastic syndrome (MDS). Responsible for the disease are biallelic or hemizygous mutations in any one of at least 15 genes (FANC-A, -B, -C, -D1/BRCA2, -D2, -E, -F, -G, -I, -J, -L, -M, -N/PALB2, -O/RAD51C, -P/SLX4) whose products interact with each other and with related proteins in the FA/BRCA DNA pathway for genomic maintenance. Here we report on a FA patient with previously unknown mutations of the most recently identified FA gene, SLX4/FANCP. We used Whole Exome Sequencing (WES) that demonstrated a nonsense mutation and an unusual splice mutation resulting in the partial replacement of exonic with intronic bases which, without truncating the open reading frame, removes a putative nuclear localization signal. Further analysis such as cellular fractionation and immunoprecipitation failed to show residual SLX4/FANCP protein which was consistent with lacking SLX4 interactions with XPF/ERCC1 and MUS81/EME1, and deficient ERCC1 nuclear foci formation. Our cellular findings were not reflected by a more severe clinical phenotype than of FA patients in general. This study exemplifies the versatility of WES for the detection of hidden mutations in patients with heterogeneous disorders like FA and may increasingly replace classical genetic approaches.

P-Compl-150

Genome-wide methylation array analysis in inflammatory bowel disease

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Inflammatory bowel diseases (IBD) including Crohn's disease (CD) and Ulcerative colitis (UC) are characterized by severe inflammation of the small bowel and/or the colon, leading to recurrent diarrhea and abdominal pain. The cause of disease is described as an interaction between different environmental, immunological, microbiological and genetic factors. To date, more than 100 genetic susceptibility loci associated with IBD have been identified that, taken together, explain no more than 20% of the genetic risk. Recent data suggest that there is a major contribution of epigenetic factors to the pathogenesis of IBD. In a first study analysing the methylation levels of the promoter regions of several IBD candidate genes, three genes (MUC6, MUC15 and IL17REL) displayed significant methylation changes in non-inflammatory (NI) and inflammatory (I) colon biopsies from IBD patients in comparison to colon biopsies of non-IBD patients (controls) with an increase of methylation in the IBD samples for the MUC6 and IL17REL gene and a decrease of methylation in the IBD samples for the MUC15 gene.

Now, 24 of the previously analyzed samples (8 controls, 8 NI-UC, 8 I-UC) were further studied for genome-wide methylation levels at more than 485,000 potentially methylated sites using the Infinium HumanMethylation450 BeadChip. Statistical analysis revealed 895 sites with significant methylation changes in I-UC samples in comparison to control samples. The approximately 480 genes containing differentially methylated CpG sites were prioritized using the degree of methylation differences and literature data on known association with UC or IBD. The methylation data of five prioritized genes (BACH2, COL1A1, STK4, STAT3 and STAT4) are currently validated using bisulfite pyrosequencing assays covering the differentially methylated CpG sites on the bead chip and other putative regulatory CpG sites. To verify if the detected

methylation changes influence the expression of the respective genes, quantitative real time RT-PCR experiments of the same tissue samples are performed in parallel. A comprehensive understanding of the epigenetic mechanisms contributing to IBD will likely enable development of new therapeutic agents and strategies targeting epigenetically dysregulated genes.

P-Compl-151

Identification of genes potentially involved in dyslexia development on chromosome 18 in Germans

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Introduction:

Dyslexia is an impairment which affects the ability to read and write and has a prevalence of ~5%. Studies estimated a heritability of 50-70% for the development of dyslexia. Genetic analysis identified several loci affecting dyslexia but the majority of the variance is still unexplained. One important chromosome in this context is chromosome 18. Scerri et al. (2010) investigated this region to narrow down the relevance to certain genes and identified eight potential dyslexia associated SNPs in seven genes which replicated in any of their independent samples. We explored if these SNPs are of relevance for dyslexia in a German population.

Methods:

We evaluated SNPs at eight loci in a cohort comprised of 388 dyslexia cases and 364 controls. We carried out case – control analysis for all SNPs separately via Chi-square analysis stratified for severity. Additionally, we performed quantitative analysis for different sub-traits of dyslexia via a linear regression approach, investigated haplotypic effects, and explored effects of allelic combinations of multiple SNPs.

Results and conclusion:

Case – control analysis revealed nominal association with dyslexia for three SNPs belonging to two different genes. Two of these SNPs are protective (odds ratio=0.72 (95% confidence interval=0.6–0.9); p=0.007 and 0.74 (95%CI=0.6–1.0); p=0.017) and one is risk associated (OR=1.31 (95%CI=1.1–1.6); p=0.011). The protective SNPs were in strong LD (R^2=0.96) and associate as haplotype, as well. The two genes belonging to the three SNPs are known to be involved in the regulation of sodium channels and plasma membrane metabolism. Quantitative analysis gave a nominal significant signal for one SNP (ß=1.50 (SE=0.74)). Further work will focus on (i) studying effects of those variants on gene expression and (ii) evaluating their usefulness for inclusion in prediction models for dyslexia.

P-Compl-152

Investigation of selected cytokine genes suggests that IL-2RA and the TNF/LTA locus are risk factors for severe alopecia areata

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Alopecia areata (AA) is the second most common cause of hair loss in humans, and has a genetically complex inheritance. The hair loss disorder affects approximately 1-2 % of the general population. AA affects both sexes and all age groups. Affected individuals typically present with a nonscarring, circumscribed hair loss, which has a sudden onset and a recurrent course. The hypothesis that AA is autoimmune in nature is supported by previous studies and a genome-wide association study. These report association with specific HLA alleles, as well as genetic variants of other genes implicated in autoimmunity, such as various cytokine genes. However, most of the cytokine genes cannot yet be considered proven susceptibility loci, since many of these association findings were derived from small patient samples. Accumulating knowledge of shared pathways in autoimmune diseases and findings of association between AA and other autoimmune disorders highlight the importance of investigating the role of other common autoimmune susceptibility alleles in AA. We therefore genotyped variants of cytokine genes with a possible involvement in AA, and investigated their association with AA using a sample of 768 AA patients and 658 controls of Central European origin. We genotyped eleven single nucleotide polymorphisms (SNPs) from cytokine genes implicated in previous AA studies using the MassARRAY system and a Sequenom Compact MALDI-TOF device (Sequenom Inc., San

Diego, CA, U.S.A.). These genes were IL-1B, IL-1A, IL-1RN, MIF, IFNG, and the TNF/LTA gene region. We also genotyped 15 SNPs selected from cytokine genes that have shown significant association with other autoimmune diseases. These genes were IL-10, IL-1F5, IL-12B, IL-6, IL-2, IL-23, IL-2RA, and IL-4R. Significant association was found for two variants within both IL-2RA and TNF/LTA. In the overall sample, the most significant results were obtained for the IL-2RA variant rs706778 (P = 0.00038), and the TNF/LTA locus variant rs1800629 (P = 0.0017). In subgroup analyses, according to severity, age of onset, and family history these effects were stronger in the severely affected patients, with the lowest P-values being obtained for rs706778 (P = 3.8*10-6). Our results therefore point to the involvement of IL-2RA and the TNF/LTA region in the aetiology of AA - in particular severe AA -, and provide further support for the hypothesis that AA is autoimmune in nature.

P-Compl-153

Target enrichment and next-generation sequencing in Fanconi anemia diagnostics

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Fanconi anemia (FA) is a rare autosomal recessive or X-linked disorder that is characterized by considerable clinical and genetic heterogeneity. Major clinical problems include progressive bone marrow failure, congenital anomalies and malformations as well as an increased cancer risk. Causative for FA are biallelic or hemizygous mutations in any of so far 15 identified FA genes (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, -P) which are components of the FA/BRCA DNA damage response pathway and essential for maintaining genomic stability. Due to their defect in DNA repair, FA cells show increased chromosomal instability, reduced survival rates and accumulate in the G2 phase of the cell cycle, in particular in response to DNA cross-linking agents. As mutations of FANCA are most frequent in FA patients, this gene is usually analyzed first, either by MLPA or by Sanger sequencing. Another approach to detect causative mutations is to first determine the complementation group of FA patients by retroviral transfection or by Western Blot.

Next-generation sequencing techniques make it now feasible to analyze a huge amount of genes simultaneously. Here, we tried to develop a diagnostic tool comprising massively parallel sequencing of all reported FA disease genes as well as some selected candidate genes. To enrich those regions of interest we used a solution-based NimbleGen SeqCap EZ Choice library where we also included probes covering several bases up- and downstream of all coding exons in order to cover all splice sites. Patient DNAs were multiplexed during hybridization with the probes and then sequenced on a Roche GS Junior. Data analysis was performed using the NextGENe software. In patients with known mutations in diverse FA genes we were able to detect those mutations after target enrichment. In a patient with a known deletion in FANCA, we were able to confirm the deletion and to detect the hitherto unknown second disease-causing nonsense substitution. We were also able to analyze some regions with a high GC content which we previously failed to amplify by sequence-specific PCR. Several smaller insertions and deletions up to 69 bp were found repeatedly, which is probably due to the long 454 sequencing reads.

The workflow is not completely optimized yet, but we think that this approach is a promising tool for complementation group assignment and mutation analysis in a single step to facilitate future FA diagnostics.

P-Compl-154

The Genetic Background for Stroke in the Persian Population

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Background and Purpose —Stroke is major cause of morbidity and mortality around the world and is the most common cause of adult disability. According to World Health Organization estimates, nearly 5.5 million people died of stroke in 2002 and more than half of these deaths occurred in South Asian countries and this organization estimated in 29 October 2011, one in six people worldwide will have a stroke in their lifetime. Based on the first population-based study of stroke during a 12-month period (2006–2007), in a Middle East region of Iran (Mashhad), the incidence of stroke in Iran is considerably greater than in most Western countries. Studies in twins, families, and animal models demonstrate substantial evidence for a genetic background to stroke disease. Long lists of candidate gene pathways and genes have been studied for a possible association with stroke. Among the most widely investigated genes are those involved in inflammation, coagulation, lipid metabolism, nitric oxide release, and renin-angiotensin-aldosterone systems, hemostasis, etc. However, anyone didn't study of genetic background that may be able to use of Excessive

and earlier Incidence of Stroke in Persian population. In this Study at the first time we analysis the genetic background for Stroke in Persian Population.

Methods—Stroke subtypes were diagnosed by computed tomography (CT) scans and magnetic resonance imaging (MRI) in 413 Stroke Patient. Additionally, 413 control subjects were matched in some stroke risk factors for example age, sex, hypertension, diabetes mellitus, lipid profile, etc. In our gene association study, was selected genes related with dependent systems in stroke, such as Factor V Leiden and Factor II 20210G>A,ACE I/D and AGTR1 ,IL6 -572 &-174 G>C and IL10 -1082 G/A,APOE e2/e3/e4 and eNOS 4a/4b in, coagulation, renin-angiotensin-aldosterone systems, inflammation, lipid metabolism, and nitric oxide release I. In these variation detected with RFLP-PCR-genotype discrimination, Real Time PCR, semi nested PCR, multiplex tetra primers ARMS PCR and PCR techniques. Data were coded and entered in SPSS Software (version 11.5).

Results—The ACE I/I and I allele, ApoEe2/e3, and IL10 -1082 G/A genotypes distribution in stroke patients differed significantly from controls (P_0.031, 0.026, 0.015, respectively).Also, there are strong association between hemorrhagic subtype and e2/e3, ACE I/I polymorphisms (p_0.001,0.024). Finally, ACE I/I and I allele associated between all subtype of stroke in the rage rang of 35 till 55 (age range of incidence).There are no statistical correlation between another selected genes and Persian stroke's Population.

Conclusion— our population-based longitudinal study is the first gene association study for stroke in Persian population. We report that there is a significant correlation between the ACE I/I, APOEe2/e3, IL10 -1082 GG and East Persian population and Stroke. There are the high possibilities that I/I and e2/e3 Genotypes play a role in the development of the hemorrhagic Stroke. Furthermore in these genotypes may be one of the reasons for the earlier Incidence of Stroke in the East Iran (Khorasan) population. Finally, this study will create new Road in understanding the new solution for this common multifactorial complex disorder.

P-Compl-155

Comparison of human MCPH1-deficient cell lines

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Microcephaly is a rare heterogeneous neurodevelopmental disorder that is characterized by markedly reduced brain size and mental retardation. Biallelic mutations of the human gene MCPH1 are the cause of autosomal recessive primary microcephaly (OMIM 251200) associated with a unique cellular phenotype that is marked by premature chromosome condensation, PCC. The MCPH1 gene has 14 Exons and is located on the short arm of chromosome 8. Its coding sequence consists of 2508 bp. Its product, microcephalin is a multifunctional protein that was reported to be involved in brain development, the DNA damage response and the regulation of chromosome condensation. It also contains three BRCT domains, which are predominantly found in proteins that are involved in cell cycle functions responsive to DNA damage.

In this project we compared different patient-derived MCPH1-deficient cell lines with different methods in respect of the DNA damage response and the PCC syndrome.

Western blot analysis showed that the MCPH1-deficient cells are actually missing microcephalin.

Then we prepared chromosomes to compare the level of PCC. 1000 cells per cell line were counted. The rate of prophase-like cells (PLC) of normal control cells was about 1.2% and the rate of the MCPH1-deficient cells up to 10 times higher.

Next we looked at two different types of DNA damage repair foci. After irradiation with 1 Gy we stained the cells with antibodies against 53BP1 and RAD51. We showed that the MCPH1-deficient cells are expressing 53BP1 and RAD51 at similar rates and with a similar time course as control cells.

Earlier reports noted that MCPH1-deficient cells show a delay in G2 checkpoint release. Therefore we irradiated the MCPH1-deficent cells along with controls with 1 Gy and determined the mitotic index by phospho-histone-H3 flow cytometry. We observed a delayed checkpoint release in all analyzed MCPH1-deficient cell lines, featuring the way in which DNA damage response to IR is defective.

P-CompI-156

Glutamatergic candidate genes in autism spectrum disorders

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Autism spectrum disorders (ASD) are heterogeneous disorders characterized by qualitative impairment in social interaction and communication, stereotyped behaviour and restricted interests. Despite a high heritability of ASD (around 90%) and approximately 1% prevalence (Freitag et al., Eur Child Adolesc Psychiatry, 2010), only a few mutations and common variants have been replicated as risk factors. Common variants are not only proposed to impact on disease risk but also particularly on phenotypic presentation in complex traits and they have directly been associated with ASD.

Fragile-X-syndrome (FXS) is one of the most studied monogenetic disorders with core symptoms of ASD caused by a trinucleotide expansion in the promoter region of the fragile X mental retardation gene 1 (FMR1). The reported dysregulated mRNA translation due to disturbances in the FMR1 signaling pathway leads to altered synaptic function and loss of protein synthesis-dependent plasticity (Lüscher et al., Neuron, 2010). The gene product of FMR1 (FMRP) is a downstream target of the metabotropic glutamate receptors 1 and 5 (GRM1/5).

The aim of this study was to assess common variants in candidate genes upstream and downstream of this pathway as risk factors for ASD. Therefore, we selected a subset of genes due to evidence as susceptibility genes for ASD from linkage, association or CNV studies. Subsequently, we performed a SNP analysis in those genes based on the following criteria: i) minor allele frequency (MAF) >10%; ii) location in potentially functional domains or tag-SNP regions.

In total, 14 annotated SNPs were genotyped in a detection sample of 192 parent-child trios and in additional 254 parent-child trios as a replication sample. Besides marker and haplotype analysis, we performed a risk allele score based approach in cases and pseudo-controls to study the combined effect of risk alleles on ASD.

In the detection sample we discovered a nominal association with ASD for a single SNP (rs7170637 CYFIP1), which was not replicable in the second set. Combination of both samples increased power to detect smaller effects, and we confirmed the result for CYFIP1 variant rs7170637. In addition, we observed nominal association with ASD for CAMK4 SNP rs25925 and GRM1 SNP rs6923492.

When combining the risk alleles into one additive risk score, this score was able to predict the case-pseudo-control status in a logit regression model in the detection and in the combined sample, but again not in the replication sub-sample.

Our results point towards the relevance of the glutamatergic system in the etiology of ASD, though with a modest effect. First, we could show that several common variants of the glutamatergic system are associated with ASD and second that they contribute in combination to the risk of ASD. Our results in the first sample were not replicated in the replication sample, but remained significant in the combined set. This is likely due to the low effect size of the common risk alleles as well as low power due to limited sample size of the detection and the replication sample. Our results in the combined sample should be replicated in an even larger sample than our combined sample. The full genetic and functional characterization of the molecular mechanisms associated with the risk alleles provide major challenges for future research.

P-CYTOGENETICS / CNVS

P-CytoG-157

Spatial Organisation of the two Parental Genomes of a BIlod/Bone Marrow Cell as tested by pod-FISH and its Promotion of Granulocytic Function

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Since Rabl (1885) the spatial arrangement of chromatin or chromosomes has been controversially described and interpreted. For many years we suggested and emphasized a “parallel order” of the two parental genomes in a diploid nucleus based on observations made on blood and bone marrow cells at

metaphase and interphase. Apparent distribution of chromosome aberrations like translocations, trisomies, polysomies or amplifications underscored such bilateral pattern (Chaudhuri and Reith Analyt Quant Cytol Histol 19:30-36, 1997; Chaudhuri and Walther Int J Oncol 23:1257-62, 2003; Acta Haematol 2012; Chaudhuri et al. Cellular Oncol 27:327-34, 2005; Anticancer Res 28:3573-78, 2008). This bilaterally symmetric genomic order, verified now by parental-origin-determination FISH (pod-FISH) (Weise et al., Int J Molecular Medicine 21:189-200, 2008), fits to the axis of nuclear bipolarity of PMN Leukocytes and favours their efficient diapedesis and chemotaxis to the focus of an injury or infection. Similar order may also be involved in the development of bipolarity of the neurons (Hsu et al. Chromosoma 34:73-87, 1971; Manuelidis PNAS 81:3123-7, 1984). These findings may also demand revisions including the karyotyping of chromosomes, the strongest tool of human genetics.

P-CytoG-158

Family with recurring CNVs resulting from a paracentric inversion in 5q

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Here we report a family with recurring structural changes in chromosome 5, including inversions, deletions, and duplications with different levels of mental retardation, short stature, and varying other features.

Our index patient is a 29 years old man with short stature (1.58 m), hypomimia, orofacial dystonia, minor ataxia of the lower limbs, irregular myoclonal postural and action tremor more pronounced on the left side of the upper limbs as well as a developmental delay. Cytogenetic and array analysis showed a deletion in 5q22.1-q23.2 of 14.22 Mb. To investigate the origin of this aberration we tested his mother and further family members from the maternal side. This revealed cytogenetically the same deletion in his uncle, who has a mental retardation and a tremor. His unaffected mother, maternal aunt and grandmother carry an inversion in 5q with putative breaking points in bands 5q22.1 and 5q33.1 [46,XX,inv(pter□q22.1::q33.1□q22.1::q33.1□qter)].

His sister, who was said to suffer since her childhood from a vasoconstriction in afferent vessels of the heart, and her two sons who show a speech delay, have a reported duplication in 5(q22.1-23.2) of 14.25 Mb (Schmidt et al., 2012). The karyograms of his half-sister and another aunt and uncle showed the same rearrangement as his sister's family. The phenotypic range of mental retardation in the duplication carriers is diverse, ranging from unaffected (aunt, uncle and sister), to mildly affected (half-sister, who requires help in daily life), and speech delay.

Despite the different types of structural changes, all family members present short stature. Mental retardation appears only in the cases with copy number changes i.e. deletions and duplications but not in the inversions.

We propose that the inversion was the original change, but at this juncture we cannot exclude additional structural rearrangements and more complex events. This and the exact break points of the mentioned changes remain to be narrowed down by FISH and further array analyses. We will suggest the mechanisms of the recurring events as well as make a phenotype-genotype correlation.

P-CytoG-159

Search for disease-associated copy number variants in common risk genes identified by genome-wide association studies in schizophrenia, bipolar disorder and major depressive disorder

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Based on family and twin studies it is well known that genetic factors are an important contributor to the susceptibility to neuropsychiatric disorders such as schizophrenia (SCZ), bipolar disorder (BPD), and major depressive disorder (MDD).

In search of the disease underlying genetic risk factors, several large genome-wide association studies (GWAS) have been performed and several genome-wide significant SNPs were identified. Researchers are aiming to follow-up these findings by searching for independent genetic support as well as gaining insight

into biological processes influenced by the risk genes/variants. The latter is tackled by detailed genotype-phenotype investigations, search for endophenotypes influenced by the genetic factors, or functional characterization using mouse models. In search of additional genetic support, replication studies of the reported risk SNPs in extended samples is one straightforward approach. In parallel, it is reasonable to explore whether a broader spectrum of risk variants is present at each risk locus in the population. Copy number variants (CNVs) represent a low frequency or rare type of variation that is testable in SNP-array data, and they have been strongly implicated in the etiology of SCZ. In the present study, we aimed to systematically screen genes with genome-wide significant SNP findings in SCZ, BPD and MDD for the presence of potentially disease-associated CNVs. Identification of disease-associated CNVs at these loci would provide strong supportive evidence for an involvement of the affected genes in disease etiology, and it would allow important insights into the genetic architecture of these psychiatric disorders.

We screened 575 patients with MDD, 882 patients with BPD, 1,637 patients with SCZ, and 1,643 controls for the presence of CNVs in the candidate genes identified in GWAS. All individuals were genotyped on HumanHap550v3, Human610-Quadv1, and Human660W-Quad arrays (Illumina, CA, USA). We did not identify a significant association between CNVs in any of the candidate genes and MDD, BPD, or SCZ. However, it is difficult to draw final conclusions from our data set because the power to find association of rare variants is limited. Extended studies using the same design are clearly warranted.

P-CytoG-160

Cystinuria in children with muscular hypotonia: an autosomal recessive contiguous gene deletion syndrome

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Muscular hypotonia in childhood is a rather unspecific sign which can be part of over 500 different genetic disorders. Hence, finding the correct diagnosis is often challenging. The fundaments of clinical evaluation of a child with hypotonia are a careful personal and family history and a thorough neuropaediatric and dysmorphological examination complemented by electrophysiological and laboratory tests. Metabolic abnormalities may occasionally yield diagnostic clues beyond individual inborn errors of metabolism. Here we report on an 8 year old boy with muscular hypotonia, mild developmental delay, mild facial dysmorphism, hypospadias, cryptorchidism, and short stature (length 117,5 cm; 1 cm <P3). During the first years of life he had a failure to thrive, followed by hyperphagia and rapid weight gain since the age of 6 years (current BMI 21,4 kg/m2, >P97). His parents are consanguineous. Molecular testing for Prader-Willi syndrome gave a normal result. However, metabolic screening including urinary amino acid analysis showed reduced absorption of the dibasic amino acids and cystine, compatible with cystinuria. Evaluation of other family members showed that the younger brother of the patient also suffers from hypotonia and cystinuria. Based on these findings hypotonia-cystinuria syndrome (HCS) was suspected. This was confirmed by molecular karyotyping which showed a homozygous deletion 2p21 with a size of 30-40 kb in both brothers (HumanCytoSNP-12v2.1 chip, Illumina). The deletion affects parts of SLC3A1-gene coding for a renal cystine transporter, mutations of which cause cystinuria type I, and the PREPL gene coding for a serine oligopeptidase supposed to be involved in secretion and/or processing of peptide hormones. Similar homozygous deletions have already been described in other patients with HCS. Clinical hallmarks of this syndrome are severe generalized hypotonia at birth, nephrolithiasis due to cystinuria, growth hormone deficiency with short stature, and initial failure to thrive followed by obesity in late childhood. Intellectual development may be normal or mildly delayed. Urinary amino acid analysis or at least a nitroprusside test (Brand reaction) should be part of the biochemical work-up in children with muscular hypotonia, in particular in those with a Prader-Willi-like phenotype who test negative for Prader-Willi syndrome.

P-CytoG-161

Prenatal diagnosis of an 18q-deletion syndrome: a case of fetal mosaicism with different 18q-deletions in chorionic villi direct (short-term) culture and long-term culture

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We present cytogenetic and molecularcytogenetic data of a prenatal detected case of distal 18q-deletion syndrome. There are no common breakpoints in patients with distal 18q-deletion, thus the size of the deletions varies widely. The largest deletion reported is 30.076 Mb, while the smallest deletion reported to cause a clinical phenotype is 3.78 Mb. Distal 18q-deletion syndrome is a rare disorder characterized by

mental retardation, growth retardation, heart abnormalities (25-30%), congenital orthopedic anomalies, cleft lip and palate (10%), kidney abnormalities and genital anomalies like cryptorchidism and hypospadia in boys. In our case chorionic villus sampling (CVS) was performed because the ultrasonographic examination at the 13th week of gestation showed double sided cleft lip and palate and a singular vein of the umbilical cord. Chromosomal analysis of a chorionic villi direct (short-term) culture showed a pathological male karyotype with deletion of the terminal long arm of a chromosome 18 with a breakpoint in the band 18q21.1. Array CGH analysis with DNA of the chorion villi revealed a terminal deletion of 18q22.3 to 18q23 with a deletion size of 5.9 Mb. Beside this, a deletion of the more proximal region 18q21.1 to 18q22.3 with a size of 25.9 Mb as well as a duplication of the region 18p11.32 to 18p11.23 spanning 7.2 Mb could be detected at a mosaic level. Chromosomal analysis of a chorionic villi long-term culture revealed a terminal 18q-deletion with a breakpoint in 18q22.3. This cytogenetic result confirms the results of the Array CGH analysis. Cells from the trophoblast layer of the chorion villi that are examined in the direct (short-term) culture show a terminal deleted chromosome 18 with a breakpoint in 18q21.1 and – as we could show afterwards with fluorescence in situ hybridization – 18p derived chromosomal material is present on the terminal long arm whereas cells from the placental mesenchym representing the fetus show the terminal deleted chromosome 18 with a breakpoint more distal in 18q22.3 but no additional 18p derived material. The parents decided to terminate the pregnancy. This case supports the fact, that pregnancies with ultrasound abnormalities are very good indications for prenatal diagnosis with array CGH.

P-CytoG-162

Familial duplication at Xq28 involving GDI1: pathogenic or benign?

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Background: Mutations of the X-Chromosome are relatively frequent causes of intellectual disability (ID) in male individuals. A copy number gain of an interval on Xq28 between position 153.20 and 153.54 Mb was detected in several families with suggested X-linked intellectual disability (XLID). It was hypothesized that the copy number of GDI1, a known XLID gene residing within this area, may drive the clinical outcome of affected individuals. Expression analysis of this gene confirmed dosage dependent mRNA levels. It was concluded that duplication or amplification of this region causes ID with dosage dependent severity.

Results: We detected a duplication of this region (153.06 to 153.53 Mb) in a mildly affected male individual. The aberration was maternally inherited and the healthy mother showed a skewed X-inactivation (92/8). Segregation analysis, however, revealed that the unaffected brother carried the same copy number variant. We were not able to detect any significant differences in expression of GDI1. Interestingly, microsatellite analysis revealed that both brothers did not carry identical X chromosomes. A meiotic recombination event took place between Xq13.1 and Xq21.32, which exchanged genetic material between both maternal chromosomes.

Conclusions: From a classical point of view the detected copy number change in our family is not the cause of ID because an unaffected male individual carries the same variant. Nonetheless, it is known that several traits have a reduced penetrance. This might be due to protective genetic features in unaffected individuals or it may require additional changes in the genetic material of affected persons: (1) the recombined fragment may play a role in terms of penetrance, (2) the recombined fragment may carry a mutation that causes ID in one of the two brothers.

P-CytoG-163

Familial occurrence of a 16p13 microdeletion including GRIN2A as a cause of developmental delay and epilepsy of the rolandic region

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Gene disruptions, point mutations and microdeletions of GRIN2A on chromosome 16p13 have been reported in a small number of patients with epilepsy and a highly variable cognitive development ranging from learning disabilities to severe mental retardation (MIM 613971). This gene codes for a subunit of the neuronal N-methyl-D-aspartate (NMDA) receptor involved in excitatory neurotransmission in the central nervous system. Here, we report on the clinical phenotype of a family with microdeletion 16p13 involving GRIN2A. The affected siblings were referred to the department of neuropediatrics because of mild developmental delay affecting in particular speech and fine motor skills. Pregnancy and birth were

unremarkable, growth measurements and cranial MRI in both children were normal, and there was only minor facial dysmorphism. However, both siblings showed an abnormal EEG pattern. The 11 year old girl had continuous subclinical sleep-induced spikes and waves in the centro-termporal region with secondary generalization corresponding to ESES (electrical status epilepticus in sleep). Her 4 year old brother showed continuous spikes/sharp-waves in the centro-temporo-occipital region without any difference between sleep and awake periods. Their father reported that he had epileptic seizures until the age of 11 years. He had learning difficulties and visited a school for children with special needs. Molecular karyotyping (HumanCytoSNP12 v2.1, Illumina) revealed a 773 kb microdeletion in chromosome band 16p13 in both children. The deletion contains 11 genes, including GRIN2A, and overlaps with the deletions in three previously described patients with intellectual disability and seizure disorders of the rolandic region. FISH confirmed that the deletion was inherited from the father; intellectual disability with or without epilepsy was reported in several paternal family members. This is the first report of the familial occurrence of a microdeletion 16p13 including GRIN2A. Our observation corroborates the clinical value of molecular karyotyping in patients with apparently monogenic epilepsy associated with variable developmental delay. Typical features of GRIN2A deficiency include epilepsy of the rolandic region which may spontaneously improve during the first 2-3 decades of life.

P-CytoG-164

A de novo 8/22 translocation with a 275 kb subtelomeric 8q microdeletion in a female patient is associated with multiple but stable white matter lesions and early onset of bilateral hearing loss.

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A young woman (now 20 years old), who at an age of 6 years has developed a rapid and complete hearing loss of her left ear and severe hearing loss of her right ear particularly of medium and high frequencies within several weeks, without any other known reason, showed also multiple white matter lesions by brain MRT-scans. No mental or any other physical impairment was observed and no further change of her disorder or of her MRT results occurred during the following more than 10 years. Because a mitochondriopathy was suspected, a muscle biopsy was performed followed by treatment withThiamine, L-karnitine and coenzyme Q10, which was applied during the following years. However after an additional muscle biopsy and by biochemical investigations finally ruling out of a mitochondriopathy due to a respiratory chain defect, this treatment was discontinued at a patient's age of 19 years. In another attempt to identify a reason for the occurrence of her hearing loss, sequencing and MLPA analysis of the GJB2- and GJB6-gene was done but failed to reveal a causative mutation. By array CGH a 275 kb microdeletion of the subtelomeric region of chromosome 8 was found. Cytogenetic and molecular cytogenetic analyses, which were also performed, revealed a translocation of nearly the entire long arm of chromosome 22 to the long arm of that chromosome 8 which shows loss of the long arm subtelomere. Subsequent cytogenetic and molecular genetic analysis of parental blood samples confirmed that the translocation in the patient occurred de novo. These findings suggest, that the observed chromosomal rearrangement and/or the microdeletion arr 8q24.3(145,975,992-146,250,824)x1 dn might be involved in the development of the disorder in the patient. Because the number of potentially affected genes is low (e.g. just 9 known genes at the subtelomere 8q, already including several members of a gene family), a number of options to identify the causative aberration, like a possible gain of function effect by gene fusion, can be considered. Since the number of reported similar aberrations and microdeletions in specific databases with well defined phenotypes is quite limited, this approach might utilize array painting, next generation sequencing of a very restricted genomic segment or even direct sequencing of a rather low number of potential candidate genes.

P-CytoG-165

Array-based genome-wide genotyping in fetuses with cerebral malformations and additional congenital anomalies

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Congenital malformations of the central nervous system (CNS) occur in about 0,5-2 of 1,000 births. CNS malformations include both, neural tube defects and cerebral malformations and may present isolated or non-isolated. For cerebral malformations no causative genetic or non-genetic factor has been unequivocally identified in humans to date. While multiple lines of evidence suggest that de novo mutations of different size including CNVs (copy number variations) are involved in the expression of some of these malformations, genetic diagnostic workup during pregnancy is mostly restricted to conventional chromosome analysis with little evidence based experience on array-based methods. Due to modern imaging techniques, most cerebral malformations are being diagnosed prenatally leading to interruption of pregnancy. In order to generate more evidence for the use of array-based analysis in the detection of putative genetic aberrations in congenital cerebral malformations, we screened a collective of 33 aborted fetuses with non-isolated CNS malformations. Since cerebral malformations are associated with structural chromosomal aberrations, it is suggested that a co-occurrence of cerebral malformations and additional congenital anomalies (non-isolated) will make it likely to detect causative CNVs.

All fetuses were sampled through the Department of Obstetrics at the University of Bonn where DNA of over 300 aborted fetuses with mostly CNS malformations was collected between 2006 and 2012. For molecular karyotyping, we used the Illumina HumanOmniExpress-12v1_H with a total of 730,525 markers. To identify potential CNVs, the SNP (Single Nucleotide Polymorphism) fluorescence intensity was analyzed with QuantiSNP using an Objective-Bayes Hidden-Markov model for calling putative CNVs.

Preliminary results revealed probably causative deletions in six fetuses including the following chromosomal regions: 18q21.2-qter (29,6 Mb), 2q35-q37.1 (12,8 Mb), 6q25.3-qter (14,4 Mb), 2q22.2-q23.3 (10,7 Mb), 1q42.3-qter (13,5 Mb) and 5p14.1-pter (23,2 Mb). The latter CNV was identified in a fetus also carrying a duplication of 24 Mb on chromosome 9q32-qter. All of these microaberrations have been previously reported in the context of cerebral malformations. For the remaining cases further investigations of minor aberrations are in progress which will be presented and discussed.

P-CytoG-166

Microdeletion 9q22.31 – a familial case report.

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Up to now, only a few cases of constitutional 9q22 deletions have been reported, most of them sporadic. Published case reports show no recurrent breakpoints and vary in size and phenotypic manifestation.

We present a familial case with a small deletion of 9q22.31 spanning 0.8 Mb, detected by array CGH (CytoChip Oligo 2x105k v.1.1, BlueGnome). The index patients, two brothers, show symptoms like developmental retardation, short stature, frequent respiratory infections, microcephaly and brachydactyly. In addition, one of the brothers is affected of obesity. The deletion of 9q22.31 is inherited from the also affected mother, who presents a milder symptomatic. Furthermore, the clinical significance of the 9q22.31 deletion is emphasized by an unaffected daughter with normal karyotype.

Deletions of 9q22 are mostly associated with the nevoid basal cell carcinoma syndrome (MIM #109400) due to haploinsufficiency of the gene PTCH1 (MIM *601309). The deletion detected in our case does not affect the PTCH1 gene. Siggberg et al. (2011) first described a familial case with a deletion 9q22.2q22.32 not affecting the PTCH1 gene. Phenotypic features of these patients are similar to those of the affected family members in our case. However, the deletion described by Siggberg et al. spans about 5.3 Mb. Therefore, our data help to narrow down the critical region thus improving the characterization of the phenotype caused by a deletion of 9q22.31.

P-CytoG-167

Heteromorphic variants of chromosome 9

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In this study, 334 carriers of heterochromatic variants of chromosome 9 were included, being 192 patients from Western Europe and the remainder from Easter-European origin. A 3-color-fluorescence in situ hybridization (FISH) probe-set directed against for 9p12 to 9q13~21.1 (9het-mix) and 8 different locus-specific probes were applied for their characterization. The 9het-mix enables the characterization of 21 of the yet known 24 chromosome 9 heteromorphic patterns. In this study, 17 different variants were detected including five yet unreported; the most frequent were pericentric inversions (49.4%) followed by 9qh-variants (23.9%), variants of 9ph (11.4%), cenh (8.2%), and dicentric- (3.8%) and duplication-variants (3.3%). For reasons of simplicity, a new short nomenclature for the yet reported 24 heteromorphic patterns of chromosome 9 is suggested. Six breakpoints involved in all four variants could be narrowed down using locus-specific probes. Furthermore, based on this largest study ever done in carriers of chromosome 9 heteromorphisms, a founder effect can be suggested for at least three of the heteromorphic variants in Western Europe. Besides, there is no clear evidence that infertility is linked to any of the 24 chromosome 9 heteromorphic variants. Supported in parts by the Dr. Robert Pfleger Stiftung, the DLR/BMBF (ARM 08/001, BLR 08/004, RUS 11/002), DAAD (D07/00070), NZZ, and the State Committee of Science of the Republic of Armenia (grant number 11-1s-0160).

P-CytoG-168

Human ring chromosomes and small supernumerary marker chromosomes - do they have telomeres?

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Ring chromosomes and small supernumerary marker chromosomes (sSMC) are enigmatic types of derivative chromosomes, in which the telomeres are thought to play a crucial role in their formation and stabilization. Considering that there are only a few studies that evaluate the presence of telomeric sequences in ring chromosomes and on sSMC, here we analyzed 14 ring chromosomes and 29 sSMC for the presence of telomeric sequences through fluorescence in situ hybridization (FISH). The results showed that ring chromosomes can actually fall into two groups: the ones with or without telomeres. Additionally, telomeric signals were detectable at both ends of centric and neocentric sSMC with inverted duplication shape, as well as in complex sSMC. Apart from that, generally both ring- and centric minute shaped sSMC did not present telomeric sequences neither detectable by FISH nor by a second protein-directed immunohistochemical approach. However, the fact that telomeres are absent does not automatically mean that the sSMC has a ring shape, as often deduced in the previous literature. Overall, the results obtained by FISH studies directed against telomeres need to be checked carefully by other approaches.

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P-CytoG-169

Severe Autosomal Recessive Osteopetrosis with Neuronal Involvement due to Homozygous Deletions affecting OSTM1

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Autosomal recessive osteopetrosis (ARO, MIM 259700) is a genetically heterogeneous rare skeletal disorder characterized by failure of osteoclast resorption leading to pathologically increased bone density, bone marrow failure, fractures, and variable neurological complications. An early identification of the underlying genetic defect is imperative for assessment of prognosis and treatment. OSTM1-related malignant infantile osteopetrosis is characterized by a very poor prognosis due to CNS involvement, which cannot be cured by bone marrow transplantation. Here we describe for the first time homozygous microdeletions affecting the OSTM1 gene in two unrelated consanguineous families with children suffering from malignant infantile osteopetrosis with severe CNS involvement and fatal outcome. Microdeletions seem to represent a considerable portion of OSTM1 mutations and should therefore be included in a sufficient diagnostic screening.

P-CytoG-170

De novo microdeletion in 16p11.2 in a boy with severe heart defect and hydrocephalus

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De novo microdeletions and microduplications play a significant role in the aetiology of intellectual disability, developmental delay, congenital anomalies and autism spectrum disorders.

We report on a male patient who was diagnosed with a complex heart defect (double outlet right ventricle, ventricle septum defect, subaortic stenosis), hydrocephalus, dysgenesis of corpus callosum, hydronephrosis, tracheomalacia, chronic respiratory insufficiency and growth retardation. The patient died at the age of seven months.

We performed genome wide copy number analysis using MLPA in order to screen for known microdeletion syndromes. This analysis revealed a copy number loss for three probes in 16p11.2 specific for the genes HIRIP3, DOC2, and MAPK3. The dosage of the MLPA probes specific for the genes MVP and MAZ in 16p11.2 was not altered. To validate and further characterize these findings, we performed high resolution molecular karyotyping using the Affymetrix CytoScan HD Array. Beside several known copy number variants, a copy number loss of 1,488 kb in 16p11.2 (chromosome 16: 29,868,724-31,356,497; hg 19) was detected. This deletion encompassed 78 genes (including MAPK3, HIRIP3, and DOC2) and overlapped distally approximately 330 kb with the known 16p11.2 microdeletion syndrome (MIM: 611913; Shinawi et al., J Med Genet. 2010). This aberration was so far neither observed in the Database of Genomic Variants (Iafrate et al., Nat Genet. 2004) nor in 820 in-house control samples. The microdeletion could not be detected in the parents, suggesting de novo origin. We consider the aberration in 16p11.2 to be disease-causing in our patient. The classical, approximately 550 kb comprising microdeletion in 16p11.2 was reported in patients with autism, congenital anomalies, psychiatric disorders, disturbances of speech and intellectual disability, but was also found in the general population, suggesting extensive phenotypic variability. The wide range of phenotypes associated with the classical 16p11.2 microdeletion, as well as additionally deleted regions (including more than 50 further genes) could explain the severe phenotype in our patient.

P-CytoG-171

Array-based genome-wide genotyping in fetuses with isolated central nervous system (CNS) malformations

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Malformations of the central nervous system (CNS) occur in about 1:200 to 1:500 live births including neural tube defects and malformations of the brain. Similar to most congenital defects, malformations of the brain occur as an isolated phenotype or in association with other defects or syndromes. While multiple lines of evidence suggest that de novo mutations of different size including CNVs (copy number variations) are involved in the expression of some of these malformations, genetic diagnostic workup during pregnancy is mostly restricted to conventional chromosome analysis with little evidence based experience on array-based methods. Nevertheless, due to modern imaging techniques, most CNS malformations are being diagnosed prenatally leading to interruption of pregnancy.

In order to identify putative aberrations we screened a collective of 35 aborted fetuses with isolated malformations of the brain. This cohort was sampled through the Department of Obstetrics at the University of Bonn were DNA of over 300 aborted fetuses with mostly CNS malformations was collected between 2006 and 2012. We performed high resolution molecular karyotyping, utilizing the Illumina HumanOmniExpress-12v1 bead-chip comprising a total number of 730,525 markers. To identify potential CNVs, the fluorescence intensity was analyzed with QuantiSNP using an Objective-Bayes Hidden-Markov model for calling putative CNVs. Preliminary results show probably causative deletions in two fetuses concerning the X-chromosomal region p22.2-p22.33 (9,1 Mb; 32 RefSeq genes) and the region 6p25.1-6pter (4,38 Mb; 30 RefSeq genes). The deletion of the short arm of chromosome 6 comprises the FOXC1-gene known to be involved in the etiology of Dandy-Walker malformations. These observations support the previously described observation of causative CNVs respectively micro-aberrations in the development of isolated malformations of the brain and suggest the use of array-based diagnostics in prenatally diagnosed CNS malformations.

Based on these findings, further elaboration of the data might show more chromosomal aberrations, probably causally related to malformations of the central nervous system.

P-CytoG-172

Microdeletions and –duplications in 3p26.3: association with non-syndromic intellectual disability and epilepsy.

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To date, terminal 3p deletions confined to the CHL1 gene have been described in patients with intellectual disability (ID) and epileptic seizures. Here, we report for the first time a 3p26.3 duplication detected by array CGH including only the CHL1 gene in a girl with non-syndromic ID and epilepsy. The microduplication was inherited from the healthy father. Reduced penetrance has also been described in previously published 3p26.3 deletions. Further studies are needed to elucidate the pathogenic mechanism of 3p26.3 imbalances and to clarify their role for genetic counseling. However, we suggest that CHL1 is a dosage sensitive gene with an important role for normal cognitive development since both CHL1 deletions and duplications seem to be associated with non-syndromic ID.

P-CytoG-173

SNP Arrays in Epilepsy: Experiences in 100 Pediatric Cases

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Copy number changes (CNVs) have been shown to play an important role in many neurodevelopmental disorders, such as schizophrenia, autism or intellectual disability. In Epilepsy, one of the most common neurologic disorders, the underlying genetic components are currently being elucidated. In about 10% of generalized epilepsies, rare CNVs collectively have been made responsible for the disease, including few recurrent CNVs at 15q13.3, 15q11.2 and 16p13.11. These recurrent CNVs are known to predispose to e.g. autism and schizophrenia, suggesting a common underlying genetic etiology in epilepsy and these neurocognitive disorders.

We present CNV data from 100 pediatric cases admitted for diagnostic work-up due to generalized epilepsy. Copy number changes were detected by use of genome-wide SNP arrays (Affymetrix 6.0, Affymetrix CytoScan HD) according to standardized protocols. All CNVs were examined manually for potential clinical relevance by a geneticist in accordance to internationally published guidelines.

In total, 489 CNVs > 200 kb were identified in our 100 cases, with in average 4 CNVs per patient. 23% of all patients carried a CNV requiring additional analysis (e.g. validation by FISH/qPCR, testing of parental inheritance). This left us with 11 copy number aberrations of clinical significance, representing 7% of all deletions and 1% of all duplications called. 70% of these pathogenic copy number aberrations were > 1 Mb in size. Half of all 10 patients with a clinically significant aberration harbored a copy number change in a recurrent microdeletion/microduplication region associated with e.g. autism. Interestingly, at least one CNV of unknown clinical significance has been detected in addition, in these cases. Recently, a putative impact of such additional larger CNVs to disease phenotype and phenotypic variability has been suggested.

In summary, molecular karyotyping by genome-wide SNP arrays enabled identification of clinically significant copy number aberrations in about 10% of 100 pediatric cases with generalized epilepsy. Despite the success rate, these pathogenic aberrations represent only 2.2% of all detected CNVs in our cases. It should be taken into consideration, that an extensive amount of clinical genetic expertise and additional testing is necessary to extract these essential conclusions.

P-CytoG-174

Chimerism as a Possible Cause For Mosaicism of Structural Chromosome Aberrations: Report on a Familial 6;16-Translocation

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Mosaicism for balanced reciprocal translocation (BRTM) in man is an extremely rare phenomenon. To date, 29 cases of BRTM have been reported to our knowledge. The prevalence, clinical significance and the mechanisms of origin of this type of chromosomal anomalies are not well known. Here we report on the clinical review and molecular cytogenetic results of familial translocation t(6;16)(q26;q24.1). It was found in unbalanced form in a male infant referred to us due to his multiple congenital malformations. Evaluation of the father revealed a balanced form. Analysis of the paternal aunt shows a mosaic state of the familial translocation.

The boy was the second child of healthy parents. He was born at the 35th week GA weighing 2,175 g. Clinical examination revealed a tetralogy of Fallot, hypoplasia of corpus callosum, hydrocephalus, microcephaly, muscular hypotonia, anal atresia, flat philtrum and widely spaced nipples. He died at the age of 11 month.

Conventional karyotyping on a 450 band level revealed a normal result. Additionally performed FISH for 22q11.2 microdeletion was negative. CGH-Array indicates a 8,76 Mb deletion on chromosome 6q26-qter. The initially diagnosed microdeletion 6q was confirmed by FISH analysis using 6qter subtelomeric FISH-probes. Cytogenetic screening of the boy's parents shows a normal karyotype of the mother and a balanced translocation t(6;16)(q26;q24.1) of the father, which was confirmed by FISH. Subsequently, the index patient was reviewed for unbalanced translocation, resulting in deletion 6q and until then not diagnosed duplication of 16q. The suspicion of 16q-duplication, which failed in aCGH detection, was confirmed by FISH. So the index patient carried the unbalanced inherited translocation of his father. Subsequently, the boy's paternal aunt underwent genetic counseling for clarification of carrier status of possible familial reciprocal translocation. Conventional cytogenetic on a 500 band level revealed a normal karyotype in 20 metaphases.

Additionally performed subtelomeric screening by FISH to confirm the cytogenetic result showed a low-level mosaic 46,XX/46,XX,t(6;16)(q26;q24.1) in lymphocytes.

This uncommon and rare diagnostic finding can result either from twin-to-twin transfusion syndrome or from a tetragametic chimera, formed by the fusion of two independent zygotes. Even if chimerism is considered to be a rarity in man, it is possible that known cases represent only a small number of the true incidence, as clinically inconspicuous chimeric individuals remain undiscovered. Nevertheless, both types of origin will be discussed as possible causes for the surprisingly found low-level mosaic.

P-CytoG-175

Mechanisms underlying non-recurrent microdeletions causing neurofibromatosis type-1 (NF1)

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NF1 microdeletions encompassing the NF1 gene region at 17q11.2 are present in 5-10% of patients with NF1. Whereas the mechanisms underlying recurrent NF1 microdeletions have been investigated in greater detail, those underlying non-recurrent (atypical) NF1 microdeletions are not well delineated. NF1 microdeletions with non-recurrent breakpoints are heterogeneous in terms of their size, breakpoint position and number of deleted genes. Furthermore, extended sequence homology is not observed in the respective breakpoint regions. In this study, we investigate 22 atypical NF1 deletions using high resolution custom made array CGH. We could assign the breakpoints to regions of a few kb. In twelve of these 22 atypical NF1 deletions, we identified the breakpoints at the level of a few basepairs. Nine of these twelve deletions were mediated by non-homologous end joining (NHEJ) as concluded by the absence of or only minor (1-2bp) homology at the breakpoints. Three of these twelve NF1 deletions exhibited microhomologies of 24 to 37 bp at the breakpoint sites indicative of microhomology-mediated end joining (MMEJ) as underlying mechanism. The other 10 atypical NF1 deletions were not yet identified at highest resolution but we were able to narrow down the breakpoint localization to a few kb according to MLPA and array results. All together, these analyses indicated that the 22 deletions are highly variable in size ranging from 518kb to more than 5.9Mb.

Moreover, 16/22 atypical NF1 deletions have their centromeric breakpoint located in SUZ12P. The enrichment of atypical NF1 microdeletion breakpoints in SUZ12P is remarkable since the breakpoints of the recurrent type-2 NF1 deletions are also located in SUZ12P. DNA DSBs seem to frequently occur in SUZ12P. We hypothesize that DSBs in SUZ12P either result in a type-2 NF1 deletion or an atypical NF1 deletion, depending upon repair protein availability, the presence of a homologous (but non-allelic) template and the occurrence of a second DSB in the NF1 gene region.

We further propose that NHEJ or MMEJ are the prevailing mechanisms underlying non-recurrent NF1 deletions that lack any complexity at the deletion breakpoint sites. This is supported by our breakpoint analyses, since the breakpoint-junctions of the twelve atypical NF1 deletions, which were characterized already at highest resolution, either showed blunt ends or microhomologies.

Nevertheless, we can not completely exclude FoSTeS/MMBIR as the underlying mechanism for the occurrence of atypical NF1 deletions. However, it seems to be less likely as these repair mechanisms are highly error prone with a high frequency of nucleotide alterations at the breakpoint junctions. As we did not observe such alterations at the breakpoint-junctions of the twelve atypical NF1 deletions, which were characterized already at highest resolution, we favour NHEJ/MMEJ as the mechanism underlying the development of atypical NF1 deletions. The characterization of all 22 atypical NF1 deletions identified by us will indicate to which extent also replication-associated mechanisms are causatively associated with non-recurrent NF1 microdeletions.

P-CytoG-176

First molecular cytogenetic high resolution characterization of the NIH 3T3 cell line by murine multicolor banding

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Since being established in 1963 the murine fibroblast cell line NIH 3T3 has been used in thousands of studies. NIH 3T3 immortalized spontaneously and became tetraploid shortly after its establishment. Here we report the first molecular cytogenetic characterization of NIH 3T3 using fluorescence in situ hybridization based multicolor banding (mcb). Overall, a complex rearranged karyotype presenting 16 breakpoints was characterized. Also it was possible to deduce the resulting gains and losses of copy numbers in NIH 3T3. Overall, only 1.8% of the NIH 3T3 genome is disome, 26.2% tri-, 60% tetra-, 10.8% quinta- and 1.2% hexasome. Strikingly, the cell line gained only four derivative chromosomes since its first cytogenetic description in 1989. An attempt to align the observed imbalances of the studied cell line with their homologous regions in humans gave the following surprising result: NIH 3T3 shows imbalances as typically seen in human solid cancers of ectodermal origin. Supported by the DFG (LI 820/17-1, 436 RUS 17/49/02, 436 RUS 17/135/03, 436 RUS 17/48/05, LI 820/19-1).

P-CytoG-177

The long shadow of parental genomes - organizing the next generation nuclei

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The distribution pattern and behavior of chromosomes follow some order that is not well studied because of technical limitations. The lack of methods to determine the parental origin of chromosomes and to follow them through cell cycle stages was recently filled by the invention of parental-origin-determination FISH (pod-FISH), based on chromosome specific copy number variants (CNVs) visualized by fluorescence intensity differences (Weise et al. 2008). Mammalian germ cells like spermatocytes as well as early embryonic cells had been studied earlier by methylation staining: parental order and genomic separation of parental chromosome sets were detected. Although this parental methylation pattern disappeared, the chromosomes remained aligned in the interphase (chromosome territories) and even in the metaphase as suggested by us previously (Chaudhuri and Reith Analyt Quant Cytol Histol 19:30-36, 1997; Chaudhuri and Walther Int J Oncol 23:1257-62, 2003; Chaudhuri et al. Cellular Oncol 27:327-34, 2005; Anticancer Res 28:3573-78, 2008¸ Chaudhuri and Walther Planta medica, 2012). Here we present application of pod-FISH to study the organization of the human nucleus on metaphase-spreads. Despite extensive investigation of metaphases of peripheral blood lymphocytes in the past, so far no approaches were available to label the chromosomes in a parental origin specific manner. In summary, we found i) bilateral symmetry of metaphases leading to haploid sorting of homologous chromosomes and ii) a parent of origin grouping of these haploid groups. This indicates i) a higher order of chromosome architecture in the cell which is caused by the parental origin of the homologous chromosomes, ii) this higher order is not limited to metaphase chromosomes but also an immanent feature of interphase nuclei, iii) the cell distinguishes homologous chromosomes by the parental origin and iv) besides horizontal sorting (equatorial plane) of chromosomes during metaphase there is a vertical sorting by parental origin. About the influence and consequences on phenotype and diseases of this parental chromosome grouping we may only speculate at this time. Supported by a grant from Carl Zeiss MicroImaging GmbH, Germany.

P-CytoG-178

Case report: A microdeletion in the RBFOX1 gene in a 15-year-old boy with autism spectrum disorder and mental retardation

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Autism spectrum disorder (ASD) is a common childhood neurodevelopmental disorder characterized by marked genetic heterogeneity. Core disturbances in children with ASD are impaired social interaction and communication and restrictive and repetitive behaviour. Patients may present with additional features such as mental retardation (in 25-70% of cases). Autism has a strong genetic basis and so far only few causative genes have been identified. Rare copy number variants in the RBFOX1 gene (a muscle- and brain specific splicing factor) have been shown to be associated with cognitive impairment, attention deficit disorder, microcephaly and ASD. RBFOX1 is an important regulator of alternative splicing in the developing and adult brain and many RBFOX1 targets are factors with known synaptic or neurodevelopmental function. The RBFOX1 gene is transcribed from multiple promoters resulting in various RBFOX1 isoforms that differ in their N-termini. A central RNA recognition motif (RRM) that mediates target mRNA binding is common to all these isoforms. Here, we report on a heterozygous ~100 kb deletion in the RBFOX1 gene in a 15-year-old boy with ASD and mental retardation (almost level 5 evidence of pathogenicity). Specifically, the promoter and first exon of the transcript variant 6 (first exon,non-coding; RefSeq: NM_001142334) were deleted in the patient. Because the promoter of this variant serves, at the same time, as a large intron for several of the other RBFOX1 variants, we hypothesize that conserved sequence blocks found in the deleted region may also act as enhancers of transcription of the other transcript variants. Multiple lines of evidence suggest that RBFOX1 is a candidate gene for ASD and mental retardation. Thus, several other patients have been described in the literature with disruptions in the RBFOX1 gene and presenting with ASD and mental retardation (Sebat et al. 2007 Science 316:445-449; Martin et al. 2007 AJMG 144B:869-876; Mikhail et al. 2011 AJMG 155A:2386-2396; Davis et al. 2012 AJMG 158A:1654-1661). This case report further establishes the role of copy number variants in the RBFOX1 gene in the etiology of ASD and mental retardation.

P-GENETIC COUNSELLING / EDUCATION / GENETIC SERVICES / PUBLIC POLICY

P-Counse-179

Clinical Utility Gene Cards and the role of genetic guidelines in the clinic

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CUGCs are disease-specific guidelines authored by international expert groups. Based on the ACCE framework they are dealing with the risks and benefits of the application of genetic tests in the clinical setting. Each document represents a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues. CUGCs offer quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers. In order to satisfy the highest standards of quality CUGCs have to pass through a carefully designed editorial process. Authors are selected on the basis of objective evidence of expertise, including their publication record. First authors are requested to invite a group of co-authors encouraging international representation. All CUGCs undergo peer review. The same procedure is applied to updates. Each CUGC is peer-reviewed and published by the European Journal of Human Genetics. CUGCs are also freely available on the websites of EuroGentest, the European Society of Human Genetics and Orphanet.

The CUGCs are well received by the scientific and clinical community: the projected rate of downloads from the journal's website per gene card and year ranges between approximately 600 and 1500, with an average above 1000. The Clinical Utility Gene Cards represent either alternatives or complements to other guideline collections serving similar purposes. A recently conducted qualitative expert survey further analyses the role of genetic guidelines in the clinic. The results show that genetic guidelines are suitable not only for disease diagnosis and management, but also the assessment of evidence for clinical validity and utility and the alteration of genetic test application and clinical management.

P-Counse-180

High throughput DNA extraction, WGA and targeted next generation sequencing (NGS)

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Biobanking and project support services play an increasingly important role in genomics research across a range of disciplines including health sciences, agriculture, and studies relating to environmental sustainability. Bringing together different techniques and applications from sample storage and preparation to analysis and data interpretation has become key in delivering services to support genomics research.

Similarly, expanded sequencing capacity is delivering new regions of interest for genome analysis, and the links between biobanks and next generation sequencing laboratories have become crucial elements in successful cohort studies as NGS technologies have grown to complement and replace Sanger sequencing in the clinic. In turn, the needs for NGS sample preparation and long term storage have become key parts of many research projects.

To meet these new challenges LGC Genomics has expanded laboratory capacity to enable delivery of high throughput DNA extraction with full QC and sample normalisation support married to follow on services for SNP and InDel marker genotyping, amplicon based targeted resequencing (aTRS)and mitochondrial aTRS. Our whole genome amplification service also plays an important role in many projects where expansion of stored DNA and the development of specific marker assays for use in future studies is required.

This publication provides a brief overview of the range of technologies and instrumentation solutions which LGC Genomics delivers for global genetic research in the areas of sample preparation, whole genome amplification, genotyping and DNA sample storage.

P-Counse-181

Drivers, Barriers and Opportunities for Genetic Testing Services in Emerging Economies: The International GenTEE Project

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Background: Due to the epidemiological transition in the emerging economies of China, East Asia, India, Latin America, the Middle East and South Africa, these economies are facing (i) an increasing proportion of morbidity and mortality due to congenital and genetic conditions, (ii) a rising need for genetic services to improve patient outcomes and overall population health. These economies are facing the challenge how: (i) to ensure the successful translation of genetic/genomics laboratory and academic research into quality assured pathways, (ii) to develop a service delivery infrastructure that leads to equitable and affordable access to high quality genetic/genomic testing services.

Objectives: (i) to document and compare current practices and the state of genetic service provision in eight emerging economies: Argentina, Brazil, China, Egypt, India, Oman, Philippines and South Africa, (ii) to identify current knowledge gaps and unmet service needs. The GenTEE international project is intended to inform policy decisions for the challenges of delivering equitable high quality genetic services and to promote international collaboration for capacity building. Methods: (i) a standardized survey that is the first of its kind worldwide that allows comparison of services internationally across a number of key dimensions by using a core set of indicators, selected by the GenTEE consortium for their relevance and comparability, (ii) capacity building demonstration projects. To date, the GenTEE project has completed its survey that maps the current state of genetic services in the participating emerging economies and identifies current drivers, barriers and opportunities for genetic services development.

Results: There is no equitable access to genetic services in all countries mainly due to financial barriers (underfunded fragmented public services, out-of-pocket expenses tend to be the norm for genetic testing services), geographical barriers (concentration of services in main cities) and skill gaps, resulting in inequitable services or delayed access. The development of services in the private sector is mostly opportunistic and technology and market driven. There is a marked lack of standard operating procedures and agreed quality assessment processes for new technologies.

Discussion: International collaborative networks can provide support for capacity building and help to strengthen the provision of quality genetic/genomic services in emerging economies.

P-Counse-182

The Heredity of Diseases and Public Policy in Germany. A Historical Retrospection.

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„Die Zeugung eines Kindes sollte dann aus eugenischer Indikation vermieden werden, wenn eine unzumutbar hohe Chance dafür besteht, dass es eine sein Wohlbefinden und seine Gesundheit ernsthaft beeinträchtigende erbliche Anomalie aufweisen würde." [1]

Reproduction should be avoided for eugenic reason, when there is an unacceptable high risk that an aberration will have great influence on health and well-being of the child. This stated the human geneticist Friedrich Vogel in 1961. [1] Through the times this was also the interest of public policy.

(A) Since the rediscovery of the Mendelian laws in 1900 there was an increasing interest on heredity of diseases in the society. The general living conditions were improving; the mortality of men in propagable age and also of children was decreasing. The consequences were an increase of hereditary diseases. Therefore there was raised the question on the biological future of mankind as a starting point for possible consequences.

(B) The next step were rules for reproduction, regarded as eugenic ones, like the Law for the Prevention of Hereditarily Diseased Offspring (Gesetz zur Verhuetung erbkranken Nachwuchses) in 1933 (active since January 1934). That meant every person with the diseases Congenital mental Deficiency, Schizophrenia, Manic-Depressive Insanity, Hereditary Epilepsy, Hereditary Chorea (Huntington's), Hereditary Blindness, Hereditary Deafness and any severe hereditary deformity must be sterilized. This law ignored at all the autonomy and as well the necessary consent of the person concerned. This was violation of existing law. Another Law for the Protection of Hereditary Health of the German (1935) said that everyone has to have a certificate of capability for marriage (Ehetauglichkeitszeugnis) for engaged couples and did forbid marriage for people with certain hereditary diseases. Both laws were said to be motivated by knowledge in human genetics.

(C) In the 1960ies human genetics had established at the universities. Based on the conclusions of the Marburger Symposium on Genetics and Society there was set up a project on genetic counselling. With the support of the Stiftung Volkswagenwerk there were established two outreach clinics for human genetic advice. Examples were Frankfurt for cities and Marburg for small towns with great catchment area. This project was successful and therefore later the health insurances paid for the counselling. This was followed by the need for genetic counselling because of the possibilities of prenatal diagnosis and DNA sequencing.

(D) After 1945 the first law that regulated the use of human genetic diagnostic was the so called Genetic Diagnostic Law (Gendiagnostikgesetz) in 2009. This is on prenatal diagnosis as well as on adults. It is said that this law should regulate the way genetic diagnostic is performed and also prevent discrimination of people because of genetic characteristics. Autonomy and Informed consent of patients is self-evident.

Conclusions:

(1) There was a great time interval between the discriminating laws in the 1930ies and the law on human genetic diagnostics in 2009. The misuse of human genetic knowledge in the Third Reich for a long time influenced the discussion in Germany and prohibited legal regulations.

(2) Also, there is continuity in using human genetic knowledge for public policy.

(3) Autonomy and Informed Consent were valid for all laws in German history. During dictatorship those were neglected.

P-Counse-183

Rare Disease Centre at Hannover Medical School: First Experiences

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A disease which affects not more than 5 in 10,000 people is considered to be rare. Therefore 7,000-8,000 of 30,000 known diseases are categorized as rare diseases, with a great majority being of genetic origin. According to WHO estimates some 30 million people suffer from a rare disease in the European Union. After the European Council recommended an action in the field of rare diseases in 2009, national strategies were set up in many European countries. In Germany the formation of many centres of expertise has taken place prior to the enacting of a national plan.

The Rare Disease Centre at Hannover Medical School was initiated by the Department of Human Genetics and opened in November 2011. An interdisciplinary facility where research and care for patients with known diagnosis and patients with chronic conditions not yet having received a definite diagnosis was set up. All patient referrals including self-referrals have been documented in detail. A patient registry is being developed. Between 30 and 50 paediatric and adult patients were referred per month with a rising tendency over time. Preliminary data suggest that the majority of requests are self referrals (82% after 1 year) and only about 14% are referrals from primary care physicians. More than half of the patients did not have a reliable diagnosis at referral. As expected most of the undiagnosed patients were asking for a diagnosis and patients with known diagnosis were asking for experts. Interestingly more than 30% of patients were seeking for information, indicating a lack of information available on rare diseases. As a unique feature the German Orphanet team is part of the Hannover Rare Disease Centre. Orphanet is the reference portal for information on rare diseases and orphan drugs particularly in Europe.

Here, we will report our experience of the first year, the organizational structure and financing of the Rare Disease Centre at Hannover Medical School including its 11 subcentres and specialized clinics, describe internal patient paths, diagnostic successes and failures, enrolment of patients in research projects, and compare our structure and experience with the few other centres in Germany and elsewhere, from which data are available. We will present an outlook into future regional, national and international networking of rare disease centres.

P-MONOGENIC DISEASE – FROM GENE IDENTIFICATION TO PATHOMECHANISM

P-MonoG-184

Results of massive parallel sequencing of 60 families with autosomal recessive intellectual disability: from mutation identification to proof of pathogenicity

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We ran systematic genome wide linkage or autozygosity mapping in 133 consanguineous families with autosomal recessive intellectual disability (ARID). We then massively parallel sequenced the index patients of 60 families in several stages of different enrichment methods (Agilent SureSelect 37 Mb, 50 MB, and Targeted Kit) and next generation sequencing (NGS) configurations (Solexa, SOLiD 4, SOLiD 5500xl, single end, paired end). 25 of the patients were sequenced more than once in order to reach highest possible coverage and to compare different configurations and strategies. We then considered the variants in linkage regions and excluded common ones based on public databases (1000genomes and Exome Sequencing Project) and internal database (over 200 exomes). We prioritized the remaining variants based on their function (splice site or non-synonymous) and in silico prediction (PhyloP, PolyPhen, SIFT, MutationTaster, and others). We then excluded technical artefacts by Sanger sequencing, tested for segregation, and genotyped the variants in a large ethnically matched control cohort.

We clarified the etiology in nine families by identifying at least one obvious pathogenic mutation in the known intellectual disability genes AIH1 (2x), ALDH5A1, CC2D1A, C12orf65, GCDH, GPR56, HGSNAT, and CRBN. In further 19 families we identified one convincing candidate mutation each (EDC3, EZR, FAR1, HMG20A, and others). In 15 families we identified more than one candidate mutation and further evaluation is thus needed before initiating functional analyses. Seven families are still in evaluation process and in 10 families the underlying mutation remained undetected. Reasons for not detecting the causing mutations are many; e. g. mutations are in non-coding sequencing or coverage is incomplete.

Eligible candidate mutations underwent exhaustive bioinformartic analyses regarding protein domains, folding, and translational modification, and working hypotheses were generated. E. g., we showed that the mutation in EZR leads to blocking growth factor induced Ras activity in NIH3T3 cells, we showed an effect of the mutation in HMG20A on expression regulation in mutant HeLa cell lines, and we support the

pathogenicity of a gene in the Glycosylphosphatidylinositol anchor biosynthesis through identification of a second mutated allele and through pathway analysis.

Our results show that linkage analysis followed by targeted enrichment and massive parallel sequencing is an effective method to identify mutations in known and novel genes in ARID. Proving pathogenicity of the identified mutations is becoming the indispensable following step. Furthermore, our data including mutations in known genes demonstrates that clinical delineation of the resulting phenotypes is of high importance.

P-MonoG-185

Skeletal dysplasia in a consanguineous clan from the island of Nias/Indonesia is caused by a novel mutation in B3GAT3

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Nias is a unique island belonging to Indonesia. The people of Nias are still organized into patrilineal descent groups or clans. In contrast to other Southeast Asia island populations, studies on genetic diversity in Nias revealed severe bottleneck or founder events in its history giving rise to a high incidence of endemic syndromes. We describe one family with disproportionate short stature and bone dysplasia in two branches. Interestingly, the affected individuals of one of the two branches displayed a milder phenotype than the other. We conducted a genome-wide linkage scan in the branch with the more severe phenotype and determined a candidate region of 10.2 cM on chromosome 11. Sequencing of individual candidate genes did not reveal the disease-causing variant. We then performed whole-exome sequencing in one affected child. By scrutinizing variants in the linkage interval on chromosome 11 we noticed a possibly damaging homozygous missense variant in the gene B3GAT3. All affected individuals of the Nias clan are homozygous for the variation that could not be detected in 350 chromosomes of ethnically matched controls. Recently, Baasanjav et al. published their results on a family characterized by joint dislocations and heart defects caused by a different mutation of the same gene, c.830G>A (p.Arg277Gln). Here we describe a second mutation of B3GAT3 associated with skeletal dysplasia, c.419C>T (p.Pro140Leu). No heart involvement was known but examination of one affected individual is scheduled to figure out if a heart phenotype is present in our family as well. B3GAT3 encodes β-1,3-glucuronyltransferase (GlcAT-I). GlcAT-I catalyzes an initial step of proteoglucan synthesis by transferring glucuronic acid onto the trisaccharide-protein linkage structure of proteoglycans. The mutation p.Pro140Leu lies within the donor substrate binding subdomain of the catalytic domain of GlcAT-I. A proline-to-leucine exchange could influence the correct folding of the protein since the affected proline residue seems to be located on the surface according to crystallographic data. A multiple alignment shows that p.Pro140 is highly conserved among mammals, amphibians and insects. Functional studies are in progress in order to analyze the degree of damage caused by the mutation at the cellular level. Preliminary results show that the recombinant GlcAT-I (p.Pro140Leu) exhibited a significant reduction in the enzymatic activity compared to the wild-type enzyme.

P-MonoG-186

Effect of Lamin B receptor deficiency on lipid metabolism

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The lamin B receptor (LBR) is a multifunctional protein of the inner nuclear membrane. It´s N-terminal part binds among others to lamin A/C and modifies chromatin. It´s C-terminal transmembrane domain exhibits sterol reductase activity. A mouse model of Lbr deficiency is the ichthyosis mouse (icJ/icJ). These mice exhibit chromatin changes, ichthyosis and alopecia among other phenotypic features. In addition, lipid metabolism seems to be effected. To investigate the effect of the sterol-reductase on lipid metabolism we determined fat distribution in abdomen and leg, composition of fatty acids in the abdomen and liver and analysed the content of lipids in the muscle using 1H MRI.

Male icJ/icJ mice (6-8 months) gain less body weight compared to wild type (WT) animals (45.5 ± 2.8 g for WT, 36.0 ±1.0 g for icJ/icJ; $p<0.01$) and have a slightly lower total fat content in the abdomen (8.2 ± 2.8

au/ml for WT, 6.1 ± 0.5 au/ml for icJ/icJ; p=0.28). In depth characterisation of lipid distribution revealed decreased visceral fat content in the abdomen of icJ/icJ mice (6.5 ± 2.3 au/ml for WT, 3.6 ± 0.5 au/ml for icJ/icJ; p=0.09) and a significantly increased superficial fat content (0.08 ± 0.04 au/ml for WT, 1.15 ± 0.16 au/ml for icJ/icJ; p<0.001) whereas deep subcutaneous fat content in icJ/icJ mice is unaltered. Total fat content, visceral and subcutaneous fat content in legs of icJ/icJ mice are increased as well albeit not significantly. To further investigate the effect of Lbr deficiency on lipid metabolism mean chain length of fatty acids in the visceral abdominal fat was determined by MR spectroscopy. No difference between WT and icJ/icJ mice was observed. Analysing the contributions of different fatty acids we found no difference in the fraction of unsaturated fatty acids, but a significant increase of monounsaturated fatty acids (21.2 ± 2.6 % for WT, 35.2 ± 7.6 % for icJ/icJ; p<0.05) and a significant decrease in polyunsaturated fatty acids (40.1 ± 5.0 % for WT, 34.2 ± 1.8 % for icJ/icJ; p=0.02). We next explored the influence of Lbr deficiency on liver fat content and observed a decrease in icJ/icJ mice compared to WT animals (9.1 ± 4.2 %fat/water for WT, 4.2 ± 1.9 %fat/water for icJ/icJ; p=0.19). To finally characterize the rate of ectopic lipid accumulation intramyocellular and extramyocellular lipid levels (IMCL and EMCL) were determined. Tibialis muscle revealed a slight increase of both IMCL and EMCL in icJ/icJ mice, whereas in soleus muscle IMCL is significantly increased (IMCL: 0.65 ± 0.18 au for WT, 0.96 ± 0.10 au for icJ/icJ; p=0.02).

Our study demonstrates an alteration of lipid distribution in Lbr deficient mice in abdominal visceral fat, abdominal subcutaneous fat and fat accumulation in legs. This is underlined by the increase in IMCL and EMCL. In addition, the accumulation of monounsaturated fatty acids and the low amount of polyunsaturated fatty acids suggests an influence of Lbr on the desaturation of fatty acids in vivo.

P-MonoG-187

De novo 45 kb microdeletion 22q13.33 comprising the major portion of SHANK3 in a girl with delayed speech development as predominant clinical feature

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BACKGROUND: The chromosome 22q13.3 deletion syndrome, also known as Phelan-McDermid syndrome (PMS, OMIM #606232), is a microdeletion syndrome characterized by haploinsufficiency of the SHANK3 (SH3 and multiple ankyrin repeat domains 3) gene, which comprises 23 exons spanning over 58 kb. The size of patients' deletions involving the SHANK3 gene either partially or entirely is highly variable, ranging from roughly 100 kb to more than 9 Mb. SHANK3 encodes a scaffold protein in postsynaptic densities of excitatory synapses, connecting membrane bound receptors to the actin cytoskeleton. Currently all described 22q13.3 deletions causing clinical symptoms involve SHANK3 disruption. Major features of PMS include neonatal hypotonia, moderate to severe intellectual impairment, severe or absent expressive language delay, autistic-like behavior, normal to mildly reduced growth rates, and characteristic facial dysmorphism.

CASE REPORT: We report on a 4-year old girl presenting with delayed speech development as predominant clinical feature as well as mild mental and growth retardation. At the age of 2 her speech production was confined to few words with no indication of limited speech perception. Her verbal skills remained on that noticeably low level up to the time of presentation. Clinical testing provided a reliable diagnosis for an unremarkable hearing ability. Her weight, height and head circumference were each at percentile 20 (P20), her hands and feet at P4 and P1, respectively. Her capacity of awareness and attentiveness to her environment was described below average. Nevertheless she attended a kindergarten for non-handicapped children. Dysmorphic aspects not in accordance with her 3 healthy siblings, 1 to 6 years old, could not be diagnosed.

Following conventional chromosomal analysis, which did not show a remarkable result, molecular karyotyping using Array-CGH (180K Microarray) revealed a de novo 45 kb microdeletion in 22q13.33, which involved about 67% of SHANK3 (58 kb) and a portion (<1 kb) of the nearby located 2 kb spanning ACR gene. Array-CGHs of the parents did both not show any loss in 22q13.33. Subsequently these results were all confirmed by FISH analysis with a SHANK3-specific 40 kb probe. Quantitative PCR (qPCR) reconfirmed the extensive SHANK3 deletion and helped locating one breakpoint inside the gene. According to DECIPHER Database queries only 2 other children have been described carrying microdeletions only in SHANK3 and ACR. In both cases these microdeletions are more extended (approx. 60 kb) and comprise a longer portion of ACR, which is not known to cause any developmental retardation. Those index patients were diagnosed with significant mental retardation, one of them with speech delay and microcephaly as well.

CONCLUSION: Our results show the currently smallest SHANK3 deletion described in literature and provide new evidence for this gene´s crucial role in speech development. Array-CGH proved to be a powerful method to narrow down suspicious genomic regions to specific candidate genes for developmental

delay. Subsequent FISH analysis with a probe covering most of SHANK3 was an elegant way to confirm our findings. However, further investigations with similar deletions and precise clinical descriptions are necessary to improve the understanding of SHANK3 genotype-phenotype correlations.

P-MonoG-188

Effects of lamin B receptor deficiency on the immune system in mice

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The lamin B receptor (Lbr) is a transmembrane protein of the inner nuclear membrane which binds lamin A/C and chromatin with its N-terminal domain and exhibits sterol reductase activity with its C-terminal membrane spanning domain. An established animal model for the analysis of Lbr function is the icJ/icJ-mouse. Loss of Lbr in these mice results in ichthyosis and premature death. Aim of the study was to investigate the influence of Lbr deficiency on the immune system in the icJ/icJ-mice. Therefore, frequency and composition of lymphocytes from spleens were determined by flow cytometry. Proliferation activity in the presence and absence of anti-CD3+ antibody stimulation as well as the production of IL-2, TNFα and IFNϒ with and without stimulation by SEB was additionally assessed.

Analysis of the phenotype demonstrated a lower weight of the icJ/icJ-animals (p=0.001 compared to wild types (wt) and heterozygotes (+/icJ)). Surprisingly, the total number of lymphocytes derived from spleens increased from wt over +/icJ to icJ/icJ-animals (wt: 9.3*107 ± 1.8*107 cells; +/icJ: 12.0*107 ± 2.5*107 cells; icJ/icJ: 16.8*107 ± 1.8*107 cells/ml). Flow cytometric analysis showed an up-regulation of CD73, but not of CD39 in the CD4+ T-cell population of +/icJ-mice with C57BL/6 background (compared to the wt). Both CD39 and CD73 are located at the cell surface and convert ATP to adenosine, which has an anti-inflammatory effect. Comparing the proliferation rate of lymphocytes from the three different genotypes after stimulation with anti-CD3+ antibody, a higher number of dead cells were found in icJ/icJ-mice. Moreover, CD4+ and CD8+ cells from icJ/icJ-mice showed a significantly lower proliferation rate than those from +/icJ- and wt-animals ($p < 0.001$). The inflammatory state of the lymphocytes determined by ELISA demonstrated in unstimulated lymphocytes a significantly higher IL-2 secretion in the +/icJ- (3.81 ± 2.00 pg/ml; $p = 0.01$) and the icJ/icJ-mice (5.62 ± 0.18 pg/ml; $p = 0.01$) when compared to wt cells (0.41 ± 0.56 pg/ml). After stimulation with SEB secretion of IL-2 did not significantly differ between the three genotypes. The IFN secretion followed the same pattern as IL-2 secretion, but did not reach significance. In contrast, unstimulated lymphocytes from +/icJ- (3.69 ± 1.92; $p = 0.04$) and icJ/icJ-spleens (2.22 ± 1.98 pg/ml; $p = 0.02$) showed a significantly decreased level of TNFα secretion compared to T cells from wt-animals (12.84 ± 7.62 pg/ml). This effect is even more pronounced in SEB-stimulated lymphocytes (wt: 26.76 ± 11.99 pg/ml; +/icJ: 4.43 ± 0.62 pg/ml, $p = 0.005$; icJ/icJ: 7.79 ± 3.66 pg/ml, $p = 0.03$).

Taken together our findings demonstrate an influence of the Lbr deficiency on the lymphocyte repertoire and activity which is characterized by an increased number of lymphocytes, but also by an altered activation as shown by the changed secretion of inflammatory cytokines.

P-MonoG-189

A mosaic intragenic EFNB1 deletion in a female with Craniofrontonasal syndrome (CFNS)

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Craniofrontonasal syndrome (CFNS [OMIM 304110]) is a X-linked disorder characterized by a more severe manifestation in heterozygous females than in hemizygous males. This genetic paradox has been explained by random X inactivation in females leading to cellular interference of mutant and wildtype cell populations which appears to be the main reason for variable expressivity and for the severity of disease manifestation in females. Hemizygous males show hypertelorism and occasionally cleft lip and palate. In contrast the manifestations in heterozygous females include frontonasal dysplasia commonly including hypertelorism, bifid nasal tip and coronal craniosynostosis. Extracranial manifestations such as body asymmetry and skeletal abnormalities can be observed.

Previously, an intragenic deletion encompassing exons 2-5 in the EFNB1 gene (OMIM 300035) has been identified in a family with CFNS (Wieland et al. 2004, AJHG 74, 1209-1215). Clinically affected members included the mother, a daughter and a granddaughter. Two mutation-carrying sons showed no clinical signs beside hypertelorism. Hemizygous deletion of exons 2-5 of EFNB1 was detected by PCR and Southern blotting in these males and a disease haplotype was determined.

We now extended the analysis in the family by introducing Multiplex Ligation-dependent Probe Amplification (MLPA) for the exons 1, 2 and 5 of EFNB1. This revealed that the signal for the MLPA probes in exon 2 and 5 was reduced by approximately 50% in the affected daughter and granddaughter. In the affected mother, however, a mosaic pattern of about 75% compared to normal could be detected by MLPA analysis. Mosaicism was confirmed by single nucleotide polymorphic sites (SNPs) located either within or outside the deletion interval. This result demonstrates that the intragenic deletion in EFNB1 occurred postzygotically in the mother. While varying levels of mosaisicm have been previously observed in the blood for small sequence changes in EFNB1 in affected females and males, this analysis shows the first mosaic intragenic EFNB1 deletion in blood of an affected female.

In conclusion, this investigation shows the possibility of identifying postzygotic mosaisicm by MLPA analysis. Therefore, it would be interesting to reinvestigate the blood of other affected patients with this method.

P-MonoG-190

A novel homozygous mutation in BMPR1B causes acromesomelic chondrodysplasia

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We present a 2 year old female patient with clinical and radiographic findings consistent with acromesomelic chondrodysplasia. Sequencing of candidate genes revealed a novel homozygous missense mutation in Bone Morphogenetic Protein Receptor type 1B (BMPR1B). Heterozygosity of the consanguineous parents was confirmed; the mother showed mild brachydactyly type A2, the father did not show any brachydactyly on clinical examination or X-rays.

Acromesomelic chondrodysplasias – Grebe, Du Pan, and Hunter Thompson type - have been shown to be caused by homozygous or compound heterozygous mutations in Growth Differentiation Factor 5 (GDF5). GDF5 belongs to the family of Bone Morphogenetic Proteins (BMPs) and signals predominantly via BMPR1B. So far, only one patient was described with a homozygous mutation in BMPR1B with a subtype of acromesomelic chondrodysplasia and additional uterus hypoplasia and absent ovaries (Demirhan et al 2005). In contrast, our patient had an apparently normal uterus on ultrasound examination; the ovaries could not be evaluated.

Functional analysis revealed that the mutated receptor was located at the cell membrane. A reporter gene assay showed no receptor activation by GDF5 indicating a loss of function of the mutation. A loss of function mechanism is also predicted for the mutation reported by Demirhan (c.361_368delGGACCTAT), whereas BMPR1B mutations causing classical autosomal dominant brachydactyly type A2 are shown to be dominant negative. Therefore, we propose a loss of function effect of the mutations causing recessive acromesomelic chondrodysplasia, as reported here, which is further supported by the only very mild brachydactyly or even non-penetrance in the heterozygous parents.

P-MonoG-191

A novel mutation in the TGFBI (BIGH3) gene in a german patient with hereditary corneal dystrophy

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Introduction:

The human transforming growth factor beta-induced (TGFBI) gene, located on the long arm of chromosome 5, was identified as the gene responsible of distinct autosomal dominant corneal dystrophies (CD). Mutational hot spots in exon 4 and exon 12 are described and lead to defined corneal dystrophies such as Reis Buckler CD (MIM 608470), Avellino CD (MIM 607541), CD Groenouw Type I (MIM 121900) and CD Lattice Type I (MIM 122200). Common for all types of TGFBI dependent corneal dystrophies are painful and recurrent corneal erosions combined with of visual declining.

We report on a novel mutation in exon 12 leading to a granular like phenotype of corneal dystrophy.

Patient and methods:

After clinical slit lamp examination of a 64 year old woman with granular corneal deposits on the right eye and corneal opacity following corneal rejection after keratoplasty on the left eye genomic DNA was extracted from peripheral EDTA blood. The exons of the TGFBI gene were amplified by polymerase chain reaction and directly sequenced in both directions. Histopathological examination was performed at the corneal button of the left eye.

Results:

A novel heterozygote mutation in exon 12 on position c.1640T>G, p.Phe547Cys was detected. The mutation results in granular superficial and deep stromal deposits comparable to those in granular corneal dystrophies.

Conclusion:

Next to the granular opacities found in our patient with the novel mutation c.1640T>G, p.Phe547Cys another mutation (c.1640T>C, p.Phe574Ser) at the same region was reported following to a lattice like corneal dystrophy, characterized by a network of delicate interdigitating branching filamentous opacities within the cornea. As described in other mutations sharing the same codon, even in the here reported case different mutations in the same codon lead to different phenotypical outcome in patients with TGFBI associated CD.

P-MonoG-192

Pathomechanism of the FAM134B-related Hereditary Sensory and Autonomic Neuropathy (HSAN2)

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We previously identified loss-of-function mutations in FAM134B as causative for severe autosomal-recessive sensory loss in patients, classified as hereditary sensory and autonomic neuropathy type 2 (HSAN2B, OMIM #613115). Loss of touch, temperature and pain perception in the affected individuals causes mutilations of hands and feet and often necessitates surgical intervention. FAM134B belongs to a gene family with three members and is predominantly expressed in sensory ganglia neurons. Fam134b is enriched in intracellular membranes of the early secretory pathway, i.e. the endoplasmic reticulum (ER) and Golgi apparatus. Our functional studies demonstrate a role for the protein in the shaping of biological membranes. Mice with a targeted disruption of Fam134b show an age-dependent decrease in the sensory nerve conductance velocities and reveal signs of a distal axonopathy. Further analyses of the KO-mice are needed to address structural alterations of the membranes of the ER/Golgi compartment in sensory neurons. We postulate that defects in the membrane-shaping of intracellular organelles contributes to the sensory loss in the FAM134b-related neuropathy. Since mutations in membrane-shaping proteins have been demonstrated as causative for other neurodegenerative disorders like e.g. hereditary spastic paraplegias (HSPs), altered membrane-shaping emerges as one of the common themes in hereditary axonopathies.

P-MonoG-193

Mutation spectrum in AAGAB and genetic heterogeneity of punctate palmoplantar keratoderma

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Punctate palmoplantar keratodermas (PPK) belong to the multifaceted family of keratoses that affect solely or predominantly the skin of palms and soles. Punctate PPK is a rare autosomal dominant disease characterized by hyperkeratotic papules, which are irregularly distributed on palms and soles. Lesions usually start to develop in late childhood to early adolescence but may also first manifest up to the fifth decade in life. Punctate PPK is clinically heterogeneous as many patients do not even report on symptoms, others, however, have strong and extended hyperkeratotic plaques over pressure points that can make walking particularly painful. Here we have analysed a large cohort of 31 unrelated patients with punctate PPK, most of these originating from Germany and including a large pedigree from Irish descent that we had mapped to chromosome 15q22-q23 before. Haploinsufficiency for AAGAB, encoding α- and γ-adaptin-binding protein p34, a cytosolic protein with a Rab-like GTPase domain, was described recently as a cause

for punctate PPK. In our patients, we have found deleterious mutations in AAGAB in 17/31 cases (55%). The 17 patients carried 8 different mutations, including 3 nonsense and 4 splice site mutations and one mutation of the translation initiation codon. Of these, only nonsense mutations p.Arg124* and p.Arg161* were described before. All the mutations were only found in one or two patients except for p.Arg124*, which was present in 8 cases from Germany. Furthermore, we identified a number of missense variants, which are all known as polymorphisms. The mutations are supposed to lead to lack of α- and γ-adaptin-binding protein p34, thus our data confirm that haploinsufficiency of AAGAB underlies punctate PPK. Moreover, the results demonstrate extended allelic heterogeneity with an accumulation of p.Arg124*, at least in German patients, and corroborate the genetic heterogeneity of punctate PPK. On the other hand, our findings do not yet explain the clinical heterogeneity and the inter- and intrafamilial variability of the phenotype, which will be further specified by re-evaluating the patients and refined clinical, histopathological, and cell biological investigations.

P-MonoG-194

A novel transgenic rat model for SCA17 recapitulates neuropathological changes and supplies in vivo imaging biomarkers

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Spinocerebellar ataxia 17 (SCA17) is an autosomal-dominant, late-onset neurodegenerative disorder caused by an expanded polyglutamine (polyQ) repeat in the TATA-box-binding protein (TBP). To further investigate this devastating disease, we sought to create a first transgenic rat model for SCA17 which carries a full human cDNA fragment of the TBP gene with 64 CAA/CAG repeats (TBPQ64). In line with previous observations in mouse models for SCA17, TBPQ64 rats show a severe neurological phenotype including ataxia, impairment of postural reflexes, hyperactivity in early stages followed by reduced activity, loss of body weight, and early death. Neuropathologically, the severe phenotype of SCA17 rats was associated with neuronal loss, particularly in the cerebellum. Degeneration of Purkinje-, basket- and stellate cells, changes in the morphology of the dendrites, nuclear TBP-positive immunoreactivity and axonal torpedos were readily found by light and electron microscopy. While some of these changes are well recapitulated in existing mouse models for SCA17, we provide evidence that some crucial characteristics of SCA17 are better mirrored in TBPQ64 rats. Thus, this SCA17 model represents a valuable tool to pursue experimentation and therapeutic approaches which may be difficult or impossible to perform with SCA17 transgenic mice. We show for the first time PET and DTI data of a SCA animal model that replicate recent PET studies in human SCA17 patients. Our results also confirm that DTI are potentially useful correlates of neuropathological changes in TBPQ64 rats and raise hope that DTI imaging could provide a biomarker for SCA17 patients.

P-MonoG-195

Altered ciliogenesis associated signal transduction in short rib-polydactyly syndrome type Majewski

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Defects of ciliogenesis have been implicated in a wide range of human phenotypes and play a crucial role in different signal transduction pathways and cell cycle coordination. We previously identified mutations in NEK1 as the underlying cause of short rib-polydactyly syndrome type Majewski (SRPS II) (Thiel et al. 2011) and also postulated a digenic diallelic inheritance in the NEK1 and DYNC2H1 genes. In screenings of additional SRPS Type Majewski patients we and others (Hokayem et al. 2012) identified further nonsense, missense and splice-site mutations located in the kinase, basic and coiled-coil domains of NEK1. The absence of full-length NEK1 leads to a severe reduction of primary cilia length (1.7 ±0.7 µm patient cilia vs. 6.2 ± 1.2 µm control cilia) and a significant decrease of ciliated fibroblasts (27% patient cells vs. 92% control cells). The primary cilium acts as a chemosensor for important developmental pathways like hedgehog, wnt and the platelet-derived growth factor (PDGF) pathways.

To further understand how the loss of NEK1 and therefore disturbed cilia formation can negatively affect signal transduction in the various pathways involved, we performed quantitative real-time RT-PCR experiments to elucidate changes of expression level of NEK1 and genes encoding key members of the

hedgehog (PTCH1 and SMO), Wnt (CTNNB1, DVL2 and FZD2), and PDGF (PDGFRA) pathways. We compared fibroblasts from the patient with the NEK1 nonsense mutation with 9 control fibroblast cell lines under normal conditions. The expression level of NEK1 was decreased to 55% in the patient and the overall expression levels of the pathway genes were increased up to 3.8-fold in our patient fibroblasts in comparison with controls. We further evaluated the changes of expression levels from normal to starvation conditions initiating ciliogenesis as ciliary defects are more pronounced under these conditions. Here, expression levels in control fibroblasts for all analyzed genes including NEK1 but not CTNNB1 and FZD2 were increased. Compared to control fibroblasts expression ratios in patient fibroblasts were further increased up to 2-fold for all pathways but not for NEK1 itself.

These results suggest a compensatory upregulation of the pathways involved in chemosensing caused by disturbed cilia formation due to loss of functional NEK1. Further evaluation of the examined pathway genes at protein level using immunofluorescence analysis did not reveal any obvious changes in intracellular protein localization. Here, future experiments like quantitative western blot and knock-down experiments will substantiate our data. Our data suggest that ciliary signal transduction is altered in short rib-polydactyly syndrome type Majewski due to lack of functional NEK1.

P-MonoG-196

Mutations in ANKH and SOST can cause highly similar skeletal phenotypes

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Craniotubular skeletal dysplasias belong to the group of disorders with increased bone density and metaphyseal and/or diaphyseal involvement according to the nosology of genetic skeletal disorders. Although craniometaphyseal dysplasia (CMD; OMIM 123000) can also cause complications, especially cranial nerve damage and hearing impairment, craniodiaphyseal dysplasia (CDD; OMIM 122860) is the most severe disorder of this spectrum. We present here two patients with overlapping features including severe cranial hyperostosis, dysplastic nose, paranasal bossing, progressive visual impairment, and diaphyseal cortical thickening, but only minor metaphyseal widening. In addition, patient 2 developed increased intracranial pressure and skull deformity. In patient 1 we identified a mutation in the SOST gene leading to the amino acid change p.Val21Met in the sclerostin protein, while patient 2 harboured a deletion p.Ser375del in the protein ANK encoded by the ANKH gene. Both variants occurred de novo. This is the second description of the p.Val21Met sclerostin mutation in a CDD case confirming the specific effect of this variant. The findings in patient 2 indicate that the p.Ser375del mutation in the membrane protein ANK can lead to a severe form of CMD that overlaps with CDD. Our results imply a hitherto unrecognized relation of the CMD and CDD pathomechanisms.

P-MonoG-197

Identification and functional characterization of a formerly uncharacterized gene mutated in a human progeroid syndrome

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Organismal aging is a complex phenomenon of increasing biomedical relevance, however the genetic and cellular mechanisms regulating aging are still incompletely understood. In a patient from a consanguineous family affected by a segmental progeroid syndrome with chromosomal instability and hepatocellular carcinoma, exome sequencing revealed just a single non-annotated sequence change with a severe impact on protein structure within the homozygous regions: a homozygous frame-shift mutation in a formerly uncharacterized gene. We showed that this protein is recruited to sites of DNA damage after treatment with genotoxic agents and is crucially involved in various DNA damage response (DDR) pathways. Further, siRNA-mediated depletion in cultured cells resulted in chromosomal instability and increased sensitivity towards genotoxic agents comparable to the findings in blood cells of the patient. Moreover, we

observed a siRNA-mediated distortion of the nuclear envelope like seen in other types of segmental progeria, which could be rescued by expression of human wildtype but not by a construct expressing the mutant isoform. In addition, we cloned the zebrafish ortholog and could show that morpholino-based silencing resulted in an increase of γH2AX foci, as a marker of DNA damage, as well as in developmental defects and increased mortality. The latter could be partially rescued by expression of human wildtype but not by a construct expressing the mutant. In summary, we identified the cause of a novel segmental progeroid syndrome and a crucial role of this formerly uncharacterized gene in the regulation of aging.

P-MonoG-198

Detection of novel de novo mutation in CHD7 for an adult male with CHARGE syndrome

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CHARGE syndrome is a rare, nonrandom combination of multiple anomalies. Major clinical features of CHARGE syndrome are ocular Coloboma, Heart malformalities, Atresia of the choane, Retardation of growth, Genital hypoplasia and Ear abnormalities.

Major cause of CHARGE syndrome is mutations in the CHD7-gene. CHD7 encodes for the chromodomain helicase DNA binding protein 7 (CHD7). The functions of CHD7 include methylated histone binding, transcriptional regulation, cell cycle regulation, regulation of apoptosis, chromatin remodeling and embryonic stem cell pluripotency.

Here we report the case of a nineteen year old male with clinical features that led to the suspicion of CHARGE syndrome as a diagnosis. The molecular analysis of CHD7 for the patient revealed a so far unpublished microdeletion in exon 3 at position c.1938 (c.1938delG) in heterozygous state. This mutation results in a frameshift, a premature stop codon and thus a truncated CHD7-protein most likely associated with a functional impairment or loss of function. The healthy parents and one healthy brother do not present this alteration.

Clinical features of the affected patient are discussed in the context of our molecular findings.

P-MonoG-199

Disease-associated mutations in Bestrophin-1 lead to degradation of the mutant protein via the endo-lysosomal pathway

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Bestrophin-1 (BEST1), the gene associated with at least four distinct retinopathies including Best macular dystrophy (MIM 153700), adult-onset vitelliform macular dystrophy (MIM 608161), vitreoretinochoroidopathy (MIM 193220), and autosomal recessive bestrophinopathy (MIM 611809), encodes an integral membrane protein specifically expressed at the basolateral membrane of the retinal pigment epithelium. So far, in Best macular dystrophy alone over 120 different BEST1 mutations have been identified although the molecular pathology of these mutations has not yet been resolved.

MDCKII cells provide a well-established model for studying protein trafficking and polarity in epithelial cells. We therefore generated eight stable lines constitutively expressing wildtype BEST1 and seven disease-associated mutants (T6P, L21V, W93C, R218C, L224M, Y227N, F305S). Cell surface biotinylation of wildtype BEST1 confirms localization to the plasma membrane, whereas for example in Y227N only 10% of biotinylated protein can be recovered. To determine the half-life of wildtype and mutant BEST1, a cycloheximide decay assay was applied. We now show that the protein level of wildtype BEST1 remains stable even after 24h treatment with cycloheximide, whereas six out of seven mutants degrade within 3 hours after arrest of protein synthesis. Mutation R218C degraded more slowly within 12h. To investigate the underlying degradation pathway we used potent and selective inhibitors of the three major degradation systems, i.e. the proteasomal, endo-lysomal and autophagy pathway. We demonstrate that wildtype BEST1 is degraded via the endo- lysosomal pathway, inhibited by the weak basic agents ammonium chloride and chloroquine. This is typical for the majority of integral plasma membranes and channel proteins. As most misfolded proteins are targeted for degradation via the ubiquitin-proteasome system, we tested three distinct proteasomal inhibitors such as Lactacystin, MG132 and ALLN. Interestingly, all seven mutants do not reveal degradation via the proteasomal pathway but instead demonstrate the characteristics of an endo-lysosomal pathway degradation. As a consequence, a chaperon-mediated rescue as a means of treating the disease may not be a therapeutic option for Best macular dystrophy.

P-MonoG-200

A de novo nonsense mutation of GLI3 in patient with Pallister-Hall syndrome

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Pallister-Hall syndrome is a pleiotropic autosomal dominant disorder comprising hypothalamic hamartoma, pituitary dysfunction, central polydactyly, and visceral malformations. Major cause of Pallister-Hall syndrome are mutations in the GLI3-gene. GLI3 is a member of the zinc finger gene family related to Kruppel and a transcription factor implicated in mediating the hedgehog signaling pathway thus being important for developmental regulation.

Here we report the case of a three year old male with clinical features that led to the suspicion of Pallister-Hall syndrome as a diagnosis. The molecular analysis of GLI3 revealed a so far unpublished nonsense mutation c.2893G>T in heterozygous state. It leads to a stop codon at amino acid position 965 (p.Glu965*) resulting in a truncated GLI3-protein and thus most likely to haploinsufficieny. The healthy parents do not present this alteration.

Clinical features of the affected patient are discussed in context of our molecular findings.

P-MonoG-201

Genotype phenotype correlation in congenital muscular dystrophies with defective O-glycosylation of α–dystroglycan

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Introduction: Congenital muscular dystrophies with defective O-glycosylation of α-dystroglycan (dystroglycanopathies) are a heterogeneous group of autosomal recessive inherited disorders resulting in a broad clinical spectrum: Patients with the early lethal Walker-Warburg syndrome (WWS) today are typically identified prenatally during sonographic examination due to characteristic cerebral malformations with marked hydrocephalus, agenesis or hypoplasia of the corpus callosum and hypoplastic brain stem and cerebellum. Additional associated clinical features include highly elevated creatine kinase values, disturbed neuronal migration with cobblestone lissencephaly and various eye abnormalities. Milder phenotypes as congenital muscular dystrophy (CMD) or limb girdle muscular dystrophy (LGMD) are also associated with milder cerebral malformations and/or cognitive impairment. Aim of the study is to further characterize the genotype phenotype correlation of patients with identified mutations diagnosed in our genetic laboratory over the past 9 years.

Methods: Evaluation of the patients medical reports and MR imaging. Linkage analysis for suitable families and stepwise sequence analysis of POMT1, POMT2, POMGnT1, Fukutin, FKRP, LARGE, and ISPD, respectively.

Results: 51 patients from 45 families with mutations associated with defective O-glycosylation were identified. Homozygous or compound heterozygous mutations were found in: POMT1 (16 families), POMGnT1 (13), FKRP (5), Fukutin (4), LARGE (1) and ISPD (1), respectively. Only one mutation was recognized in 5 cases (POMGnT1: 2; FKRP:2; LARGE: 1). No POMT2 mutation was identified. 17 patients from 12 families were referred to us with the clinical diagnosis of WWS; in this cohort mutations were predominantly identified in POMT1 (7/12 families) and only one family each with mutations in either POMGnT1, FKRP, FKTN, LARGE or ISPD. Interestingly, among WWS patients with POMT1 mutation exclusively homozygous or compound heterozygous truncating mutations were observed. In all three WWS families with two or more affected offspring a uniformly severe clinical presentation was observed. In 3 WWS families prenatal diagnosis was requested in subsequent pregnancies. POMGnT1 mutations in our cohort resulted in either Muscle-Eye-Brain disease (MEB; 12 families) or WWS (1 family). In 10 patients from 9 families with the clinical diagnosis of an LGMD POMT1 mutations were identified, thus confirming the diagnosis of an LGMD2K. Three patients with the milder phenotype of a POMT1 associated CMD or

LGMD2K were compound heterozygous for one missense mutation and one truncating POMT1 mutation. A rather uniform LGMD2K phenotype with proximal muscle weakness and cognitive impairment was observed in patients from 6 families homozygous for the POMT1 mutation p.Ala200Pro.

Summary: In our patient cohort mutations were predominantly identified in POMT1 and POMGnT1. Our data support a genotype phenotype correlation for POMT1 with homozygous or compound heterozygous truncating mutations resulting in WWS, while at least one missense mutation was observed in patients with milder manifestations as CMD or LGMD. Clinically well characterized families without identified mutation will now be included in genome wide studies to identify further candidate genes involved in dystroglycanopathies.

P-MonoG-202

Novel form of X-linked nonsyndromic hearing loss caused by a mutation in a type IV collagen gene

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Hereditary hearing loss is one of the most common human diseases worldwide with an incidence of approximately 1 in 1000 newborns. Nonsyndromic hearing loss is extremely heterogeneous with more than 50 associated genes known to date. In most cases, nonsyndromic hearing loss is inherited in an autosomal recessive pattern while X-linked forms are very rare accounting for only 1 to 5%.

Here, we present a three-generation family in which only males suffer from profound congenital hearing loss due to cochlear malformations while some females show a mild hearing impairment in later life. Due to the pedigree, an X-linked inheritance was most likely. After exclusion of the known X-linked deafness genes POU3F4 and PRPS1, next generation sequencing of the whole X-chromosomal exome was performed in the index patient, his mother and his affected cousin. Filtering and comparison of about 10.000 exonic variants per person revealed only a few shared unknown variants in which a missense mutation in a type IV collagen gene perfectly co-segregated with the disease in the family. Bioinformatic analyses including prediction of functional effects, splice site prediction as well as in silico analysis of protein stability and structure confirmed the causality of the detected variant. In situ hybridization in zebrafish embryos and immunostaining in the mouse inner ear demonstrated expression of the type IV collagen in the otic vesicle of zebrafish and in the spiral ligament of the murine ear, respectively. Expression data in combination with the cochlear malformations visible in the male patients provide evidence for an essential role of the type IV collagen in normal ear development and function. Although mutations in type IV collagen genes are known to be associated with Alport syndrome, a nephropathy often combined with hearing loss, there is no evidence for the presence of an Alport syndrome in the examined family.

In conclusion, our results suggest one of the X-chromosomal collagen genes as being the fourth gene associated with X-linked nonsyndromic hearing loss.

P-MonoG-203

A mosaic maternal splice donor mutation in the EHMT1 gene leads to aberrant transcripts and to Kleefstra syndrome in the offspring.

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Background: The euchromatic histone-lysine N-methyltransferase 1 (EHMT1) gene was examined in a 3-year-old boy with characteristic clinical features of Kleefstra syndrome.

Methods and results: Sequencing of all 27 EHMT1 exons revealed a novel mutation, NM_024757.4:c.2712+1G>A, which affects the splice donor of intron 18. Whereas the index patient is heterozygous for that mutation, his phenotypically normal mother shows tissue-specific mosaicism. Sequencing of EHMT1 RT-PCR products revealed two aberrant transcript variants: in one variant, exon 18 was skipped; in the other, a near-by GT motif was used as splice donor and intronic sequence was inserted between exons 18 and 19. Both transcript variants were found in the patient as well as in his mother. The latter had lower amounts of these transcripts, which is consistent with her mosaic status. This is the first description of an EHMT1 point mutation being inherited from a parent with verified mosaicism.

Conclusions: A constitutive c.2712+1G>A splice site mutation in EHMT1 is fully pathogenic and the transcript variants produced as consequence of the mutation do not attenuate the severity of the disease. Tissue-specific mosaicism, however, may not have phenotypic consequences, depending on the degree of the mosaicism and on the tissues that are affected.

P-MonoG-204

Two New Patients With GRIP1 Mutations Causative For Fraser Syndrome

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Fraser syndrome (FS; OMIM 219000) is a genetically heterogeneous condition characterised by cryptophthalmos, syndactyly and anomalies of the respiratory and urogenital tract. The malformation syndrome is inherited as an autosomal recessive trait and caused by mutations in FRAS1 and FREM2. Both genes encode extracellular matrix proteins that are essential for the adhesion between epidermal basement membrane and the underlying dermal connective tissues during embryonic development. Recently, also GRIP1 mutations were identified as cause of classic Fraser syndrome in humans (Vogel et al. J Med Genet. 2012;49(5):303-6). GRIP1 encodes for a scaffolding protein that interacts with FRAS1/FREM proteins and Grip1 mutations in mice are known to cause FS-like defects. To date, there were only three published patients with GRIP1 mutations causing Fraser syndrome.

Here we report on two new patients with Fraser syndrome and underlying GRIP1 mutations. We identified a homozygous nonsense mutation c.1860C>A, p.(Tyr620*) in exon 16 in a patient from a consanguineous family, with both parents being heterozygous for this variant. Another apparently homozygous nonsense mutation c.2120C>A, p.(Ser707*) in exon 18 of GRIP1 was found in a second patient. We tested both parents but only the father was carrier of the variant. Further testing revealed the mutation in a hemizygous state in this patient and a deletion on the second allele which was inherited from the mother and encompasses at least exon 18. Our observation expanses the GRIP1 mutation spectrum and indicates that the associated phenotype in indistinguishable from the one caused by FRAS1 or FREM2 mutations.

GRIP1 as the third Fraser syndrome gene adds another piece to the clinically and pathogenetically overlapping group of FRAS-FREM complex.

P-MonoG-205

Autosomal dominant aggressive periodontitis in a Tyrolean family

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Aggressive periodontitis (AgP) is characterized by destruction of the periodontal attachment apparatus by rapidly progressive inflammation and, if untreated, loss of all teeth by the age of 35 years. Familial aggregation of isolated AgP with different modes of inheritance has been reported but the genetic basis of isolated monogenic AgP remains unknown. Abnormalities of neutrophil granulocyte migration, phagocytosis, and oxidative burst, as well as increased levels of cytokines in gingival crevicular fluid and peripheral blood have been reported. Loss of teeth may be delayed by thorough mouth hygiene; treatment may include antibiotic therapy and surgical intervention. We report on a large family with 14 individuals in 3 generations affected by aggressive periodontitis. Affected individuals showed normal dentition in childhood. Chronic periodontal inflammation and loss of teeth started in late adolescence or early adulthood and was complete between the age of 30 and 35. The pedigree suggests an autosomal dominant mode of inheritance of a monogenic trait with complete penetrance. Immunological studies in gingival crevicular fluid and blood in affected individuals revealed evidence for neutrophil dysfunction. Aggressive peridontitis as an inherited monogenic condition should be considered in all individuals with complete loss of teeth in early adulthood.

P-MonoG-206

Large de novo deletion in HNF1α as cause of maturity diabetes of the young type 3 (MODY3)

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Maturity onset diabetes of the young (MODY) is a group of monogenic forms of diabetes mellitus (MODY1-11) and characterized by autosomal dominant inheritance and early onset at age 25 or younger. MODY is the cause of about 5% of all cases of diabetes. The genetic, metabolic, and clinical heterogeneity of MODY is determined by mutations in currently known eleven different genes. In five of these genes mutations are the genetic cause for nearly 90% of all cases with MODY. MODY3 is associated with mutations in the HNF1α gene. MODY3 is characterized by reduced secretion of insulin and progressive hyperglycaemia. The patients are responsive to a treatment with sulfonylurea or small dosages of insulin.

Here we report the case of a 33 year old male who has signs and symptoms of MODY since the age of 15. Direct sequencing of HNF1α and GCK revealed no indications for alterations. The molecular analysis with MLPA (multiplex ligation-dependent probe amplification). presented a so far unpublished large deletion of exon 4 and exon 5 (ex04ex05del c.714-?_1107+?del) of HNF1α in heterozygous state. The healthy parents and two healthy sisters did not present this alteration.

We report the family history as well as clinical signs and symptoms in detail and discuss the relevance of the identified alteration.

P-MonoG-207

Functional involvement of the monogenic Parkinson disease gene VPS35 in heavy metal homeostasis

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The identification of disease genes in monogenic forms of Parkinson disease (PD) like α-synuclein, Parkin or ATP13A2 has provided important insights into the pathophysiology of this severe neurodegenerative disorder. Very recently, missense mutations in VPS35, encoding a component of the retromer complex which is involved in intracellular vesicle transport, have been reported in autosomal dominant PD, however the underlying pathomechanism is still elusive. Because of the known relevance of heavy metals (HMs) in PD and the importance of intracellular vesicles/vesicle transport in HM homeostasis, we wanted to analyze a putative role of VPS35 for HM toxicity. To achieve this aim, we analyzed the effect of selected HMs in yeast cells in which the ortholog of Saccharomyces cerevisiae was inactivated by a genomic deletion (Δvps35). In addition we also analyzed the effect of HMs in yeast strains with knockouts of other known components of the retromer complex like vps26 and vps29. Both in spot tests and liquid cultures we could indeed observe an increased toxicity of one HM in Δvps35 cells as compared to wildtype yeast. This effect was dose-dependent and could be rescued by transformation with the yeast wildtype vps35 gene. On the contrary, there was no complementation of this cellular phenotype if we transformed Δvps35 cells with a yeast vps35 gene harboring the most commonly identified PD mutation in VPS35. Taken together we could show a functional role of this novel PD gene in heavy metal homeostasis and established a cellular assay system to test the possible effect of putative PD mutations in VPS35.

P-MonoG-208

Metal responsive elements as a new point of view in the pathogenesis of Wilson Disease

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Background: Wilson Disease (WD) is a monogenic autosomal recessive disorder leading to toxic copper accumulation mainly in liver tissue. It is caused by mutations in the ATP7B gene (OMIM#606882). Currently, more than 500 disease-related ATP7B mutations are known, providing a basis for molecular genetic diagnosis of WD. In patients who exhibit clinical WD symptoms but lack a detectable ATP7B mutation, differential diagnosis remains difficult. Our previous studies revealed a decrease in liver ATP7B mRNA

expression in molecular genetically proven WD patients compared to controls. Decreased ATP7B mRNA expression was also observed in patients with typical WD symptoms but without an ATP7B mutation. Metal responsive elements (MREs) are likely to be linked to this downregulation. MREs (a, c, d and e) can be found in the ATP7B promoter region and modulate the promoter activity by binding transcription factors in a metal ion concentration-dependent mechanism. An interaction partner in the ATP7B gene is already known for MRE a. Ultimate aim of our study is to unveil and characterize further MRE-interacting proteins that might orchestrate the aberrant ATP7B mRNA expression in patients with or without genetically detectable WD.

Methods: Nuclear proteins were extracted from human hepatocellular carcinoma cell line cells (HLE). To screen for protein-DNA interactions, an electrophoretic mobility shift assay (EMSA) was performed by incubating the nuclear extract with biotin-labeled double-stranded 31 bp probe corresponding to ATP7B MRE c sequence. To confirm the specificity of protein-DNA binding and to narrow down the protein binding site, excessive amounts of wild-type and mutated unlabeled MRE c oligonucleotides were used as competitors. The protein-DNA complex was isolated by means of magnetic streptavidin microbeads. Eluted proteins were identified by MALDI-TOF/TOF mass spectrometry.

Results: A highly specific protein binding on the MRE c probe was revealed by EMSA experiments. The use of mutated MRE c competitor oligonucleotides also indicated that not only nucleotides of the consensus MRE c sequence, but also up to six nucleotides downstream of the MRE c are involved in protein binding. Among the isolated proteins are proline- and glutamine-rich splicing factor (SFPQ) and poly(U)-binding-splicing factor (PUF60), which are not only involved in splicing, but also in regulation of transcription.

Conclusion: Our findings demonstrate that the regulatory MRE c sequence of the ATP7B gene is specifically bound by at least one protein. Candidate binding partners are the two factors SFPQ and PUF60. Specific interactions with either of these proteins have to be confirmed by supershift assays and their effects on transcriptional activation will have to be elucidated by luciferase assays. In prospective studies, liver samples of WD patients and possible WD patients without an ATP7B mutation could then be analyzed regarding validated MRE c binding transcription factors. More precisely, expression and mutational analysis of the transcription factors as well as mutational analysis of the MRE c itself are of immediate interest. A downregulation of transcription factors or disturbed interaction between transcription factors and MRE c may explain the decreased ATP7B mRNA expression in WD. These findings could provide new insights into the pathogenesis of WD but most of all enhance WD diagnosis in patients with disease symptoms but without an ATP7B mutation.

P-MonoG-209

Segregation of an intronic variant in a family with Idiopathic Short Stature (ISS)

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Short stature is a developmental, multifactorial condition with a strong genetic component. Approximately three per cent of the population worldwide is affected. Short stature can have many different causes; one of the most prevalent was shown to derive from variants affecting the SHOX gene (short stature homeobox gene; MIM 312865). Alterations of the coding region of SHOX cause a wide spectrum of short stature phenotypes including Leri-Weill, Langer and Turner syndrome, but mutations are also found in patients with idiopathic short stature (ISS).

Here we present a 6 year old girl with ISS. Karyotype analysis from lymphocytes revealed a normal female karyotype (46, XX). A molecular analysis of the SHOX-gene was performed using MLPA (multiplex ligation-dependent probe amplification) and no aberrations were detected with this method. Sequencing of the complete coding region of the SHOX-gene revealed an alteration in intron 4 c.544+45C>A.

We report the family history as well as clinical signs and symptoms in detail and discuss the relevance of the identified alteration under consideration of database information.

P-MonoG-210

Novel splice-site mutation in RUNX2 causes cleidocranial dysplasia

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Cleidocranial dysplasia is a skeletal dysplasia characterized by delayed closure of cranial structures, mid-face hypoplasia, hypoplasic or aplastic clavicles, multiple dental abnormalities, and hand abnormalities such as brachydactyly, tapering fingers, and short, broad thumbs. The phenotype may vary among

individuals in the same family. Manifestations range from isolated dental anomalies to fully manifested disease with poorly ossified cranium and absent clavicles.

Mutations in the runt-related transcription factor 2 (RUNX2) gene are the genetic cause of cleidocranial dysplasia. Mutations in the gene are associated with a high penetrance of symptoms and an extreme variability.

Here we report the index case, a woman of 60 years and her sister of 56 years. Both present with hypoplastic clavicles, hyperdontia and mid-face hypoplasia. Molecular analysis of RUNX2 revealed for index patient and her sister a so far unpublished nucleotide substitution in intron 4 (c.685+2T>G). Computer assisted analyses indicate that splicing will be affected by this mutation since the donor splice-site is destroyed. Clinical features are discussed in the context of our molecular findings.

P-MonoG-211

Single-stranded nucleic acids promote SAMHD1 complex formation

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SAMHD1 (SAM domain and HD domain-containing protein 1) is a dGTP-dependent triphosphohydrolase that degrades deoxyribonucleoside triphosphates (dNTPs) thereby limiting the intracellular dNTP pool. Mutations in SAMHD1 cause Aicardi-Goutières syndrome (AGS), an inflammatory encephalopathy that resembles congenital viral infection and phenotypically overlaps with the autoimmune disease systemic lupus erythematosus. Both disorders are characterized by activation of the antiviral cytokine interferon-α initiated by immune recognition of self nucleic acids. Here we provide first direct evidence that SAMHD1 associates with endogenous nucleic acids in situ. Using fluorescence cross-correlation spectroscopy we demonstrate that SAMHD1 specifically interacts with ssRNA and ssDNA and establish that formation of SAMHD1 complexes and nucleic acid-binding are mutually dependent. Interaction with nucleic acids and complex formation do not require the SAM domain, but are dependent on the HD domain and the C-terminal region of SAMHD1. We finally demonstrate that mutations associated with AGS exhibit both impaired nucleic acid-binding and complex formation implicating that interaction with nucleic acids is an integral aspect of SAMHD1 function.

P-MonoG-212

Independent identification of OTOG mutations as a cause of autosomal recessive hearing-loss

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Sensorineural hearing loss represents a heterogeneous group of disorders and advances in molecular genetics have largely expanded our knowledge about the molecular pathogenesis of this common neurosensory disorder. To date mutations in more than 60 genes involved in different forms of autosomal dominant or autosomal recessive non-syndromic hearing loss have been identified. Very recently, biallelic mutations in the Otogelin (OTOG) gene have been identified in two unrelated families with non-syndromic autosomal recessive hearing impairment. Moreover, mutations in the murine Otog ortholog have been described causing a severe hearing loss and additional vestibular dysfunction in mice. By positional cloning, we independently identified compound heterozygosity for two causative mutations in OTOG (c.2500C>T (p.Gln834*); c.3493C>T (p.Arg1165*) in a non-consanguineous German family with 4 affected children and one unaffected sibling. We provide detailed clinical data on the audiologic and vestibular phenotype in this family expanding the knowledge of this rather rare genetic form of hereditary non-syndromic hearing loss.

P-MonoG-213

A novel 3' cryptic splice site mutation in GRHL2 in a German family with autosomal dominant non-syndromic hearing impairment

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Non-syndromic hearing loss (NHSL) is a heterogeneous disorder characterized by both genetic and environmental causes. Genetic etiologies account for approximately half of NSHL, are estimated to affect approximately 1 in 1000 newborns, and typically follow standard Mendelian inheritance patterns, whereby monogenic mutations confer recessive and dominant inheritance. To date, there are 41 autosomal recessive, 27 autosomal dominant and three X-linked non-syndromic hearing loss genes, with many additional loci identified without specific genes determined (Hereditary Hearing Loss Homepage).

In a study, we employed a deep sequencing panel to screen for genetic mutations in 80 known and associated deafness genes in an individual of German descent affected with autosomal dominant non-syndromic hearing loss. We discovered a splice site mutation in the autosomal dominant gene GRHL2 (Grainyhead-like) (DFNA28, MIM 608641) c.1258-1G>A that co-segregates in three generations. GRHL2 is known to have an essential role in epithelial morphogenesis and epidermal development, and a zebrafish mutant line in which grhl2b expression is interrupted by an insertion of a Tol2 transposon element is shown to affect developing ear structures and swimming positions. cDNA sequencing of GRHL2 in one of the affected individuals revealed that this mutation activates a cryptic splice signal, whereby the 3' acceptor recognition sequence is disrupted, facilitating a deletion of the first nucleotide of exon 10, which results in a frameshift and eventually a premature termination codon. Furthermore, inheritance of the splice site mutation was detected at the genomic level through Sanger sequencing in seven family members. All other sequencing variations were filtered out and Illumina Omni1-Quad v1.0 chip and Human CGH 3-plex ISCA Plus Cytogenetic Array analyses were able to exclude copy number variations that are associated with hearing loss. In agreement with a previously reported segregating GRHL2 (c.1609-1610insC) frameshift mutation in a large North American family (Peters et al., 2002), it is hypothesized that GRHL2 haploinsufficiency may result in the impaired maintenance of epithelial cells and is thought to be an underlying pathological reason for post-lingual onset. To our knowledge, this is the first report of a family with an autosomal dominant form of progressive hearing loss associated with a splice mutation in the GRHL2 gene.

P-MonoG-214

Identification of mutations in patients with retinal dystrophies using a Panel-based Next Generation Sequencing approach

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Retinal dystrophies (RD) constitute a group of blinding diseases that are characterized by clinical variability and pronounced genetic heterogeneity. The different forms of RD can be attributed to mutations in more than 100 genes. Consequently, next generation sequencing (NGS) technologies are among the most promising approaches to identify mutations in RD. We screened 40 patients with different forms of RD (autosomal reccesive and autosomal dominant Retinitis Pigmentosa, cone dystrophies, cone-rod dystrophies and Achromatopsia) for mutations in 105 RD-associated genes using hybridization-based enrichment and SOLiD-based NGS. Most of these patients had been tested negative upon screening for mutations in frequently affected genes applying Sanger Sequencing and/or high throughput mutation microarrays (Asper chips). All putative disease-associated variants identified by our NGS approach were validated and tested for segregation with the phenotype in available family members by Sanger sequencing. In addition, exons with a coverage less than 10 reads of the NGS data were analyzed by Sanger sequencing. Whole exon deletions were validated by duplex PCR. In total, we detected mutations in 16 different RD genes. Mutations explaining the disease phenotype were identified in 18 cases. In the remaining 22 patients, we did not detect sequence alterations that explain the disease phenotype. Among these, we found several autosomal recessive cases that carry sequence alterations in one of the frequently affected genes (e.g. USH2A or ABCA4), but lack a second clearly pathogenic variant. It cannot be excluded that deep intronic variants exist which were not detected due to the targeted enrichment of the exonic regions for NGS-based genetic testing. Another explanation is of course that these patients harbor mutations in yet unknown disease-genes. To elucidate this, we are currently analyzing the unsolved cases by whole-exome-sequencing.

To summarize, we used a targeted resequencing approach in a genetically pre-selected cohort of patients with RD and were able to identify causative mutations in 45% of cases. Non-solved cases are promising candidates for the identification of novel disease-genes using whole-exome sequencing.

P-MonoG-215

Towards identifying the gene associated with North Carolina Macular Dystrophy in 6q14-q16.2

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North Carolina Macular Dystrophy (NCMD) is an autosomal dominant macular dystrophy with complete penetrance but variable expressivity. The phenotype is classified into drusen-like lesions in the central macular (grade 1) to larger lesions in the retinal pigment epithelium (grade 2) to severe pigmentation lesions (grade 3). Following genetic linkage analysis, the gene for NCMD was mapped within chromosome 6 in q14-q16.2 and was named Macular Dystrophy, Retinal, 1 (MCDR1). Further refinement localized the gene to an interval of 1.8 Mb of DNA between D6S1716 to D6S1671.

Sanger sequencing of all RefSeq protein-coding genes within the 6q14–q16.2 region of interest has not revealed NCMD-associated mutations. By next generation sequencing of the entire candidate interval we analyzed two affected patients (mother and daughter) and the unaffected father. Bioinformatic evaluation and sophisticated filtering of the raw data revealed 13 unique sequence variants specifically associated with the disease locus. These candidate variants were verified by Sanger sequencing and range from single base pair substitutions to deletions and insertions of up to twenty base pairs. None of these variants was localized within a RefSeq gene. These findings suggest that untranslated gene regions, intronic sequences or non coding elements at the 6q14-q16.2 locus are likely candidates for NCMD-causing effects.

Five variants were localized to intronic regions of an as yet unknown spliced gene highly expressed in retina and brain but no other tissue analyzed so far. In a first step, transcription will be analyzed on the background of the normal and the NCMD-associated haplotype. This may provide insight into the role of the newly discovered gene in the pathogenesis of NCMD.

P-MonoG-216

A number of lucky coincidences to find the needle in the haystack

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During the last years whole-exome sequencing has become a powerful technique for identifying novel genes in Mendelian disorders. Although this has led to an enormous increase in our understanding of human diseases and their underlying genetic causes, sometimes also fortunate coincidences do help identifying new disease-causing genes and -mutations. Initially, we started our analysis with a single German patient born to non-consanguineous parents and presenting with phenotypical features of the Dubowitz/Seckel spectrum, including growth retardation (-5 SD), primary microcephaly (-9 SD), developmental delay, mild mental retardation and mild facial dysmorphisms. Screening of known genes causing Seckel syndrome led to the identification of a heterozygous missense mutation, p.Ser529*, in CENPJ, but we were not able to detect a second mutation in CENPJ.

Incidentally, we got into contact with the family of a Turkish patient not only presenting with an identical phenotype, but also bearing only one heterozygous mutation, p.Met21Phe, in CENPJ. For both patients, we initiated whole-exome sequencing followed by standard filtering protocols, e.g. for reaching minimal quality (>5 reads) and excluding annotated SNPs, but we were not able to identify the disease-causing gene in both patients.

We tried alternative filtering methods utilising the protein-protein-interaction (Interactome) database STRING and we filtered for known genes of the microcephaly-Seckel-MOPDII spectrum including their interaction partners and also considering poorly covered variants or variants that have been annotated as SNPs. This approach led to the identification of LIG4 as disease causing gene in both patients. The underlying compound heterozygous mutations in LIG4 were a heterozygous nonsense mutation (p.Arg814*), which was initially excluded due to its database-annotation as a SNP (rs26489), and a heterozygous frameshift mutation (p.Lys424ArgfsX19), affecting a position that was previously excluded due to poor coverage. Both patients carried the identical mutations on different haplotypes. Taken together, our results gave new insights into possible reasons for unsuccessful whole-exome sequencing and show alternative and elegant strategies for handling these difficulties.

P-NORMAL VARIATION / POPULATION GENETICS / GENETIC EPIDEMIOLOGY / EVOLUTIONARY GENETICS

P-NormV-217

The imprinted NPAP1 gene in the Prader-Willi syndrome region belongs to a POM121-related family of retrogenes

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We have recently shown that the human NPAP1 (C15orf2) gene encodes a nuclear pore complex-associated protein. This gene is one of several paternally expressed imprinted genes in the Prader-Willi syndrome (PWS) region in 15q11q13. Loss of NPAP1 expression may contribute to PWS. Interestingly, in contrast to all other genes in 15q11q13, NPAP1 has, however, no orthologue in rodents. Since it appears to be under strong positive Darwinian selection in primates it, therefore, might code for important functions in primate neurobiology.

Here, we report on our investigation of the evolutionary origin of NPAP1. We have found that NPAP1 is absent from the 15q11q13 orthologous regions in all non-primate mammals. We identified homologous sequences, some of which match Ensembl-annotated genes, in all sequenced placental mammals except rodents. Phylogenetic analysis revealed the existence of a gene family, with one mammal-specific and two primate-specific genes, that are related to the vertebrate transmembrane nucleoporin gene POM121. In contrast to POM121, however, the members of this gene family have no or small introns, suggesting that their common ancestor arose by retrotransposition from POM121 during early mammalian evolution. Furthermore, the predicted proteins lack a transmembrane domain. In some mammalian lineages, the mammal-specific gene, which we call NPAP1L, was segmentally duplicated, whereas it was lost in the rodent lineage. In the human genome, two copies of NPAP1L are located on chromosome 9, although these loci are considered pseudogenes. The two primate-specific genes NPAP1 (on human chromosome 15) and UPF0607 (on human chromosome 10), most probably arose by retrotransposition from one of the two NPAP1L copies, before these became pseudogenes in the primate lineage. Our results show that the primate specific NPAP1 gene originates from a vertebrate nucleoporin gene, and propose a new, and so far unrecognized, gene family of possible mammal- and primate-specific nuclear pore-associated genes.

P-PRENATAL DIAGNOSIS / REPRODUCTIVE MEDICINE

P-Prenat-218

Equal contribution of parental M2/ANXA5 carriage to recurrent pregnancy loss risk

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The M2 haplotype of the ANXA5 gene is a confirmed hereditary risk factor for recurrent pregnancy loss (RPL). This factor is comprised of four consecutive nucleotide substitutions in the core promoter of ANXA5 and generally results in reduced expression levels of the protein in chorionic placenta. Reduced ANXA5 expression has been previously noted for the antiphospholipid syndrome, pregnancies complicated by preeclampsia or fetal growth restriction and in non-pregnant women with previous recurrent fetal losses. Later studies demonstrated that this decrease can be specifically due to the carriage of the M2 allele, that there is no genetic compensation through the wild type allele in heterozygous placentas, and that the lowered expression observed in embryonic tissue results of parental transmission. M2/ANXA5 apparently acting embryonally, we designed an initial study to evaluate maternal and paternal carriage of M2/ANXA5 as abortion risk factors in RPL couples.

The prevalence of M2 was estimated in a group of RPL couples, presenting at the Hormone and Fertility Center, LMU Munich, and compared to three independent control groups. Women were prescreened negative for carriage of the Factor V Leiden (FVL) and prothrombin (PTm) mutations and thirty RPL couples with three or more unexplained pregnancy losses were included in this study. Fertile female controls were from the same center (n = 90) and also from the resource of the Institute of Human Genetics, WWU Muenster (n = 500). Population controls were from the PopGen biobank, UKSH Kiel (n = 533). Incidence of M2 carriage was estimated in the patients and control groups, odds ratios were calculated and RPL risk was evaluated.

RPL risks associated with M2 carriage in RPL couples were between 1.7 and 3.8 as compared to the control groups of fertile women and equaled 2.3 when compared to population controls. Paternal and maternal contributions were about equal with 10 male vs. 9 female carriers in the patient group. Thus calculated risk rates directly apply for male as well as female carriers of M2 from the RPL couples.

Our genetic analysis of RPL couples indicates that paternal as well as maternal carriage of the haplotype M2/ANXA5 contributes an equal risk for pregnancy losses. This finding should be considered, when assembling improved diagnostic algorithms for idiopathic recurrent pregnancy loss and thrombophilia related obstetric complications. Both partners in RPL couples should be screened for M2 carriage.

P-Prenat-219

Counseling women with Recurrent Pregnancy Loss – Lessons from the ETHIG II Study

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Beside the common use in daily practise currently exists only insufficient evidence to routinely recommend low molecular weight heparin (LMWH) to women with a history of unexplained recurrent pregnancy loss (RPL) independent of thrombophilia status. In the only small number of prospective randomized trials LMWH could not improve the pregnancy outcome. We report here the prospective randomized ETHIG II multicenter trial, comparing the efficacy of the combination dalteparin (5.000 U) and multi-vitamins against vitamins alone, in a subsequent pregnancy of women with at least two early or one late miscarriage in the history. The primary outcome measure was the number of ongoing pregnancies at 24 complete weeks of gestation, whereas live birth rate and the prevalence of pregnancy complications regarded as secondary outcome measures.

450 women with a vital pregnancy <8 completed weeks were randomized (224 to dalteparin, 226 as controls) in 12 trial centers in Germany and Austria from November 2007 to August 2012. At least 432 pregnancies could be analysed (99% of the calculated sample size). Thrombophilic risks were reported in 53% of the cases (factor V Leiden 5,8%, prothrombin mutation 1,8%, lipoprotein (a) 28%, hyperhomocysteinaemia 14%).

Until now, more than 350 women had a complete follow up.

Last patient out will be at the end of December 2012. The primary outcome analyses will be presented.

P-Prenat-220

Determination of fetal fraction from cell-free DNA derived from maternal blood – a quality control application for massively parallel sequencing (MPS) based non-invasive aneuploidy detection.

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A critical issue of MPS based aneuploidy detection from cell-free DNA isolated from maternal plasma is the amount of fetal DNA in relation to total amount of DNA. The sensitivity of aneuploidy detection is limited by a certain amount of fetal DNA in the background of maternal DNA and thus it is important to determine the fetal percentage of the respective cell-free DNA samples. In case of male fetuses the fetal percentage can be measured by relative quantification of Y-chromosomal sequences using sequences specific for total DNA as reference, but for female fetuses autosomal markers are required. CpG-methylation is a powerful marker to discriminate fetal DNA from maternal DNA in a gender independent fashion. The usage of placental markers that are differentially methylated compared to cell-free DNA derived from the maternal genome allows PCR assay design covering methylation dependent restriction enzyme target sequences. Hypermethylation of the respective genomic area in trophoblasts prevents digestion of the targeted region and enables the determination of the fetal percentage using a relative quantification PCR approach including a pretreatment with methylation sensitive restriction enzymes.

P-Prenat-221

Investigation of maternal effect mutations in the NLRP gene family in women with spontaneous abortions

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The NLRP (Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing) genes are attributed to have several roles in the innate immune system as well as in the reproductive system of mammals. Maternal effect mutations in NLRP7, for example, have been associated with recurrent hydatidiform moles. To investigate whether mutations in any of the NLRP genes could be associated with spontaneous abortions, we screened 46 women with abortions (range: 1-6 abortions, median: 2) for mutations in all coding exons of the 14 NLRP genes by using RainDance technology followed by next generation sequencing. A total of 33 sequence variants passed the applied filter from which 10 were known SNPs. The remaining 23 sequence variants were validated by Sanger Sequencing which confirmed presence of 10 (43%) of the predicted variants. Comparison with the latest release of the 1000 Genomes Project showed 6 of these 10 variants to be SNPs leaving a total of 4 potentially pathogenic changes which all are missense mutations. These four mutations were detected in four different women with 2, 6, 1 and 5 abortions. In three women we identified mutations in the NACHT domain of the NLRP genes 4, 9 and 11, respectively. The fourth mutation was located in the LRR domain of the NLRP1 gene. None of the four mutations could be detected in a control cohort of 50 individuals. In conclusion we identified potentially pathogenic mutations in a NLRP gene in 4/46 women with spontaneous, in part recurrent abortions. Further studies have to show, whether the changes in the respective NLRP proteins can be functionally linked to an increased risk for miscarriages.

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P-Prenat-222

PGD for monogenic disorders on polar bodies and trophectoderm - impact of clinical parameters on PGD outcome

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Polar body diagnosis (PBD) has been applied at our center in Regensburg since 2001 in 111 ICSI cycles for 59 families requesting preimplantation genetic diagnosis (PGD) for monogenic disorders. In 9 of these PBD cycles in addition trophectoderm biopsy (TEB) samples were analyzed with the family specific PBD assay during a legal time window between July 2010 and November 2011. 91 Transfer cycles resulted in 30 clinical pregnancies in 28 families (clinical pregnancy rate 33,7%). In order to further optimize PGD outcome, we evaluated our PGD cycles for critical treatment parameters including the number of oocytes obtained, maternal age and impact of disease specific factors on pregnancy rates and general outcome.

As expected, the number of obtained mature oocytes was identified as one important parameter with highest clinical pregnancy rates per transfer cycle (8/17 = 47,1%) for cycles with 16 to 20 retrieved mature oocytes, which now will be targeted directly during adapted hormone stimulation in order to obtain an optimal number of mature oocytes per cycle. Poor treatment results were obtained in cycles with less than 7 retrieved mature oocytes (1/12 = 8,3%), prompting us to routinely recommend cryoconservation of these oocytes and PGD together with further oocytes collected in an additional ICSI cycle. With this strategy we could increase our rate of transfer cycles from 74,6% (47/63) to 91,7% (44/48) within the last 24 months.

Maternal age was confirmed as another important parameter with highest clinical pregnancy rates in patients between 26 and 30 years (7/18 = 38,9%), while a female age between 36 and 40 years resulted in a clinical pregnancy rate of 28,0% (7/25). In our cohort an abortion occurred in 6 clinical pregnancies, so far we did not observe an increased abortion rate with increasing maternal age.

Furthermore, our data confirm an important impact of the underlying genetic diagnosis primarily prompting PGD. In particular, PGD cycles for Myotonic Dystrophy type 1 (MD1: 14 oocyte retrieval cycles in 6 families) as well as for female Fragile X premutation carriers (9 oocyte retrieval cycles in 4 families) resulted in a lower average number of retrieved mature oocytes (MD1: 11,7; FMR1-Pre: 8,7 vs. overall cohort 13,3) and a lower clinical pregnancy rate when compared to our overall PGD cohort (MD1: 2/10 = 20%; FMR1-Pre: 1/7 = 14,3% vs. overall cohort 30/91 = 33,7%). In contrast, higher numbers of retrieved mature oocytes (mean 15,2) and an unexpectedly high clinical pregnancy rate of 47,6 % (10/21) per transfer cycle could be obtained for female CFTR mutation carriers.

Overall PGD results for monogenic disorders at our center are at least comparable with the results of day 3 blastomere biopsy obtained in the ESHRE PGD consortium. Our data confirm an important impact of clinical parameters on individual PGD outcome and should be considered early in genetic counseling as well as for ovarian stimulation and PBD itself in order to further improve PGD outcome.

P-Prenat-223

Validation of microarray comparative genome hybridization in polar bodies for prediction of the status of the corresponding oocyte in translocation carriers

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Introduction: Structural chromosome abnormalities occur with a frequency of ~ 0.2% in the human population. The frequency may be as high as 5% among subfertile individuals. Balanced chromosomal rearrangements represent one of the most frequent indications for preimplantation genetic diagnosis (PGD). The purpose of this study was to validate the use of array comparative genome hybridization (aCGH) for prediction of the status of the corresponding oocyte in translocation carriers. Furthermore a comparison was made between clinical pregnancy rates achieved after PB diagnosis by aCGH and by Fluorescent in situ hybridization (FISH).

Material and methods: 20 Patients (mean age 36.5 years) included in the study underwent one to three cycles of IVF or ICSI plus preimplantation genetic screening (PGS). The patients were balanced carriers for reciprocal translocations or Robertsonian translocations. All were treated in one centre. Polar body biopsy was performed immediately after fertilization check. Genetic analysis of the polar bodies from 27 cycles was performed with aCGH. For array CGH the SurePlex Amplification DNA Amplification System (BlueGnome, Ltd. UK) was used. Amplification products and SureRef Male DNA were labelled and cohybridized onto 24sure V3 microarrays (BlueGnome, Ltd. UK). For comparison the data of 31 cycles of patients treated in the same center using appropriate FISH probes were analyzed.

Results: Using aCGH the pregnancy rate per embryo transfer was 33.0% and per cycle 25.9%. In cases with 5 or more fertilized oocytes (22 cycles) the respective numbers were 41.2% per transfer and 35.0% per cycle whereas no pregnancies were achieved in cases with ≤ 4 oocytes (5 cycles).

Using FISH the observed pregnancy rate per embryo transfer was 18.2% and per cycle 12.9%. In the youngest age group (< 36y, FISH N=19, aCGH N=10) the respective figures for pregnancy rate per embryo transfer were 30.8% (FISH) vs. 57.1% (aCGH) and for pregnancy rate per cycle 21.1% (FISH) vs. 40.0% (aCGH).

As compared to PGD in single cells of the embryo, the prediction of the status of the corresponding oocyte in translocation carriers by aCGH in polar bodies is hampered by the fact, that approximately 95% of segregation errors in meiosis I is due to premature chromatid separation. Our results sustain the notion that this applies to chromosomes involved in structural aberrations to the same extend.

Conclusions: Our data from 27 cycles from 20 balanced translocation carriers demonstrate that aCGH in polar bodies can be successfully applied for the diagnosis of the oocytes produced by carriers of chromosome rearrangements, providing a single protocol applicable to most patients of this type and thus eliminating the need for developing patient specific protocols. In addition the data show the ad-vantage over the standard strategy using FISH, indicating a higher reliability of aCGH detecting mal-segregation and in addition to providing a comprehensive chromosome screening.

P-Prenat-224

Non-invasive prenatal determination of gender in women with pregnancies at risk of congenital adrenal hyperplasia (CAH) and X-linked disorders

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Early determination of fetal gender can clinically be indicated for pregnancies with risk of CAH, where early prenatal therapy can reduce the degree of virilization in affected female fetuses, and in X-linked disorders where male fetuses are primarily at risk.

We present results on prenatal determination of fetal sex in maternal plasma from eight women from 8th weeks of gestation onwards on cell-free fetal DNA (cffDNA).

Non-invasive prenatal diagnosis was performed using real-time polymerase chain reaction analysis of the Y chromosomal markers DYS14 and SRY and a fetal epigenetic marker RASSF1A. Results were confirmed by analysis of umbilical cord blood or invasive prenatal testing.

P-Prenat-225

Mutations of LHX1 and HNF1B are a rare cause for disorders of the Müllerian ducts

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Malformations of the Müllerian ducts including the different manifestations of unicornuate uterus, uterus didelphys, bicornuate uterus and uterus septus or subseptus, occur with a incidence of approximately 5% in infertile women. The so-called Mayer-Rokitansky-Küster-Hauser syndrome is characterized by congenital aplasia of the uterus and the upper part of the vagina in women with usually unaffected ovaries and normal female karyotype. MRKH can occur as an isolated form (type I) or in combination with various malformations as a type II MRKH. To date in most of the cases the underlying etiology remains unclear. Recently, in approximately 4-6% of patients with disorders of the Müllerian ducts deletions of chromosomal region 17q12 has been identified. Two strong candidate genes are located into the common deletion interval: LHX1 and HNF1B. The LHX1 gene has been suggested to be a strong candidate because targeted inactivation of Lhx1 in mice causes a complex phenotype including aplasia of the Müllerian ducts. Mutations of HNF1B are typically associated with renal cysts and diabetes (OMIM 137920), but have been reported also to cause in few cases also malformations of Müllerian ducts in females By sequence analysis of LHX1 and HNF1B in a cohort of 174 patients with disorders of the Müllerian ducts (123 patients with MRKH and 51 patients with fusion disorders of the Müllerian ducts) we detected in the LHX1 gene a heterozygous frameshift mutation resulting in a premature stop codon and a missense mutation in two MRKH patients. Sequence analysis of HNF1B revealed the detection of two possible splice site mutations in two patients with fusion disorders of the Müllerian ducts. We conclude that heterozygous mutations of LHX1 and HNF1B are a rare cause for disorders of the Müllerian ducts. LHX1 is to our knowledge the first gene shown to be involved in isolated MRKH.

P-Prenat-226

Diagnostic accuracy of PrenaTest® for non-invasive prenatal detection of common autosomal aneuploidies

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Objective: Recent advances in non-invasive prenatal diagnosis show that massively parallel sequencing (MPS) of maternal plasma DNA allows an accurate detection of common fetal aneuploidies. Here, we describe the results of a collaborative clinical study with the aim to validate the diagnostic accuracy of the non-invasive PraenaTest® based on MPS for detecting common autosomal aneuploidies. Furthermore, we report on the first experiences of clinical application of PrenaTest® in Germany, Austria, Liechtenstein and Switzerland.

Methods: In the study maternal blood samples were collected from 517 pregnant women with risk for aneuploidies in conventional EDTA blood collection tubes (BCT) or Cell-Free DNATM BCT. Extracted cell-free plasma DNA was analysed using Illumina sequencing platform HiSeq2000 in a multiplexed fashion. Fetal aneuploidies were identified using a Median Absolut Deviation based z-score equation (PreanTest®DAP.T21). After unblinding study data, a new bioinformatics algorithm based on GC normalization (PrenaTest® DAP.plus) was applied. Results of MPS based technique were compared with those from invasive procedures.

Results: Overall, 39/40 samples were correctly classified as trisomy 21-positive (sensitivity: 97.5%; one-sided confidence interval: 88.7%) and 427/427 samples were correctly classified as trisomy 21-negative (specificity: 100%; one-sided confidence interval: 99.3%). Furthermore, 5 of 5 T13 cases and 8 of 8 T18

cases were correctly identified using PrenaTest® DAP.plus. The overall detection rate of trisomies 13, 18 and 21 is 98.11% (52/53).

Conclusion: Due to the high accuracy, the PraenaTest® allows detection of common autosomal trisomies and has the potential to decrease the use of invasive procedures during prenatal care. The test is especially suitable for women at risk in addition to first trimester screening to reduce the false-postive-rate of this method. However, future clinical studies are required to validate the use of MPS for the detection of broader spectrum of fetal chromosomal abnormalities and fetal genomic imbalances.

P-Prenat-227

RBM8A variations are rarely associated with disorders of the Mullerian ducts

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Malformations of the Mullerian ducts encompass the so-called Mayer-Rokitansky-Küster-Hauser syndrome (congenital absence of the uterus and vagina) and fusion anomalies of the Mullerian ducts such as unicornuate uterus, didelphic uterus, bicornuate uterus, septate uterus, and vaginal septum. Array-CGH analysis in patients with disorders of the Mullerian ducts showed deletions of the so called TAR (Thrombocytopenia–absent radius) susceptibility locus in 1q21.1. In a few cases with TAR-syndrome an association with Mullerian anomalies has been described. It is known from the TAR syndrome that the deletion may be inherited by an unaffected parent, suggesting incomplete penetrance as well as variable expressivity in the phenotypical outcome. Very recently, the additional presence of rare intronic regulatory variants of the RBM8A gene, which is located into the smallest common TAR-deletion interval, showed that TAR syndrome is caused by compound heterozygosity. Furthermore two TAR-patients without deletion of the TAR-susceptibility locus carry besides one of the non-coding SNPs in the RBM8A gene a truncating and a frameshift mutation causing a premature stop codon, respectively in the second allele. Because of these findings RBM8A seems to be a strong candidate gene for disorders of the Mullerian duct.

Sequential analysis of RBM8A in a total of 153 patients, 101 patients with MRKH syndrome and 52 patients with disorders of Mullerian ducts has been performed.

In total, we identified 3 different RBM8A variants in 13 patients with disorders of the Mullerian ducts. One of the TAR-associated rare intronic variants (c.67+32G>C) was detected in heterozygous state in a MRKH patient, while heterozygosity for the second TAR-associated variant (c.-21G>A; rs139428292) was found in 8 MRKH patients and 3 patients with disorders of the Mullerian ducts, respectively. Furthermore, we noticed a heterozygous missense mutation of RBM8A in a patient with MRKH syndrome.

Since we detected TAR-associated variants with increased frequencies in patients with disorders of the Mullerian ducts in comparison with the general population and a potential pathogenic missense mutation in a MRKH case, we conclude that variants of the RBM8A gene seem to be associated with disorders of the Mullerian ducts.

P-Prenat-228

Lipoprotein(a) as a thrombophilic risk factor for recurrent pregnancy loss

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Objectives: Thrombophilic disorders are known risk factors for recurrent pregenancy loss (RPL) and complications in late pregnancy. The relevance of elevated lipoprotein(a) [Lp(a)] level as risk factor for chorionic vascular complications is well known but under discussion in regard of placental dysfunction and RPL. Elevated Lp(a) levels > 30 mg/dl has a prevalence of 5 % in normal population, but 20 % in patients with thrombo-embolic events (Nowak-Göttl et al., 2003). The aim of this study is the evaluation of Lp(a) as risk factor for early and late miscarriages.

Patients and Methods: Lp(a) measured of 170 women with two or more early or at least one late unexplained pregnancy losses during human genetic counselling together with other thrombophilia from December 2008 – August 2010. The detection of Lp(a) was performed using an in vitro turbidimetric immunoassay.

Conclusion: An increased Lp(a)-level is the most common abnormal parameter in thrombophilia diagnostics of women suffering from RPL. Our analyses show that RPL can be found three times more often among women with Lp(a)-elevation than among normal population. Especially at later pregnancy complications in many cases a vascular-induced placental insufficiency may play a major pathogenic role.

P-TECHNOLOGY AND BIOINFORMATICS

P-Techno-229

Quality evaluation of NGS analyses for clinical use as routine diagnostics of maturity onset diabetes of the young (MODY): Implications for translation of the method

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During the last years the development of sequencing methods showed a remarkable dynamic that led to several techniques designated as so called “next-generation sequencing” (NGS) methods. However, if NGS methods are applied as a diagnostic procedure in a clinical context, in case of the detection of a mutation, the results are usually proven by a second method such as conventional Sanger sequencing.

Essential requirements for a translation of NGS as a reliable diagnostic tool for the clinical routine are standardization and streamlining of the preanalytical processes, and establishment and evaluation of the analytical accuracy. In order to enable this, our aim was to decrease hands-on time and the development of protocols that are suitable for processing stepwise new molecular genetic diagnostic requests by a targeted sequencing approach with the Roche GS Junior System. Simplifying amplicon preparation, protocol standardization and accurate data analysis have been addressed before NGS implementation could be accepted as a routine in our diagnostic setting.

We report about our successful efforts concerning the establishment of a standardized amplicon preparation protocol that is suitable for the continuous introduction of new multi-gene-panel analyses in diagnostic quality and the considerable benefits concerning reduced costs and time. We present our findings regarding library quality, average coverage, detection rates and evaluation of the improved approach by comparison of its reliability with conventional Sanger sequencing by reanalyzing patients with known disease causing variants in one of the MODY genes.

P-Techno-230

High-coverage next-generation sequencing for retinal dystrophies: High diagnostic yield, CNV detection and novel insights into disease mechanisms

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Retinal dystrophies (RD) such as retinitis pigmentosa (RP), early-onset severe retinal dystrophies (EOSRD; including Leber congenital amaurosis, LCA) and macular dystrophies (MD) are genetically very heterogeneous, hampering comprehensive genetic testing. Using a next-generation sequencing (NGS) approach, we currently target all coding exons of 31 autosomal recessive (ar) RP genes (413 exons), 23 autosomal dominant (ad) RP genes (248 exons) and 16 EOSRD genes (215 exons). Currently, we have analyzed 120 patients and identified causative mutations in most of them with different detection rates in the subgroups (arRP: >70%; adRP: 80%; MD: >55%; EOSRD: >50%). High exon coverage was obtained with the Illumina MiSeq system, allowing for quantitative analysis of reads to detect copy number variations (CNVs) in most target regions.

Detected CNVs were confirmed by MLPA analysis and were particularly beneficial for the elucidation of the genetic basis of disease in the following scenarios: 1. CNVs in recessive RD genes in trans to heterozygous point mutations: a) In a consanguineous EOSRD family, a deletion of the last CRX exon

revealed that an apparently homozygous nonstop mutation of the gene's natural termination codon in trans was in fact hemizygous. In this constellation representing a potential pitfall for Sanger sequencing, CNV analysis uncovered unexpected compound-heterozygosity in offspring from related parents – with major consequences for genetic counselling. b) Discovery of novel candidate exons with a potential role in transcription regulation, illustrating the benefit of including 5´-UTR regions in NGS of disease gene or exome panels: A complete deletion of the non-coding exon 1 of EYS in trans with a truncating mutation in the coding region segregated with disease in an arRP family.

2. CNVs in adRP with incomplete penetrance: Heterozygous partial or complete PRPF31 deletions were found in patients with sporadic and thus presumably recessive RP but without point mutations in any recessive RD gene, altering genetic counseling for these families.

Beyond CNV detection, our findings underscore the necessity to consider the full variant load of all tested RD genes to avoid false interpretation of mutations in singular genes: For example, truncating RP1 mutations may cause either adRP or arRP, and RP1 was the most prevalent arRP gene in our study. However, truncating C-terminal RP1 alleles appeared to be non-pathogenic in two families with arRP and EOSRD who carried clearly causative mutations in secondary arRP genes (TULP1 and PROM1).

P-Techno-231

Moving on to the next generation – first experiences with the Roche GS Junior in BRCA1/2 diagnostics

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During the last few years, the number of reports successfully applying massively parallel sequencing in diagnostic approaches increased dramatically. Along with this, studies were conducted to prove the robustness of the technique. The benefits of next generation sequencing for diagnostic tasks are convincing: analysis of multiple patients and/or multiple loci per patient per run, the ability to find mutations in small subclones due to a high sequencing coverage and, last but not least, decreased analysis costs.

Our diagnostic core areas are 1) molecular genetics of hereditary cancers, e.g. breast and ovarian cancer and colon cancer, and 2) molecular genetics of leukemia. Considering the advantages given above, an NGS device can be used in both areas, on the one hand by speeding up the analysis of known cancer genes (e.g. BRCA1/2) which is done at the moment by High-Resolution Melting analysis, DHPLC and Sanger sequencing, and on the other by being able to analyze small leukemic subclones for MRD diagnostics or mutation detection.

Criteria for choosing the right instrument were: 1) amount of samples to be analyzed on a regular basis, 2) sensitivity and specificity of the instrument, 3) easy integration into existing workflows, and 4) costs. The MASTR assay from Multiplicom (Niel, Belgium) is widely used for BRCA1/2 analysis. Using this approach, it was possible to adopt some of the established workflows, as the amplicon-based kit from Multiplicom does not differ much from the techniques used in Sanger sequencing. The compatibility of the MASTR assays and the relatively low costs finally led us to use a Roche GS Junior (V2.7).

During the validation process, a complete run for BRCA1 sequencing was prepared and executed. Six different HBOC samples with BRCA1 mutations (c.187_188delAG, c.213-12A>G, c.564A>G, c.962_965delCTCA, c.9257-1G>C, dup Ex13) were used as the initial set for a multiplexed run. The second run was prepared using seven samples with mutations in BRCA2 (c.295+1G>C, c.353A>G, c.426-7_426-4delATTT, c.654-12_654-8delGTTTT, c.658_659delGT, c.4206_4207insTGCT, c.10322_10323insGAATTATATC). The results were very satisfactory. All mutations, UVs and most SNPs known from Sanger sequencing could be found again. Differences were only due to different amplicon design of the Multiplicom kit in comparison to our in-house procedure.

Along with the hardware, we were also looking for a software solution that can be used for daily routine data analysis. The software should be easy to use, allow a patient-based analysis, give clear results and integrate flawlessly into our workflow. Besides the Amplicon Variant Analyzer delivered by Roche together with the instrument, we tested NextGene from Softgenetics, USA, and SeqNext from JSI Medisys, Germany. The detection rate of all solutions was equal, but because of its focus on diagnostic use and the perfect integration with the other software modules for Sanger sequencing and MLPA, we focused on SeqNext for data analysis. SeqNext is also audit-trail capable and therefore well suited for use in an accredited diagnostic lab.

Taken together, our first experiences with the Roche GS Junior platform are quite promising. After having completed the validation process, the GS Junior platform is integrated into routine diagnostics.

P-Techno-232

Estimating exome genotyping accuracy by comparing to data from large scale sequencing projects

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Next-generation sequencing based methods, such as exome analysis, are now being introduced into routine diagnostics as a tool for mutation detection for many clinical settings. Various solutions for exome enrichment and DNA sequencing exist, and numerous algorithms for sequence read mapping and variant detection are in use. However, while there are platform specific guidelines for sequencing depth and recommendations for filtering single genotype calls based on quality scores, there are no criteria for assessing whether the variants identified by exome sequencing represent a comprehensive list.

We developed a way of assessing the exome genotyping accuracy by analyzing the composition of the detected variant set. We compare the identified variant set of a test sample to exome data that was generated in large scale sequencing studies such as the 1000 genomes project by means of a distance metric. The basic idea of our approach is that in the same population variant sets of comparable quality are more similar than variant sets of very different.

When two samples disagree in their genotypes at an exome position that is highly conserved this contributes more to the total distance than different genotype calls at a highly variable site of the exome, as such an event has a higher probability of being wrong. The differences in variant sets are therefore weighted by the genotype frequencies of the appropriate background population. The detection of disproportionally many rare variants in a test sample will then point to a high ratio of false positive genotype calls. On the other hand, if many variants that are common in a population are not detected in the test sample, this indicates a high proportion of false negative errors.

First, we computed the distances of all individuals analyzed in the 1000 genomes project that we use as a high quality reference set. The distribution of these distances describes the genomic variability and indicates also an upper bound for the accuracy that we can estimate. We then simulated for each individual sets of exome variants of reduced accuracy by introducing genotyping errors. For these different accuracy groups we computed the distances to the original variant data. As the distributions of these distances are clearly distinguishable from the distance distributions of the unperturbed data, we computed a reference curve for a standardized distance score. Based on this reference curve we are able to estimate the exome genotyping accuracy of an unknown sample.

P-Techno-233

Diagnostic Whole Exome Sequencing in patients with clinically suspected, genetically heterogeneous syndromes

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Identification of disease causing mutations in genetically heterogeneous conditions by targeted Sanger sequencing is time-consuming, costly and often unsuccessful. The advent of next generation sequencing (NGS) techniques is paving the way for novel large scale approaches with an unforeseen diagnostic power. Targeted NGS of limited disease specific gene panels enable very deep sequencing likely ensuring low false negative results for the genes investigated, but is not useful if the clinically suspicion was not correct or if the affected gene was not in the panel. We therefore investigated the diagnostic power of whole exome sequencing as a mutational screening tool in patients with clinically suspected, genetically heterogeneous syndromes. In order to establish an optimized work-flow for diagnostic whole exome sequencing using the SOLiD 5500XL platform we used DNA samples of 7 patients with a variety of known disease causing mutations. The known mutations were in the dominant genes ZEB2 (c.657delC/=), BRAF (c.1455C>A/=), MEF2C (c.43C>T/=), NSD1 (c.1894C>T/=), NF1 (c.1504_1505insAAATTCATG/=) SLC6A1 (c.452delT/=) and in the recessive gene RMRP (c.4C>T/, c.35C>T). Exomes were captured with the Agilent SureSelect Kit (SureSelectXT HumanAllExon V3) and analyzed with the NextGene software from Softgenetics. In the initial approach with pooling of 8 samples on one flowchip resulting in an average 40x coverage 3 mutations out of 5 were not detected due to low coverage. We therefore changed the procedure to pool only 4 samples onto one flowchip resulting in an average 100x coverage and optimized the analysis setting by permutation of the various alignment and filter parameters. We then used an optimized protocol with 16% aberrant allele calling in at least 3 reads and a minimal coverage of 6 to analyze three patients with suspected genetically

heterogeneous syndromic diagnosis and unknown mutation. Disease causing missense and indel mutations were identified in all three patients for Joubert syndrome, Fraser syndrome and Noonan syndrome. Our results thus demonstrate that whole exome sequencing is a powerful diagnostic tool in patients with suspected genetically heterogeneous syndromes.

P-Techno-234

High Throughput Primer and Assay Design Pipelines for Epigenetics

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Epigenetics refers to the study of heritable changes in gene expression that are flexible enough to respond to environmental influences without changes in the underlying DNA sequence. Occurring epigenetic abnormalities are playing an important role in e.g. cancer, genetic disorders, autoimmune diseases, pediatric syndromes and aging. Therefore, epigenome-wide association studies are increasing to complement genome-wide association studies and to search for novel disease genes, as well as clinically relevant biomarkers. However, feature selection in highly multivariate data (e.g. from genome-wide screening methods) often leads to a large proportion on false positive results. Thus, it is critical to put forward a large number of identified features for independent validation in larger sample cohorts. As a result we have developed several validated assay design pipelines for 100-1000 targets from high-throughput experiments. The pipelines provided can be used for primer design of methylation-sensitive restriction enzyme-based qPCR (MSRE), methylation-specific PCR (MSP), targeted deep bisulfite sequencing (TDBS), SNP-testing (SNP qPCR) and targeted resequencing. They are integrated in the XworX platform, a user-friendly workflow-based software which can be downloaded from the XworX website (http://www.xworx.org). This systematic approach to epigenomic-wide screening by using bioinformatics pipelines can significantly increase the speed and success rate of biomarker development.

P-Techno-235

XworX - Cloud-Ready High Performance NGS Data Analysis Framework for Biomarker Screening & Disease Diagnostics

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High throughput molecular profiling technologies such as Next-Generation Sequencing (NGS) play an increasingly important role in disease diagnostics, and will also allow medicinal products to be used more efficiently allowing cost-efficient remuneration based on drug performance. We present XworX, a cloud-ready, user-friendly NGS data analysis framework that enables even small laboratories to accomplish standardized state of the art diagnostic data analysis, interpretation & reporting of highly complex genomics data on affordable desktop servers behind local firewalls.

P-Techno-236

Clinical resequencing using semiconductor technology

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Next generation sequencing (NGS) technologies are reshaping our diagnostic procedures. In particular, targeted resequencing of multiple amplicons represents an interesting novel diagnostic approach. We tested initially a targeted resequencing approach using PCR pooled amplicons. Experiments varied between 91 (BRCA), 111 (HNPCC) and 150 amplicons (Intellectual Disability, ID) representing up to 73 kb of target sequence and were derived from patient's DNA samples used in Sanger sequencing based routine diagnostics. After pooling in equimolar concentration, libraries were sequenced on Ion Torrent 314 chips on a PGM instrument. Between 200,000 and 385,000 reads with a mean length of 110 bases were obtained. All coding bases were fully covered. SNVs were called with the Variant Caller software (Life Tech) and all SNVs previously identified with Sanger could be confirmed (e.g. a total of 50 SNVs for the ID panel). Subsequently we switched to multiplex PCR based Ion AmpliSeq Custom Panels (200bp design) ranging from 19 (Parkinson Disease) to 27 genes (HNPCC panel) and 453 to 723 amplicons, respectively. Each patient was individually panel-amplified and sequenced on Ion Torrent 316 and 318 chips with average coverage of 4,000x. More than 97% of the target sequence was covered >20x. Due to this excess of coverage we later introduced barcoding and pooled 4 patients on each 318 chip (5-6 million reads with mean length of 136bp) resulting in an even distribution of sequences between all 4 barcoded samples and with a still redundant

coverage. For variant calling we introduced the SeqNext module of the Sequence Pilot software (JSI medical systems). For the HNPCC panel 8 samples had been previously Sanger sequenced for 3-7 genes. Again 100% concordance between Sanger and semiconductor sequencing was observed. The entire process is easy to handle and takes one person less than one week from DNA to called SNVs. Variant calling with SeqNext software is especially well suited for diagnostic purposes with the familiar features from Sanger Sequencing. Only smaller problems with automated panel design were observed. One gene could not be scored (PMS2) due to the co-amplification of a pseudogene and exon-intron boundaries were sometimes only partially covered. With the novel 300bp design, though, this problem should be minimized.

Our experience indicates that amplicon resequencing using PCR-based NGS approach represents an alternative to conventional Sanger sequencing. The availability of pre-designed Ion AmpliSeq Ready-to-use panels as well as self-designed Ion AmpliSeq Custom Panels with up to 3,072 primer pairs per pool for amplification of target regions enables rapid and accurate identification of disease-causing variants using high-throughput NGS technologies. Currently, we are establishing gene panel sequencing on PGM and Proton semiconductor sequencing platforms for several disease groups.

P-Techno-237

New Patient-Based Locus Specific Mutation Databases of Metabolic Disorders

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The aim of databases is to share knowledge about variants found in patients, and to facilitate interpretation of mutations detected by molecular genetical methods and not yet described in the literature. For many diseases the phenotype varies depending on genotype, hence the importance to classify the variants according to their functional effects is obvious.

We developed genotype (mutation) variation databases for inborn errors of metabolism (IEM) and some other diseases which connect genotypes to clinical phenotypes including: ACAD8, ACADSB, AUH, HMGCS2, HSD17B10, DHCR7, FKBP14 and ROGDI. Phenotypic descriptions and biochemical data are included as detailed as possible. For DHCR7 genetic modifier data (maternal ApoE and ABCA1 genotypes) are added. Some of the genes have recently been associated with diseases as is the case for FKBP14 and ROGDI. Mutations in the FKBP14 gene cause one type of Ehlers-Danlos syndrome (EDS) (Baumann et al., 2012). Mutations in the gene ROGDI (Schossig et al., 2012) cause Kohlschütter-Tönz syndrome, a rare autosomal recessive disorder characterized by the combination of epilepsy, psychomotor regression, and global amelogenesis imperfecta of the hypoplastic or hypocalcified type affecting primary and secondary teeth. The exact functions of the protein coded by these genes are unclear.

Our databases are lovd2.0 (http://LOVD.nl) based. This open-access software allows flexible adjustment of content details. The future lovd version 3.0 will give the opportunity to link data of several genes in the same patient and storage of NGS (next generation sequencing) data. All of our databases are patient-based with detailed phenotype description where possible. Mutations and genotypes are described as proposed by HGVS at cDNA and protein level. Submissions of new variants are controlled by the curators of the database which are experts in this field. A detailed concept how to handle new mutations was designed by our laboratory (submitted to Human Mutation). Curation will be done according to published guidelines (Human Mutation 33:291-297,2012). Submitters are encouraged to add their patients' data to the databases. For submission of data registration is necessary. Data for research are only available after request. The herein described databases are accessible via the HGVS homepage (http://www.hgvs.org/dblist/glsdb.html) where nearly all actual existing databases are listed.

P-Techno-238

Hyperspectral Imaging of Chromosomes: a Novel Concept for Marker Free Karyotyping

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Staining techniques like GTG banding are routinely used to characterize metaphase chromosomes based on their unique banding pattern. Advanced molecular cytogenetic techniques like Fluorescence-In-Situ-Hybridization (FISH) provide a more sensitive tool for complex and small structural aberrations. However, these methods sometimes lack reproducibility and expert knowledge is needed to understand and diagnose diseases.

We have made efforts to substitute the time consuming state of the art karyotyping techniques with a fast and label free karyotyping procedure using a low cost diode array spectrometer in the visible range which

can easily be integrated into a standard light microscope. With this system we measure the stray light interference pattern of unstained chromosomes or chromosome-substructures. The complex spectra can be interpreted as spectra of “nanostructured particle arrays” with particles of different size and refractive indexes. The information is the result of the superposition of the morphological and chemical structure of the “array”. It represents the interference pattern of the chromosome thicknesses, the spectral interference of the band pattern, changes in refractive indexes along the chromosome axis as well as the absorption of chromophores in different spectral regions of the chromatin condensation. The complex data can easily be analyzed by means of multivariate data analysis (e.g. Principal Component Analysis, Multivariate Curve Resolution). The chromosomes are then classified in the multidimensional space of the principle components analogous to the pattern of the GTG banding or FISH.

Besides using a low cost diode array spectrometer we also use a hyperspectral imaging system based on a Pushbroom Imaging device. Here, more than 150 individual spectra can be registered along the chromosome axis. The spatial pixel resolution of the spectra can be as small as 50 nm. The spatial location is of course limited to the diffraction limit of the classical microscope (equally to the size of about 5 Mb). However the inherent information of sub-chromosome defects well beyond this resolution still remain in the spectra. These features are confirmed using Scanning Near-Field Spectroscopy (SNOM). In this case, optically resolved images and spectra can be obtained of sub-structures of chromosomes as small as about 50nm. Thus label free karyotyping is competing with the resolution of the FISH technique but with the advantage of getting the spectral information fast and without the need of expensive and time consuming staining techniques. The results are confirmed with model particle array measurements and show a strong correlation with calculated Mie interference spectra. Furthermore, FDTD (Finite Difference Time Domain) simulations of model chromosomes confirm the photon diffusion pattern as well.

The paper will outline the theoretical background of the new technique, the design of the instrument for fast and label free karyotyping as well as the design of the near field instrument.

P-Techno-239

G1 phase arrest improves the interpretability of array-CGH profiles

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Array-based comparative genomic hybridization (array-CGH) as a powerful method to detect genomic copy number alterations is a valuable tool in clinical diagnostics, e.g. for the discovery of microdeletions or microduplications in patients with unexplained mental retardation syndromes. Moreover, in tumor genetics and stem cell research, there is a rising need to prove genomic integrity of in vitro engineered cells like induced pluripotent stem cells or retrovirally transduced hematopoietic stem cells.

The analysis of genomic profiles generated from rapidly dividing cells is challenging due to characteristic patterns of data points oscillating around the array-CGH baseline, as we show here. We further demonstrate that this phenomenon can be overcome by arresting cells in the G1 phase. Two functionally well defined cell lines, the murine IL3-dependent pro-B cell line BA/F3 and the human AML cell line HL60 were cultured for 24 hours in IL3-free and FCS-free medium, respectively. These cultivation conditions could lead to an enrichment of relative G1 phase populations of about 33% and 12% for BA/F3 and HL60 cells, respectively. To test the impact of specific cell cycle inhibition, we applied the mammalian target of rapamycin (mTOR) inhibitor Torin1 on murine T-ALL cells sensitive to mTOR inhibition. This treatment led to about 15% more cells residing in the G1 phase. The S phase population was almost undetectable.

The comparison of genomic profiles from treated and untreated cells gave rise to a remarkably straightened array-CGH profile for cells arrested in the G1 phase. Thus, to gain high quality array-CGH data from rapidly dividing cells and to avoid possible false-positive as well as false-negative results, we strongly recommend reducing proliferation rates prior to DNA extraction.

P-Techno-240

Development of NGS panels for indepth analysis of Hereditary Peripheral Neuropathies

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We compiled a gene panel for Next Generation Sequencing (NGS) comprising 63 of the most relevant genes responsible for hereditary neuropathies. This panel includes hereditary motor and sensory neuropathies, as well as other rare disorders like giant axon neuropathy. We evaluated this panel on a pilot cohort of 50 patients, 12 controls with known mutations (missense, nonsense, small deletions and indels),

and 38 patients with an unclear genetic diagnosis after single gene testing with Sanger sequencing. Sample preparation was performed with the HaloPlex enrichment system. We targeted 500 Kilobases of genomic sequence including all exons, 100 bp of flanking exon regions, as well as the 3' and 5'-UTR regions of each gene within the panel. Samples were sequenced in four runs of a MiSeq sequencing technology (Illumina) using 2x150 paired-end sequencing cycles, which produced a total of 22 Gigabases. Data were analyzed using an "in-house" bioinformatics pipeline based on BWA, SAMtools, and ANNOVAR. We used Sanger sequencing to validate the results and analyzed the sensitivity and specificity of the detected variants. Preliminary analysis revealed that we were able to generate sufficient sequencing data for mutation analysis using our diagnostic panels. We identified all expected mutations in our control samples. One mutation in the MPZ gene (c.182A>G, p.Asp61Gly) was identified in five patients of the same geographical origin in Germany. This may point to a rare founder effect for MPZ. Overall this panel has proven to fulfill diagnostic criteria and will offer a fast and reliable procedure, which will help to clarify the molecular genetic defects underlying peripheral neuropathies.

P-Techno-241

Network information improves cancer outcome prediction

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Motivation: Disease progression in cancer can vary substantially between patients. Yet patients often receive the same treatment. Recently, there has been much work on predicting disease progression and patient outcome variables from gene expression in order to personalize treatment options. Despite first diagnostic kits on the market, there are open problems such as the choice of random gene signatures or noisy expression data. One approach to deal with these two problems employs protein-protein interaction networks and ranks genes using the random surfer model of Google's PageRank algorithm.

Results: In this work we created a benchmark dataset collection comprising 25 cancer outcome prediction datasets from literature and systematically evaluated the use of networks and a PageRank derivative, NetRank, for signature identification. We show that the NetRank performs significantly better than classical methods such as fold change or t-test. Despite an order of magnitude difference in network size, a regulatory and protein-protein interaction network perform equally well. Experimental evaluation on cancer outcome prediction in all of the 25 underlying datasets suggests that the network-based methodology identifies highly overlapping signatures over all cancer types, in contrast to classical methods that fail to identify highly common gene sets across the same cancer types.

Conclusion: Integration of network information into gene expression analysis allows the identification of more reliable and accurate biomarkers and provides a deeper understanding of processes occurring in cancer development and progression.

P-Techno-242

UPDtool: a tool for detection of iso- and heterodisomy in parentchild- trios using SNP-microarrays.

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UPDtool is a computational tool for detection and classification of uniparental disomy (UPD) in trio SNP-microarray experiments. UPDs are rare events of chromosomal maldistribution and describe the condition of two homologous chromosomes or homologous chromosomal segments that were inherited from one parent. The occurrence of UPD can be of major clinical relevance. Though high-throughput molecular screening techniques are widely-used, detection of UPDs and especially the subclassification remains complex. We developed UPDtool to detect and classify UPDs from SNP-microarray data of parent-child-trios. The algorithm was tested using five positive controls including both iso- and heterodisomic

segmental UPDs and 30 trios from the HapMap project as negative controls. With UPDtool we were able to correctly identify all occurrences of non-mosaic UPD within our positive controls, whereas no occurrence of UPD was found within our negative controls. In addition,

the chromosomal breakage points could be determined more

precisely than previously by microsatellite analysis. Our results were compared to both the gold standard, microsatellite analysis, and SNPtrio, another program available for UPD-detection. UPDtool is platform-independent, light-weight and flexible. Because of its simple input format UPDtool may also be used for other high-throughput platforms (e.g. Next-Generation-Sequencing).

P-Techno-243

VARBANK: leveraging the power of exome sequencing

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In recent years exome sequencing has become an invaluable tool for disease variant discovery with a major impact on oncology and rare disease genetics. The combination of efficient enrichment protocols with low-cost next-generation sequencing technology made this amazing success possible. The technological breakthrough was accompanied by an avalanche of software developments, adapted to the particular challenges of assembling short reads and reliably calling variants from this sort of DNA sequences. The requirements for the IT infrastructure and the complexity of software workflows are very demanding. Therefore, outsourced cloud-based analysis pipelines are becoming popular strategies.

VARBANK is such a new exome sequence analysis pipeline, which has been developed at the Cologne Center for Genomics in cooperation with the Regional Computing Center Cologne. The list of features combined in VARBANK is unique and superior to many other pipelines. Its first part is implemented on a 100 Tflop/sec HPC-system and includes the following modules: i) SNV and short INDEL calling, ii) structural variant (SV) analysis (CNV, ROH, split reads, paired-end mapping distance), iii) phasing and combining SNVs in codon distance, iv) de novo variant detection using parents-child trios, v) integration of data and functional interpretation including tests for canonical and cryptical splice sites, splice enhancers and silencers, branch points, and poly(A) sites.

The second part of VARBANK is a new database including web-based tools to allow researchers to browse, filter and rate rare variants. The features of the database comprise i) fast access to variant lists, at any time and from any place, ii) numerous filter options including pedigree and linkage information, iii) user-editable database fields for pedigree and phenotype information, iv) query of external databases for gene-centred information including orphaned rare disease and mouse genome databases, v) implementation of a graphical alignment viewer to display all reads that cover the variant of interest, vi) graphical and tabular tools for a gene- or exon-focussed coverage analysis, and vii) export of variant lists to Excel.

In summary, VARBANK combines software to analyse the full spectrum of variants with an easy-to-use graphical interface. When developing VARBANK, we had users in mind, with or without skills in bioinformatics, who work in a clinical environment and want to have full control and permanent access to their genomic variant data that they may easily but selectively share with their colleagues.

P-Techno-244

Creation of an SQL Based Variant Analysis Pipeline for Ultra Deep Sequencing

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For several years now, Ultra Deep Sequencing methods have become established as the mainstay of variant analysis in Human Genetics. With ever growing amounts of data produced, the bioinformatics methods to evaluate this data, however, still have to match this development. While, with established exome sequencing methods, the typical result yield is in the order of 50,000 variants, the most difficult task is to identify those few of these which warrant further examination. Using a combination of proprietary, open source and self-created software, we have established an analysis pipeline based on an SQL server with a dedicated frontend, so that researchers may comfortably employ different filtering approaches that suit the specific models they are employing in their projects. The Server allows for filtering according to annotations from various public databases (1000genomes, ESP5400 and others) as well as to all other variants identified in our labs and thus contained in the database. Up to now, this amounts to about 450,000 distinct variants from well over 200 exome samples, enabling very efficient filtering. The frontend is a Microsoft .NET application, which can, as the needs of the researchers evolve, easily be adapted to additional tasks. With this custom-made pipeline for human genetic variant analysis, we have further reduced the development gap between the deep sequencing wet lab and human genome bioinformatics.

P-Techno-245

MutAid- A comprehensive sequence variance analyses tool for pipelined human molecular genetic diagnostics

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Molecular genetic techniques have gathered a very important role for diagnostic testing of hereditary human diseases. Today there are more than 5000 known genetic conditions and the molecular basis is known for almost 2000 human mono-genic disorders. DNA sequencing has become the gold standard for human genetic diagnostics and genome-sequencing approaches are currently transferred from research to diagnostic labs.

Although the NGS technologies are already well established from the technical and laboratory point of view, data analyses remains a major hurdle and might be a major reason for human geneticists to postpone implementation of novel highly paralleled sequencing technologies.

Using a server-client architecture we have set-up and designed a software solution for analyses of multiplexed- and targeted amplicon re-sequencing. A tool repository enables generation of standard- and customized- pipelines for analyses of predefined gene-panels. All sequence variants are automatically matched, justified and annotated versus human reference genomes and genetic databases. All functions are implemented within a user-friendly docking framework, and alignments are visualized within the implemented UCSC Genome and the Integrated Genome Browser. A data repository and logging of analyses parameters and settings provides complete documentation of sequence analyses and generation of analyses reports.

MutAid thus enables fast, efficient and documented analyses of complex sequencing data which is a prerequisite for implementing genome sequencing technologies in human genetic diagnostic laboratories.

P-THERAPY FOR GENETIC DISEASE

P-Therap-246

Olesoxime improves specific features of the Huntington disease pathology in a transgenic rat model

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Olesoxime, a cholesterol-oxime, is a neuroprotective compound initially developed for the treatment of ALS. It interacts with proteins on the outer membrane of mitochondria and inhibits the opening of the mitochondrial permeability transition pore. Furthermore, it was found to accelerate oligodendrocyte maturation, thereby enhancing myelination. Since both mitochondrial function and myelination are impaired in HD, we evaluated the effect of olesoxime on the behavioral and neuropathological phenotype of the BACHD rat. BACHD rats and their wild type littermates were fed ad libitum with either an olesoxime-containing or a control diet beginning at 5 weeks of age. Behavioral observations were carried out during a 12-months study period and neuropathology and mitochondrial function were investigated subsequently. Olesoxime treatment improved the cognitive and psychiatric phenotype in BACHD rats, which might be associated with a reduced mhtt accumulation found in the prelimbic cortex that is involved in learning and emotionality. It further increased the width of axon bundles in the striatum, which was significantly decreased in BACHD rats compared to wild types, possibly due to an improved myelination. Olesoxime was further capable of restoring mitochondrial respiratory chain function, rescuing a deficit in the expression of OMM proteins and normalize mitochondrial membrane fluidity. In conclusion, olesoxime did not improve motor and metabolic function but it ameliorated the cognitive, psychiatric and mitochondrial pathology in the BACHD rat.

P-Therap-247

From gallstones to liver transplantation - Long term follow-up and success of liver transplantation in patients with familial intrahepatic cholestasis: is there an association between genotype and outcome?

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Study and Objective

Infants with autosomal recessive inherited progressive familial intrahepatic cholestasis (PFIC) suffer from liver cirrhosis progressing to liver failure in the first or second decade and ultimately require liver transplantation. Milder manifestations with intermitted cholestasis include benign recurrent intermittent cholestasis (BRIC), intrahepatic cholestasis in pregnancy (ICP), drug-induced liver injury and gall stones. Three causal genes necessary for bile flow across the canalicular membrane have so far been identified: ATP8B1, ABCB4 and ABCB11.

Our aim was to further characterize the genotype-phenotype relationship and to search for clinical and genetic parameters important for long term outcome, pharmacological and surgical response as well as the success of liver transplantation in order to improve individual treatment options.

Method

A total of 72 index patients from 70 families with familial intrahepatic cholestasis were screened by direct sequencing at our center between 2008 and 2012 for mutations in ATP8B1, ABCB4 and ABCB11. Long term follow-up data of 6 patients with genetically confirmed PFIC was retrospectively analyzed.

Results

In our cohort 69% of the patients clinically presented with PFIC. Most pathogenic mutations were identified in ABCB11 (56%), followed by ATP8B1 (32%) and ABCB4 (12%). In BRIC patients (15%) the majority of mutations was also found in ABCB11 (60%), followed by ATP8B1 and ABCB4 (both 20%). Two patients presented with an intermediate BRIC/PFIC phenotype, in these patients mutations were identified in both ABCB4 and ABCB11. In patients with milder cholestasis associated symptoms (ICP, gall stones) the majority of heterozygous mutations was found in ABCB4 (80%) and ABCB11 (20%). Overall missense mutations were the most frequently detected type of mutation (73%), followed by truncating mutations (17%), splice mutations (7%) and small in-frame deletions (3%). In addition 9 novel mutations were identified.

Follow-up data of 6 PFIC patients is presented to illustrate the specific problems in interdisciplinary medical care (follow-up range 2-35 years). Response to pharmacological therapy was insufficient in 5 patients. Thus, biliary diversion was performed in 3 cases and liver transplantation in 5 patients (mean age 6.5 years). Liver transplantation was complicated by: bleeding and coagulation problems (2), severe rejection requiring retransplantation (1) and death (1). Interestingly, both patients with life threatening complications were compound heterozygous for one truncating and one missense mutation. After liver transplantation the most severe long term complications were observed in ATP8B1 mutations carriers and included: severe chronic diarrhea, renal failure (1 requiring renal transplant), missing catch-up growth, pancreatitis, deafness and polyneuropathy.

Conclusion

Truncating mutations may be associated with a higher rate of complications in liver transplantation. However, further studies are required to confirm this hypothesis. With an ongoing interdisciplinary study we address two major objectives (1) evaluating the individual response to UDCA medication, biliary diversion as well as liver transplantation in a larger cohort and (2) using whole genome sequencing to search for further PFIC candidate genes in mutation negative patients.

P-Therap-248

Detection of the positive regulator of the alternative complement pathway in age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause for irreversible vision loss in the aging population in industrialized countries. The growing population of elderly emphasizes the need for effective treatment options and the development of preventive programs. Currently, monoclonal anti-VEGF antibody therapy slows down neovascular age-related macular degeneration, a late complication in about 10-15% of AMD patients. Importantly, there is no effective therapy option for atrophic AMD, the most common phenotype with slow bilateral progression of the disease. The various phenotypes of AMD result from contributions of genetic, environmental and aging factors. Complement factor H, a known risk-associated

genetic factor in AMD, is a negative regulator of the alternative complement pathway while properdin is the sole positive regulator.

The aim of the present study was to establish properdin detection systems from human and murine samples. Commercially available antibodies were used for sandwich immunoassays and Western blot analysis. Validation of the ELISA assays showed sensitive and specific detection of human properdin from serum with a lower limit of detection of 5-10 ng/mL in different settings. Consequently, we compared properdin concentrations in serum samples from AMD patients and a matched control group. Murine properdin was not detected from serum samples with the methods used for human specimen. To overcome this difficulty several immunization strategies were used to generate antibodies against human and murine properdin, which then can be used in AMD animal models and humans in parallel. These initial results encourage further investigation into the role of properdin in the development and progression of age-related macular degeneration.