VORTRÄGE

KEYNOTE LECTURE

Wie wird man zum Spitzenmusiker? Höchstleistung im Spannungsfeld von Genetik, Gesellschaft und Persönlichkeit

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Bis heute ist die Frage offen, welche Bedeutung Gene für musikalische Ausnahmeleistungen haben. Jedem fällt die Familie Bach ein, die über mindestens drei Generationen Ausnahmemusiker hervorbrachte. Gegen eine einfache genetische Verankerung von Musikalität spricht allerdings die Tatsache, dass die Bach-Familie eine Ausnahme ist. Die Söhne von Mozart und Wagner erzielten als Komponisten allenfalls Achtungserfolge, Robert Schumanns Nachkommen waren nur noch in einer Generation Klavierlehrerinnen und eine große Zahl der herausragenden Komponisten blieb überhaupt kinderlos, Beispiele sind Beethoven, Schubert, Brahms, Chopin, Ravel.

Auf der anderen Seite sind Teilfertigkeiten musikalischer Ausnahmeleistungen zumindest teilweise genetisch bedingt. Dazu zählen nicht nur das absolute Gehör, nämlich die Fähigkeit Tonhöhen ohne einen Referenzton korrekt zu benennen oder zu produzieren, sondern nach neuen Zwillingsstudien auch die Fähigkeit subtiler Rhythmus-, Melodie- und Tonhöhen-Wahrnehmung. Ganz eindeutig vererbt ist das Gegenteil, eine mangelhafte Tonhöhenwahrnehmung, die als kongenitale Amusie bezeichnet wird.

Auch die motorischen Leistungen von Musikern scheinen genetisch mitbedingt zu sein. So gibt es zahlreiche Befunde, die auf eine genetische Verankerung der Grundschnelligkeit des sensomotorischen Systems hinweisen. Auf der anderen Seite konnten wir gemeinsam mit der Lübecker Neurogenetik-Gruppe unter Leitung von Christine Klein nachweisen, dass die Musikerdystonie, der Verlust der feinmotorischen Kontrolle hochgeübter motorischer Fertigkeiten bei ungefähr einem Drittel familiär auftritt.

Wie sind diese Befunden einzuordnen? Die erste Schwierigkeit besteht darin, festzustellen, was musikalische Ausnahmeleistungen sind. Ist es die Kreativität, also die Fähigkeit etwas Neues in eine Domäne einzubringen, das Experten und später das Publikum als wertvolle Neuerung erkennen? Ist es die Fähigkeit als Interpret brillant zu spielen oder zu singen? Ist es die emotionale Tiefgründigkeit einer bestimmten Spielweise? Und welchen Einfluss haben die frühe Ausbildung, die sozialen Netzwerke, die Insider-Informationen, die musikalische Eltern ihren begabten Kindern mitgeben können?

In meinem Vortrag werde ich diese vielfältigen Aspekte und die zu Grunde liegenden wissenschaftlichen Evidenzen berichten, ohne eine eindeutige Antwort zu geben zu können. Vermutlich ist es für musikalische Ausnahmeleistungen wichtig, eine genetische Grundlage für die auditive, sensomotorische und emotional-soziale Lernfähigkeit zu haben und diese früh im Leben auszubilden. Für das motorische Lernen beispielsweise existieren sehr gute Hinweise, dass sehr frühes Training ein Gerüst für die spätere Trainierbarkeit der Feinmotorik anlegt. Was allerdings dann aus einem Mozart einen Mozart macht und nicht einen Salieri oder Paisiello entzieht sich vermutlich dem wissenschaftlichen Zugriff.

PLENARY SESSIONS

Plen 1

Genetic Prediction: Clinical Relevance of DNA Testing and the Future of Human Genetics

Sir John Burn

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Times of rapid change can expose fault lines in society. Just as the mass of refugees from the Middle East elicited contrasting reactions which in turn stressed the existing partnerships in Europe, so the arrival of high throughput sequencing and point of care DNA testing will test the assumptions of Europe's nation states. Termination of pregnancy and population screening for genetic disorders test the diversity of opinion from the more individualistic and secular traditions of the North and West of Europe across to the countries of the East and South of Europe where traditional Catholic teaching holds sway. Memories of misuse and fears about impact on insurance deter many from whole genome sequencing and genetic databases while others, notably in the UK, press ahead with industrial scale sequencing in healthcare.

The emergence of non-invasive testing based on fetal DNA in maternal circulation will increase terminations of pregnancy for genetic disorders. Similarly, the availability of molecular targeted cancer treatments such as PARP inhibitors, PD1 blockers and BRAF specific therapy will prompt adoption of testing for germline defects in high penetrance “cancer genes” routine.

Point of care DNA testing for pharmacogenetic susceptibility, DNA/RNA tumour markers and the presence of pathogen sequences will democratise genomics. By testing cheaply and quickly for diagnostically relevant information at the clinical interface it becomes possible to use DNA technology to improve outcomes without eliciting negative reactions. Current restrictions on use of DNA technology in Germany will soon seem out of step with pressing clinical utility. A balance must be found which allows high throughput and point of care DNA tests to be ordered by a wide range of healthcare professionals and, in some cases, by members of the public while ensuring the hard won skills of professional geneticists remain available and their services financially viable.

Plen 2

Plasma DNA as a Treasure Trove for Molecular Diagnostics

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There is much recent interest in the biology and diagnostic applications of cell-free DNA in plasma. In particular, the discovery of cell-free fetal DNA in maternal plasma in 1997 has opened up new possibilities for non-invasive prenatal testing (NIPT). Over the past 4 years, the performance for NIPT for fetal chromosomal aneuploidy detection has been performed in over 2 million pregnant women in over 90 countries. This represents one of the most rapid adoption of genomic testing worldwide. With an increase use of NIPT, a number of tested women are finding that aberration NIPT results could originate not just from the fetus, but from themselves. One of the latter possibilities is the presence of previously unknown malignancy in the pregnant woman. In this regard, we have recently developed a new technology that we have termed ‘plasma DNA tissue mapping’ that allows one to trace the tissue of origin of a genomic aberration detected in plasma. This approach has numerous diagnostic applications and would yield valuable information on the biology of circulating nucleic acids.

SYMPOSIA

S1-01

Development and Applications of CRISPR-Cas for Genome Engineering

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Novel genetic tools adapted from the microbial CRISPR-Cas system are accelerating the pace of biological research and enabling targeted genetic interrogation in almost any organism and cell type. CRISPR-Cas genetic perturbation is simple and scalable, and expands our ability to elucidate the functional organization of the genome and connect genotype to phenotype both in vitro and in vivo. Here, I give an overview of the development of the CRISPR-Cas technology and present recent work from our laboratory focusing on genome-scale screening in vitro and disease modeling in vivo, and discuss benefits and challenges of genome editing in biomedical research.

S1-02

CRISPR-Cas9 Genome Editing – a Game Changer in Genetics

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The type II clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9 (Cas9) system is an efficient tool for genome editing. Properly designed short guide RNAs are used to direct Cas9 precisely to target sites, where it functions as an endonuclease to efficiently produce double strand breaks in DNA. These breaks are repaired by two distinct mechanisms, non homologous end joining (NHEJ) and homologous directed repair (HDR). Because NHEJ is error prone, it produces unpredictable alterations

in sequence, in particular small indels, and is therefore suitable to induce random mutations. HDR as an alternative pathway can be used for precise sequence editing via the incorporation of an exogenous DNA fragment, commonly a single or double stranded DNA template. CRISPR/Cas9 can be used in embryonic stem cells (ESCs) or directly in fertilized oocytes for the generation of mutant mice. The advantages of producing SVs in ESCs are easy selection of the desired mutation and the direct use of these cells in culture to investigate the effects of induced mutations. The CRISPR/Cas technology can also be used to generate large structural variations. We applied the CRISPR technology in mouse embryonic stem cells (ESCs) and developed a 10-week protocol that we named CRISVar (CRISPR/Cas-induced structural variants), targeted at two different positions of a chromosome, to efficiently produce deletions, inversions, and duplications in mice. We were able to rearrange targeted genomic intervals ranging from 1 kb to 1.6 Mb using the CRISPR/Cas system in mouse embryonic stem cells (ESCs). ESCs harboring these mutations can be used to create chimeric animals. The efficiency of CRISPR/Cas in introducing mutations can be considered a game changer in genetics. Variants or unknown significance can now be tested quickly in vitro or in vivo. This is of particular importance for extremely rare diseases/variants for which a second patient/variant is difficult to find.

S1-03

Correction of Dystrophin Mutations by CRISPR/Cas9

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The advent of genome engineering technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise editing and regulation of endogenous human genes and epigenetic states. We have applied these tools to the correction of mutations that cause genetic disease and also adapted them to manipulate the epigenome and control cell fate decisions. For example, we engineered CRISPR/Cas9-based nucleases to correct the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients. When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored in vitro and following cell transplantation into mouse models in vivo. When delivered directly to a mouse model of this disease, gene editing by the CRISPR/Cas9 system led to gene restoration and improvement of biochemical and mechanical muscle function. In other studies, we have engineered CRISPR/Cas9-based tools to regulate the expression of endogenous genes and applied these tools to control genes relevant to medicine, science, and biotechnology. Genome-wide analysis of the DNA-binding, gene regulation, and chromatin remodeling by these targeted epigenome modifiers has demonstrated their exceptional specificity. We have recently applied these technologies to control the decisions of stem cells to become specific cell fates and reprogram cell types into other lineages that could be used for drug screening and disease modeling. Incorporating methods to dynamically control the activity of these proteins, such as optogenetic control of the proteins with light, has allowed us to pattern gene expression both temporally and spatially. Ongoing efforts include designing strategies to manipulate specific epigenetic marks that would enable deciphering the influence of epigenetics on gene regulation and disease states. Collectively, these studies demonstrate the potential of modern genome engineering technologies to capitalize on the products of the Genomic Revolution and transform medicine, science, and biotechnology.

S2-01

Epigenetic Clock and Biological Age

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The DNA methylation based biomarker of aging known as the "epigenetic clock" can be used to measure the DNA methylation (DNAm) age of any human (or chimpanzee) tissue, cell type, or fluid that contains DNA with the exception of sperm. DNAm age of blood has been shown to predict all-cause mortality in later life, even after adjusting for known risk factors, which suggests that it relates to the biological aging process. Similarly, markers of physical and mental fitness are also found to be associated with the epigenetic clock (lower abilities associated with age acceleration).

DNA methylation age has the following properties: first, it is close to zero for embryonic and induced pluripotent stem cells; second, it correlates with cell passage number; third, it gives rise to a highly heritable measure of age acceleration; and, fourth, it is applicable to chimpanzee tissues.

I illustrate the utility of this novel biomarker of aging by studying obesity, HIV infection, Alzheimer's disease, Parkinson's disease syndrome X, and supercentenarians.

Analysis of 6,000 cancer samples from 32 datasets showed that cancer types exhibit significant positive and negative age acceleration. Low age-acceleration of cancer tissue is associated with a high number of somatic mutations and TP53 mutations, while mutations in steroid receptors greatly accelerate DNA methylation age in breast cancer.

These results suggest that we are close to achieving a long standing milestone in aging research: the development of an accurate measure of tissue age or even biological age.

Main reference
Horvath S (2013) DNA methylation age of human tissues and cell types. Genome Biology 2013, 14:R115
Correction: http://genomebiology.com/2013/14/10/R115/comments
Wikipedia: https://en.wikipedia.org/wiki/Biological_clock_(aging)

S2-02

Epigenetic Effects of Glucocorticoids Exposure During Hippocampal Neurogenesis and Their Implication in Stress Related Psychiatric Disorders

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Exposure to early life stress (ELS) is a well-known major risk factor for developing psychiatric and behavioural disorders later in life. Both prenatal (ex. maternal stress during pregnancy) and postnatal (ex. child maltreatment) stressors have been shown to have a long-lasting impact on adult pathological states. Epigenetic mechanisms have been shown to be in part responsible for the embedding of these long-term changes. It was also shown that the differences in these epigenetic mechanisms depend on the type and timing of stress exposure as well as tissue specific vs. more global changes. One of the mechanisms that might lead to epigenetic alterations in multiple tissues is a long-lasting disruption of the stress hormone system by excessive glucocorticoids (GC) release after ELS exposure. Stress and GCs are known to regulate hippocampal neurogenesis and to induce long-lasting changes in DNA methylation in specific loci such as the glucocorticoid receptor (GR) and FK506 binding protein 5 (FKBP5) in hippocampal but also in blood cells DNAs. Using a genome-wide approach, we analysed gene expression and DNA methylation levels of immortalised human hippocampal progenitor cells (HPC) treated with dexamethasone (Dex, a potent GR agonist) or vehicle at different stages during neurogenesis. Our results revealed an effect of Dex treatment on DNA methylation of more than 27 000 CpG sites and on mRNA expression of ~ 3900 genes during hippocampal differentiation where a significant portion of these alterations where maintained after differentiation including the FKBP5 locus. These analyses provide evidence of clustered and genome-wide epigenetic effects of GC activation during hippocampal neurogenesis where the timing of the exposure seems to be critical to induce long-lasting changes. In addition, we observed that the genes showing Dex-induced DNA methylation alterations during hippocampal neurogenesis were also enriched in genes differentially methylated during fetal brain development as well as in genes previously associated with child abuse in human hippocampus and blood cells. We currently explore whether epigenetic changes associated with GR activation in the hippocampal cells could relate to GR-induced changes in blood of patients where we already shown that genetic factors predict GR-induced changes in gene expression following acute treatment with Dex (GR-response eQTL analysis). These SNPs showing an association with GR induced changed in gene expression are highly enriched among SNPs associated with psychiatric disorders and interact with child abuse to predict disease risk. We believe that our combined approach using cellular models and patients exposed to glucocorticoids, either through acute treatment with Dex or exposure to early life stress events, would provide additional insights into disease risk mechanisms. A mechanistic understanding of the long-term epigenetic consequences of stress may allow novel, targeted intervention and prevention strategies for behavioral, psychiatric and other stress-associated disorders.

S2-03

Epigenetics – why its Impact on the Development of Disease is Underestimated

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The mainstream view of development and disease is deterministic and gene-centric. It began to dominate modern genetics and developmental biology soon after Watson's and Crick's discovery of the double helix structure of DNA in 1953, because nucleic acid-based experiments were easy to perform and most funds went into such projects. Epigenetics, which had been developed by C.H. Waddington in the 1940s and which provides a system biology view on development and evolution, was ousted by the gene-

centric view and rediscovered only 30 years ago. Although epigenetics is nowadays considered as one of the hottest fields in biology, it is often regarded only as the study of covalent DNA and histone modifications. For a deeper understanding of development and disease we have to rediscover and further develop its theoretical framework that embraces system dynamics, developmental plasticity and genetic assimilation.

S3-01

Integrated Omics Technologies for Patients with Genetic Neuromuscular Diseases

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Genetic neuromuscular diseases (NMD) have a wide phenotypic spectrum, show an enormous genetic heterogeneity, are usually incurable and can be associated with severe complications including sudden death. In the past 25 years the strategies and methods applied have allowed us to identify neuromuscular disease genes mainly in larger families and for more frequently occurring genetic conditions. Using cost- and time-intense step-by-step single gene approaches only the most frequent genetic causes and patients with characteristic phenotypes are normally being tested, leaving many patients with very rare and clinically unspecific NMD without a precise diagnosis. Depending on the health system and local access to diagnostic tools, between 30-80% of neuromuscular patients in Europe remain undiagnosed. As a precise genetic diagnosis is a prerequisite for the monitoring of disease complications, the counselling of families and therefore the overall quality of life and life expectancy of a patient, it is a major challenge to identify the genetic cause for all patients with NMD. Since the identification of the first gene associated with a genetic muscle disease, namely Duchenne muscular dystrophy, an extensive body of research into genetics and pathogenesis has resulted in the identification of genetic defects responsible for over 750 NMD, revealed an increasingly varied phenotypic spectrum, and exposed the need to move towards a new systems-based understanding of the conditions in terms of the molecular pathways affected. New omics technologies including whole-exome and whole-genome sequencing are continuing to expand the range of genes and phenotypes associated with NMD, and new computational approaches are helping clinicians move into this new genomic medicine paradigm. However, 30 years on from the DMD gene discovery, no curative therapies exist for any form of NMD, and systematic exploration of their natural history is still lacking. In order that basic research can be more rapidly translated to the clinic, well-phenotyped and genetically characterized patient cohorts are a necessity, and appropriate outcome measures and biomarkers must be developed through natural history studies. International collaborations are now addressing these translational research issues and are already showing that the identification of novel NMD genes can facilitate personalized medicine, advance and standardise NGS diagnosis, reduce ineffective interventions and burdensome investigations, improve standards of care for patients and the development of target–driven therapies.

S3-02

Parkinson Syndromes

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Several genes are well validated as causes of a typical Parkinson's disease (PD) phenotype. However, known monogenic causes and genetic risk factors only partly explain the observed familial aggregation of PD. Not surprisingly, the application of new techniques such as next generation sequencing (NGS) and GWAS meta-analyses have allowed for the discovery of new genes and genetic risk factors for PD. In addition, clinico-genetic studies have been used to improve genotype-phenotype correlations and to reveal the earliest disease signs. Furthermore, there has been significant progress in the development of new disease models, particularly through the use of induced pluripotent stem (iPS) cell-derived neurons.

According to a recently revised system of the genetic nomenclature of PD, there are ten confirmed monogenic forms of parkinsonism with a PARK designation. Three follow an autosomal dominant mode of inheritance, and seven are recessively inherited. The most common forms are late-onset autosomal dominant parkinsonism with mutations in the LRRK2 gene and early-onset parkinsonism caused by mutations in the Parkin gene. The three dominant forms and three of the seven recessive forms of parkinsonism (Parkin, PINK1, DJ-1) are associated with a clinical picture closely resembling that of idiopathic PD with its cardinal motor features of bradykinesia, resting tremor, rigidity and postural instability. The remaining four recessive forms (ATP13A2, FBOX07, DNAJC6, and SYNJ1) usually have a juvenile onset and present with atypical, multisystem features including early dementia, eye movement abnormalities, pyramidal signs etc.

Genome-wide association studies (GWAS) have been a major advance in genetic research, enabling the assessment of genetic risk factors associated with PD and other disorders via large-scale, population-based studies. The third and most comprehensive meta-analysis included data from seven million polymorphisms originating either from GWAS datasets and/or from smaller-scale PD association studies and was supplemented with unpublished data to include >100,000 patients and controls. Many previously reported risk loci, including SNCA and MAPT, were confirmed and evidence for a new risk variant in the ITGA8 gene was found. The risk factors identified by these studies provide clues to the underlying molecular mechanisms and offer potential targets for novel treatments.

Both the monogenic and the idiopathic forms of Parkinson disease share common pathophysiological mechanisms converging on oxidative modification, impaired protein degradation, and mitochondrial dysfunction. Therefore, monogenic forms of Parkinson disease can serve as human model diseases for the idiopathic form.

S4-01

Genome-Embedded Ribonucleotides and Genome Instability

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Our identification of biallelic hypomorphic mutations in three RNase H2 genes1 in the neuroinflammatory disorder, Aicardi-Goutières syndrome led us to investigate enzyme complex they encode. We subsequently established this is an important genome surveillance enzyme that removes over 1,000,000 ribonucleotides embedded in the genomic DNA of every replicating mammalian cell. Such ribonucleotides represent the most common non-canonical nucleotides incorporated into the genome by replicative polymerases and are an important potential source of genome instability.

S4-02

Systemic DNA Damage Responses in Development and Aging

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The causal contribution of DNA damage in driving the aging process has become evident in a variety of progeroid syndromes that are caused by defects in DNA repair systems. Congenital defects in genome maintenance mechanisms cause complex disease phenotypes characterized by developmental growth failure, cancer susceptibility, and premature aging. The distinct human disease outcomes of DNA repair defects become particularly apparent in syndromes caused by mutations in nucleotide excision repair (NER). While transcription-coupled (TC-) NER defects lead to growth and mental retardation and premature ageing in Cockayne syndrome (CS) patients, global-genome (GG-) NER mutations lead to highly skin cancer prone Xeroderma pigmentosum (XP). Intriguingly, the distinct outcomes of NER deficiencies are conserved in the simple metazoan C. elegans. TC-NER deficiency renders worms highly susceptible to DNA damage during developmental growth and with aging, while GG-NER defects give rise to genome instability in proliferating germ cells.

We employed the nematode model to investigate distinct response mechanisms to genome instability in somatic tissues and in the germline. DNA damage that persists in somatic tissues leads to activation of the insulin-like growth factor signalling (IIS) effector DAF-16. The FoxO transcription factor DAF-16 is efficiently activated in response to DNA damage during development while its DNA damage responsiveness declines with aging. We demonstrated that DAF-16 alleviates growth arrest and enhances DNA damage resistance in somatic tissues even in the absence of DNA repair. We propose that IIS mediates DNA damage responses in somatic tissues and that DAF-16 activity enables developmental growth amid persistent DNA lesions and promotes tissue maintenance through enhanced tolerance of DNA damage that accumulates with aging.

DNA damage that persists in germ cells leads to enhanced stress resistance of somatic tissues. The “Germline DNA damage-induced systemic stress resistance” (GDISR) is mediated by the innate immune system that is triggered by genomically compromised germ cells and executed through elevated activity of the ubiquitin proteasome system (UPS) in somatic tissues. We propose that GDISR elevates somatic endurance to extend reproductive lifespan when germ cells require time to reinstate genome stability before resuming offspring generation.

Our findings suggest that somatic tissues adapt to distinct constraints of genome instability in the germline and the soma: Developmental growth of somatic tissues can be sustained despite genome

instability by DAF-16-mediated DNA damage tolerance, while adult tissues adapt to the requirements of genomically compromised in germ cells through GDISR.

S5-02

Pan-Cancer Patterns of Somatic Retrotransposition

Jose M. C. Tubio[1,2], Jan Korbel[3] & Peter J. Campbell[1] on behalf of the Retrotransposition Subgroup of the PanCancer Initiative

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Retrotransposons are repetitive elements that are constantly on the move. By poaching certain enzymes, they copy and insert themselves at new sites in the host genome generating structural variability of potential functional importance for the cancer cell. Retrotransposons can also promote genomic rearrangements by recombination, and mobilize coding and regulatory regions by transduction. Despite next-generation sequencing analyses have recently increased our understanding of cancer retrotransposition, little is known about the extent to which retrotransposons can generate diversity in somatic cells and contribute to the development of cancer. Within the framework of the PanCancer initiative, we aim to assess the impact and functional consequences of retrotransposons in cancer. We are surveying the topography of somatic retrotransposition in 2,600 cancer whole-genomes, integrated with transcriptomic and epigenomic data. We believe the study of this large dataset will uncover new mutational processes in cancer where retrotransposons are involved, and provide new insights on the role of retrotransposons in oncogenesis.

S6-01

Networks of Genes, Epistasis and a Functionally-Clustered Genome

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Autism is highly genotypically heterogenous disorder, to which variants in a large number of genes likely to contribute. Identifying the molecular pathways in which these genes act provides not only insight into the pathoetiology but also translational routes to diagnosis, patient stratification and targeted therapy. By combining the strengths of many different types of functional genomics data, we create sensitive functional networks that identify commonly perturbed molecular networks underlying genetic disorders. For autism, these approaches unify different classes of genetic risk variants, identify multiple distinct molecular aetiologies, and also identify the contribution of genetic interactions, which is one of the most significant challenges facing human disease genetics. Finally, these approaches suggest that the disruption of genomically-clustered functionally-related genes is a significant and common characteristic of large pathogenic structural variants.

S6-02

CNVs as Modifiers of the Cardiovascular Phenotype in 22q11.2 Deletion Syndrome (22q11DS)

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The 22q11.2 deletion syndrome (22q11DS; velocardiofacial /DiGeorge syndrome; VCFS/DGS; MIM #192430; 188400) is the most common microdeletion syndrome. The phenotypic presentation in this population is highly variable. Approximately 65% of 22q11DS patients have a congenital heart defect (CHD), mostly of the conotruncal type, and/or an aortic arch defect. The etiology of this phenotypic variability is still not known. We have hypothesized that copy number variants (CNVs) outside the 22q11.2 deleted region might increase the risk of being born with a CHD in this sensitized population. Genotyping with Affymetrix SNP Array 6.0 was performed on two groups of 22q11DS patients separated by time of ascertainment and processing. CNV analysis was completed on a total of 949 patients (Cohort 1 n=562; Cohort 2 n=387), 603 with CHDs (Cohort 1 n=363; Cohort 2 n=240) and 346 with normal cardiac anatomy (Cohort 1 n=199; Cohort 2 n=147). Our analysis revealed that a duplication of SLC2A3 was the most frequent CNV identified in the

first cohort. It was present in 18 subjects with CHDs and 1 subject without (p=3.12x10-3, two-tailed Fisher's exact test). In the second cohort, the SLC2A3 duplication was also significantly enriched in subjects with CHDs (p=3.30x10-2, two-tailed Fisher's exact test). The SLC2A3 duplication was the most frequent CNV detected and the only significant finding in our combined analysis (p=2.68x10-4, two-tailed Fisher's exact test), indicating that the SLC2A3 duplication may serve as a genetic modifier of CHDs and/or aortic arch anomalies in individuals with 22q11DS. Biological studies in zebrafish are under way to assess the functional significance of these findings. We further hypothesized that rare copy number variants (CNVs) outside the deleted region might modify the risk of being born with a CHD in this genetically "modified" population. Rare CNV analysis was performed using Affymetrix SNP Array 6.0 data from 946 22q11DS subjects; with CHDs (n=607) or with normal cardiac anatomy (n=339). Although there was not a significant difference in the overall burden of rare CNVs, an overabundance of cardiac related genes was detected in in the CNVs of 22q11DS individuals with CHDs (cases). When the rare CNVs in cases were examined with regard to gene interactions, specific cardiac networks appear to be overrepresented in 22q11DS CHD cases but not controls. Taken together with the finding of an excess of the SLC2A3 duplications in cases, these data suggest that CNVs outside the 22q11.2 region may contain genes that modify risk for CHDs in some 22q11DS patients. These findings are worthy of a replication study to determine whether they are repeated in additional subjects and are deserving of further consideration with respect to functional validation.

SELECTED PRESENTATIONS (SEL)

Sel-01

A novel molecular mechanism for progeroid phenotypes

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Segmental progeria syndromes are a class of inherited disorders characterized by the premature aging of specific tissues. Using Whole-Exome Sequencing we identified the p.G472R de novo missense mutation and the p.I611del de novo deletion in anoctamin 6 (ANO6) in two patients affected by segmental progeria syndromes of varying severity. ANO6 is a transmembrane protein with a proposed dual function as an ion channel of debated properties and as a phospholipid scramblase, which can move phospholipids across the two layers of the plasma membrane. ANO6 loss-of-function mutations cause Scott Syndrome, a bleeding disorder with no signs of premature aging. We therefore hypothesized that the p.G472R and p.I611del mutations might be gain-of-function mutations. We first investigated the ion channel function of ANO6 using whole-cell patch measurements with HEK cells overexpressing either WT or mutated ANO6 constructs. When compared to WT ANO6, both mutants showed faster activation kinetics and stronger currents after the addition of calcium. Furthermore, the ANO6 p.G472R-overexpressing cells showed the presence of currents even in the absence of calcium. We next investigated the scramblase activity of ANO6. We obtained patient fibroblasts carrying the p.I611del mutation and measured the exposure of phosphatidylserine (PS), which is normally confined to the inner leaflet of the plasma membrane, using annexin V staining and subsequent FACS analysis. The patient's fibroblasts showed a striking increase in the amount of PS exposed on the outer leaflet, indicating that the p.I611del mutation causes an increase in the scramblase activity of ANO6. We could confirm this finding using stable ANO6 knock-out HEK cells transfected with the p.I611del construct. We next performed mass spectrometry of whole cell lysates to determine the lipid composition of p.I611del fibroblasts. When compared to control fibroblasts, we detected several significant changes in the quantity of phospholipid subspecies and in the total amount of sphingomyelins. Finally, we analyzed the MEK-ERK pathway by Western blots of phosphorylated cRAF, MEK and ERK. At all three levels, we could detect an upregulation of the pathway. Consistent with the reported effects of prolonged hyperactivation of the MEK-ERK pathway, the patient's fibroblasts showed a significantly higher doubling time and an increased positivity to β-galactosidase staining, indicating cellular senescence. Taken together, our results show that different gain-of-function mutations in ANO6 cause progeroid phenotypes through an increase in

both the ion channel and the scramblase activity. We propose that such changes affect the lipid composition of the plasma membrane, which in turn causes dysregulation of the MEK-ERK signaling and premature cellular senescence.

Sel-02

Copy number variation morbidity map of congenital limb malformations reveals that the majority of pathogenic variants affect non-coding regulatory elements

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Congenital limb malformations can occur as part of a syndrome or as an isolated form and are thought to be largely genetic in origin. The extensive genetic heterogeneity of these anomalies requires a genome-wide detection of all types of genetic variation. Microarray studies in single affected families have previously demonstrated the importance of copy number variations (CNVs) in limb malformations, but no large scale study has been performed so far and the majority of cases remain undiagnosed. Here we applied high resolution array CGH to 300 patients with congenital limb malformations. All patients included had not received a molecular diagnosis after candidate gene testing. We found 31 pathogenic CNVs in known disease loci and identified 6 new loci previously not known to be associated with limb malformations. The pathogenic CNVs affected non-coding cis regulatory elements more frequently than expected (21 non-coding vs 16 coding). We performed functional studies in transgenic mice using the CRISPR/Cas9 system and/or segregation studies in these families to investigate the pathogenicity of 6 novel CNVs causing limb defects. Taken together we reached a diagnostic yield of 12% in this cohort, which is comparable to copy number studies in other cohorts such as in individuals affected with intellectual disability. However, the majority of the pathogenic CNVs (57%) were likely to result from changes in the non-coding cis regulatory landscape, while only 43% were due to gene dosage effects or haploinsufficinency. Additionally, we identified 20 rare CNVs of unknown clinical significance (7%) that were inherited from an unaffected parent. Due to reduced penetrance, a key feature of limb malformations, these rare CNVs might still have an important impact on the skeletal phenotypes of our patients.

Our results suggest that CNVs affecting non-coding regulatory elements are a major cause of congenital limb malformations.

Sel-03

Mutations in the X-linked gene TEX11 are a common cause for meiotic arrest and azoospermia

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Background The genetic causes of non-obstructive azoospermia (NOA) are unknown in the majority of infertile men. To investigate the genomic imbalances responsible for NOA, a genome-wide copy number variation study was carried out and followed-up with further analyses of the X-linked candidate gene *TEX11*.

Methods First, we performed array comparative genomic hybridisation (aCGH) in 15 NOA patients. Then, *TEX11* was screened for mutations via direct Sanger sequencing in 49 men from Pittsburgh and 240 from Münster (total 289 NOA patients) 384 controls (192 from Pittsburgh and 192 from Münster).

Results A 99-kb hemizygous deletion of three *TEX11*-exons was identified in a NOA patient. The deletion was further analysed by a targeted X-chromosome high-resolution microarray and an identical deletion was identified in another NOA patient. Our subsequent mutation screening showed five previously undescribed *TEX11* mutations: three splicing mutations and two missense mutations. These mutations, which occurred in 7 of 289 men with azoospermia (2.4%), were absent in 384 controls with normal sperm concentrations. Notably, five of those *TEX11* mutations were detected in 33 patients (15%) with azoospermia caused by meiotic arrest. Meiotic arrest in these patients resembled the phenotype of Tex11-deficient male mice. Immunohistochemical analysis showed specific cytoplasmic TEX11 expression in late spermatocytes, as well as in round and elongated spermatids, in normal human testes. In contrast, testes of patients who had azoospermia with *TEX11* mutations had meiotic arrest and lacked TEX11 expression.

Conclusions Mutations in the X-chromosomal gene *TEX11* are a frequent cause of meiotic arrest and azoospermia in humans.

Funding

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Reference

Yatsenko AN, Georgiadis AP, Röpke A, Berman AJ, Jaffe T, Olszewska M, Westernströer B, Sanfilippo J, Kurpisz M, Rajkovic A, Yatsenko SA, Kliesch S, Schlatt S, Tüttelmann F. X-linked TEX11 mutations, meiotic arrest, and azoospermia in infertile men. N Engl J Med 2015;372:2097-107

Sel-04

Ultra-sensitive mosaic mutation detection for clinical applications

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Despite the great advances in the next generation sequencing field there is still room for improved targeted re-sequencing assays that combine high throughput with ultra-high sensitivity. We have now further optimized a single-molecule molecular inversion probe (smMIP) based targeted re-sequencing approach1. Single-molecule tracing is enabled using up to 410 (1,048,576) molecular tags. Consensus calling of respective PCR-duplicates allows correction for PCR and sequencing errors. The improved assay allows low-frequency or sub-clonal variant detection with variant levels of <0.05%. This assay provides very robust genotyping accuracy, high throughput, fast turnaround and cost-effectiveness. We anticipate that this or similar assays allow novel applications in which mutations are present in very low relative abundance in any given DNA sample with important new applications beyond cancer genetics.

Here we present first successful applications that include: 1.) Accurate determination of the fraction of mutated alleles for post-zygotic de novo mutations2. 2.) Detection of previously unrecognized mosaic disease causing mutations for rare clinical syndromes. 3.) Detection of known ‘paternal age effect disorders’ causing mutations as small clonal events in dissected testis material3. 4.) First evidence for presence or absence of parental alleles in cell free DNA from plasma of pregnant women.

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1) Hiatt JB, et al. Genome Res. 2013 May;23(5):843-54.
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EDU-EDUCATIONAL SESSIONS

EDU1

Bewegungsstörungen: Welche Informationen helfen bei der genetischen Diagnostik

Moderation: Katja Lohmann (Lübeck) und Alexander Münchau (Lübeck)

Bewegungsstörungen umfassen eine Reihe neurologischer Erkrankungen und betreffen mehr als 5% der Bevölkerung. Sie sind durch Störungen der Bewegungs- und Haltungsregulation gekennzeichnet, denen eine pathologische Aktivität in motorischen Regelkreisen zugrunde liegt. Bei Patienten treten Verarmung oder Überschuss an Bewegungen auf, die nicht oder nur sehr eingeschränkt der willentlichen Kontrolle unterliegen. Häufige Bewegungsstörungen sind der essentieller Tremor, das Gilles de la Tourette Syndrom, das Restless Legs Syndrom und Parkinson-Syndrome, seltener sind Dystonien, Ataxien, und Myoklonus.

Die Parkinson Erkrankung galt viele Jahre lang als *das* Lehrbuchbeispiel einer nicht genetischen Erkrankung. Untersuchungen in den letzten drei Jahrzehnten konnten jedoch klar das Gegenteil beweisen.

Bewegungsstörungen sind klinisch, aber auch genetisch sehr heterogen, was die Diagnostik erschwert und selbst für Bewegungsstörungsexperten oft eine Herausforderung darstellt. Es gibt allerdings einige Grundkenntnisse, die die genetische Diagnostik von Bewegungsstörungen erheblich erleichtern können.

Die EDU-Veranstaltung wird sich in 5 Abschnitte gliedern und relevante Informationen präsentieren:

1 Einführung: Klinisches Bild verschiedener Bewegungsstörungen

Hierbei soll anhand ausgewählter Videos auf die charakteristischen Zeichen bei verschiedenen Bewegungsstörungen und die entsprechenden Unterschiede eingegangen werden.

2 Nomenklatur genetischer Bewegungsstörungen und Entwicklung eines online-tools zur Phänotyp-Genotyp-Prädiktion

Die Nomenklatur vieler Bewegungsstörungen basierte auf einer konsekutiven Nummerierung gefundener Krankheitsgene. So entstanden im Laufe der Jahre lange Listen von Krankheitsbezeichnungen wie z. B. *PARK1* bis *PARK20* zur Einteilung genetischer Formen eines Parkinson-Syndroms. Diese Listen enthalten allerdings eine Reihe von Fehlern. Daher wurde die Nomenklatur kürzlich revidiert. Dies wird vorgestellt werden. Obwohl für eine Reihe von Bewegungsstörungen eine Vielzahl von Krankheitsgenen identifiziert wurden - wie zum Beispiel mehr als 40 Gene für spinozerebelläre Ataxien - weisen doch viele der Bewegungsstörungen typische klinische Zeichen auf, die eine Eingrenzung der Kandidatengene erlauben. Basierend auf einer detaillierten Literaturrecherche entwickeln wir mit Unterstützung der *Movement Disorder Society* ein online-tool, das bei der Eingrenzung von Kandidatengenen helfen soll.

3 Unterschiede in der Genverteilung bei verschiedenen Ethnien

Die Mutationshäufigkeit verschiedener Gene, die bei Bewegungsstörungen von Bedeutung sind, variiert sehr stark bei verschiedenen Populationen. So erklärt z. B. eine Mutation im LRRK2-Gen bis zu 40 % der Parkinson-Erkrankungen in Nordafrika, während weniger als 1 % der Deutschen Parkinson-Patienten diese Mutation tragen. Andere Phänotypen kommen ausschließlich in bestimmten ethnischen Gruppen vor. Ein Beispiel hierfür ist das X-gekoppelte Dystonie-Parkinson Syndrom bei Filipinos aus Panay. Die Kenntnis der ethnischen Unterschiede in der Verteilung bestimmter Krankheitsursachen hilft bei der Priorisierung einer weiterführenden genetischen Diagnostik.

4 Funktionelle Charakterisierung von potentiell pathogenen Varianten

Ein häufiges Problem bei der Befundung genetischer Analysen ist die Interpretation von Varianten in bekannten Genen für Bewegungsstörungen hinsichtlich ihrer Pathogenität. Im Zeitalter des next generation sequencing ist nicht mehr das Aufspüren einer Mutation in einem Kandidatengen die Herausforderung für die Diagnostik, vielmehr die Interpretation gefundener Varianten hinsichtlich ihrer Pathogenität. Für einige Dystonia-Gene konnten relativ einfach durchführbare funktionelle Tests entwickelt werden, die bei der Unterscheidung von benignen und pathogenen Varianten helfen können. Diese sollen exemplarisch vorgestellt werden.

5 Aktuelle Erfolge und Misserfolge bei der Aufklärung genetischer Bewegungsstörungen

Während die genetischen Ursachen für einige Bewegungsstörungen wie zum Beispiel die Huntington-Erkrankung gut bekannt sind, liegen die genetischen Ursachen häufigerer Erkrankungen wie dem essentieller Tremor oder dem Tourette-Syndrome noch weitestgehend im Dunkeln. In diesem letzten Teil der Veranstaltung soll ein Überblick über den aktuellen Kenntnisstand gegeben werden. Es sollen auch Einblicke vermittelt werden, welche Diagnostik-Methoden (Kandidatengen, Panel, Exom, quantitative Analysen) bei welcher Erkrankung erforderlich bzw. sinnvoll sind.

EDU 2

DER (UN-)GELÖSTE FALL (IN GERMAN ONLY)

Moderation: Dagmar Wieczorek (Düsseldorf), Gabriele Gillessen-Kaesbach (Lübeck)

In dieser EDU soll den Teilnehmern Gelegenheit gegeben werden, ungelöste Fälle oder interessante gelöste Fälle vorzustellen. Willkommen sind auch maximal 8 Minuten dauernde Kurz-Reviews zu neuen syndromalen Krankheitsbildern. Um die differenzialdiagnostische Diskussion möglichst effizient zu gestalten, bitten wir bis zum 01. März 2015 um Zusendung von 2-3 Folien (wesentliche klinische Daten, durchgeführte Diagnostik sowie aussagefähige Fotos der Patienten) für die (un)gelösten Fälle und Anmeldung von Kurz-Reviews an folgende Email-Adressen: dagmar.wieczorek@med.uni-duesseldorf.de oder g.gillessen@uksh.de.

WS1-INTELLECTUAL DISABILITY

WS1-01

SysID: A systematic approach to the genetic and clinical heterogeneity of intellectual disability disorders

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Intellectual disability (ID) disorders are clinically and genetically extremely heterogeneous and thus represent a major challenge in clinical genetics and diagnostics. Though recent studies on specific subsets of ID and co-morbid autism spectrum disorders have indicated that convergent molecular pathways underlie common phenotypic aspects, a comprehensive and systematic understanding of ID disorders and their underlying biology is still limited.

To provide an inventory on monogenic causes of ID and to systematically unravel common molecular themes, we established SysID, a publicly available database containing 746 known ID genes and additionally 359 ID candidate genes (status Nov 2015). Further gene-related information, various functional datasets and information on associated disorders were implemented. We classified all ID genes according to their ID manifestation (syndromic with or without structural malformations, non-syndromic,) and severity and according to 27 associated core clinical features such as organic or neurological anomalies. Currently, mutations in 60% of 746 ID genes follow autosomal recessive inheritance, mutations in 27% are autosomal dominant (mainly de novo), and 15% are X-linked. This database can be exploited for various queries and clinical, diagnostic and research purposes.

Using this integrated resource we could systematically break down ID genes and disorders into biologically coherent modules. Nearly half of all ID proteins physically interact with other ID proteins and are significantly co-expressed, particularly in the hippocampus. 86% of ID genes fall into 32 common biological, gene ontology based processes: metabolism and nervous system development being the largest, and hedgehog and glutamate signalling the most enriched groups.

By combing functional and phenotype information we found that certain phenotypic subgroups can predict novel gene functions, and that vice versa, certain functional themes correlate with specific phenotypic features. The latter revealed characteristic, process-defined phenoprofiles in terms of IDopathies including “chromatinopathies” or “DNArepairopathies”. We also showed that biological coherence among ID genes is sufficient to predict ID genes, and that utilization of phenotypic information adds even more predictive power. Furthermore, we utilized custom-made datasets on ID gene function in Drosophila. Early onset behavioral and specific morphological wing phenotypes were characteristic for ID genes in general, and several fly phenotypes were particularly representative for specific human clinical phenotype classes.

SysID (http://sysid.cmbi.umcn.nl/) is a manually curated, publicly available and widely usable database of ID genes, their associated disorders, phenotypes and biological functions. Using this resource, our study provides a systematic insight into the molecular and clinical landscape of ID disorders and proves the utility of systematic phenomic analyses in a highly heterogeneous genetic disorder.

WS1-02

Exome sequencing of 311 trios confirms the importance of de novo mutations in intellectual disability

Wieland T.[1], Luedecke H-J.[2], Zink AM.[3], Graf E.[1], Bramswig N.[2], Schwarzmayr T.[1], Becker J.[3], Berutti R.[1], Czeschik JC.[2], Cremer K.[3], Meitinger T.[1,4], Engels H.[3], Wieczorek D.[2], Strom TM.[1,4]

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Intellectual disability (ID) has an estimated prevalence of 1-2%. Recently, exome sequencing of parent-child trios has shown that a large proportion of ID are caused by de novo mutations.

We sequenced exomes of 311 children with ID and of their parents to an average read depth of 114. CNVs have previously been excluded in most patients. Variant data was generated through an automated

pipeline using different variant and CNV callers (SAMtools, Pindel and ExomeDepth) and stored in a custom database. Variants were annotated in a cooperative way through a Web-based frontend and confirmed by Sanger sequencing. We identified 585 de novo variants (120 synonymous, 349 missense, 13 in-frame indels, 103 loss-of-function (nonsense, frameshift, splice site)), with an exomic mutation rate of 1.88 per individual per generation. 53 patients (17%) had at least one protein altering de novo variant in a gene already associated with a developmental disease by monoallellic mutations (291 genes from the DDG2P database version July 2015). 56 patients (18%) had no de novo variant.

These variants in combination with data from collaborating groups were instrumental in identifying two genes not yet associated with ID, HIVEP2 and CHAMP1. Additionally, we extended the phenotypic spectrum of protein altering variants in seven genes: ASXL3, KCNH1, SETD5, CTNNB1, ECHS1, IQSEC2 and TCF20.

We compared these de novo variants to a set of 58 de novo variants (22 synonymous, 31 missense, 5 LoF) identified in 50 healthy control trios, corresponding to an exomic mutation rate of 1.16 per individual per generation. 17 controls (34%) had no de novo variant. Interestingly, the difference in the synonymous mutation rate was not significant (p=0.62; Mann-Whitney-Wilcoxon test, two-sided), however the difference in the protein altering mutation rate was highly significant ($p=1.65x10^{-5}$).

When comparing only the 251 excluding cases with de novo variants in genes already associated with developmental disorders or in one of the nine genes described above, to the 50 controls, the difference between protein altering mutation rates remains highly significant ($p=6x10^{-4}$).

In summary, we show that exome sequencing in a multicenter setting with an appropriate IT environment can efficiently be used to generate clinical grade diagnoses by integrating the advantage of standardized central sequencing and distributed evaluation and annotation of the resulting data in the clinical context.

WS1-03

Whole exome sequencing in 150 consanguineous families with intellectual disability: high diagnostic yield and identification of novel candidate genes

Reuter M.[1], Tawamie H.[1], Buchert R.[1], Froukh T.[2], Hosny Gebril O.[3], Strom T.[4], Hoffjan S.[5], Wieczorek D.[6], Sticht H.[7], Reis A.[1], Abou Jamra R.[1,8]

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Autosomal recessive forms of intellectual disability (ARID) represent a significant fraction of the causes of ID, especially in consanguineous families. Several hundred ARID genes have been identified to date, and many more are presumed. We investigated 150 consanguineous families with one (29%) or multiple (71%) affected children with ID with genetic mapping followed by whole exome sequencing (WES). Almost all affected persons (94%) showed additional symptoms, mostly muscular hypotonia, epilepsy, and microcephaly. We prioritized variants based on several criteria, including localisation in a candidate region, being rare, highly conserved and predicted pathogenic in silico, as well as being in a gene with a (presumed) neurological function. We identified a relevant variant in 114 (76%) of the families, with even higher rates in cases with a more specific phenotype and in families with more than one affected person. Among these, 53 (36%) were genes that in the meanwhile were published by us (10 genes, 7%) or by others (43, 29%), and likely clarify the cause of ARID in the corresponding families. In the remaining 60 (40%), the identified homozygous variant is located in a candidate gene. Despite the high heterogeneity we found variants in two unrelated families for four already described genes, *AHI1*, *GPR56*, *PRRT2*, and *PLA2G6*. At least one patient has two distinct phenotypes because of two homozygous variants in different genes.

In order to prove the pathogenicity of non-truncating variants, we applied a wide spectrum of functional experiments including testing specific parameters in affected individuals (e.g. metal ions in serum), testing specimens of affected persons (e.g. flow cytometry of lymphocytes), and analyses of cell models on RNA and protein levels. Until now, we have shown a deleterious effect for 12 variants in 10 genes (*FAR1*, *PGAP1*, *PGAP2*, *EZR*, *EDC3*, *PTEN*, *TAF13*, *FRRS1L*, *ZIP8*, *KIAA0586*). In order to prove the causality of the candidate variants, identifying further likely pathogenic variants in similarly affected individuals is necessary. To achieve this, and since ARID is extremely heterogeneous, we initiated a Consortium of Autosomal Recessive Intellectual Disability (CARID) to share data of candidate genes and of phenotypes, through which we identified so far additional independent cases for 12 candidate genes.

On research level, further sequencing and functional experiments are ongoing to identify and characterize further ARID genes. On the clinical level, WES in consanguineous families with intellectual disability has the highest diagnostic yield of all genetic techniques and should therefore become part of a first line diagnostic approach.

WS1-04

De novo deleterious mutations in CHAMP1 cause intellectual disability with severe speech impairment

Hempel M.[1], Cremer K.[2], Oeckeloen CW.[3], Lichtenberg KD.[4], Herbert JC.[5], Denecke J.[6], Haack TB.[7], Zink A.[2], Wohlleber E.[2], Johannsen J.[6], Alhaddad B.[7], Pfundt R.[3], Fuchs S.[1], Wieczorek D.[8], Strom TM.[7], van Gassen KLI.[4], Kleefstra T.[3], Kubisch C.[1], Engels H.[2], Lessel D.[1]

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By trio whole-exome sequencing, we have identified de novo deleterious mutations in *CHAMP1* in five unrelated individuals affected by intellectual disability with severe speech impairment, motor developmental delay, muscular hypotonia, and similar dysmorphic features including short philtrum and a tented upper and everted lover lip. Recently further five unrelated patients with similar clinical features and deleterious de novo *CHAMP1* mutations have been independently reported. *CHAMP1* encodes a protein with a function in kinetochore-microtubule attachment and in the regulation of chromosome segregation, both of which are known to be important for neurodevelopment. All so far identified mutations, if resulting in a stable protein, are predicted to lead to the loss of the functionally important zinc- finger domains, which regulate CHAMP1 localization to chromosomes and the mitotic spindle, in the C-terminus of the protein, thereby providing a mechanistic understanding for their pathogenicity. We thus establish deleterious de novo mutations in *CHAMP1* as a more common genetic cause of intellectual disability than previously anticipated. In this presentation we will provide a detailed phenotypic characterisation of this novel *CHAMP1*-associated syndrome.

WS1-05

Homozygous variant in TAF13, a component of transcription factor II D, deregulating transcription in multiple CNS development pathways in a family with autosomal recessive intellectual disability

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Genetic mapping followed by whole exome sequencing (WES) in two children of consanguineous parents with mild intellectual disability, microcephaly, and growth retardation, revealed one homozygous candidate mutation in TAF13, c.119T>A; p.(Met40Lys). TAF13 encodes a highly conserved protein that assembles with the TATA-box-binding protein (TBP) and the highly selective promoter TBP-associated factors TAF11, TAF15, and TAF7 to form the transcription factor II D (TFIID).

Molecular modeling suggested that the replacement of the non-polar methionine (Met40) by the basic polar lysine (Lys40) is energetically unfavorable, and would hinder the interaction with the leucine at position 57 of TAF11, and consequently the formation of the heterodimer TAF13-TAF11. To confirm this, we performed co-immunoprecipitation in double transfected HeLa cells with plasmids encoding TAF11 and TAF13 with either wild-type or mutant constructs. This experiment confirmed that the identified variant indeed abolishes the TAF13-TAF11 heterodimer formation, which is involved in the TBP recruitment into the TFIID complex.

To obtain further understanding of TAF13 role in gene expression and neuronal functions, we performed RNA-seq following siRNA knock down of TAF13 expression in human neuroblastome cell lines (SK-N-BE). Of the expressed genes in SK-N-BE, 7.8% were deregulated in silenced cell lines in comparison to controls. The evaluation with Ingenuity Pathway Analysis (IPA) revealed that TAF13 is implicated in several pathways and functions essential for central nervous system development, e.g. neuron development, neuron recognition, and negative regulation of cell proliferation and migration. In order to functionally validate these results, we tested the TAF13-down-regulated SK-N-BE cells for proliferation, migration, and differentiation using the WST-8 colorimetric assay, scratch assay, and retinoic acid stimulation, respectively. TAF13 knock-down remarkably enhanced migration and proliferation rates of SK-N-BE cells. Further, the cells' response to differentiation stimulant, measured by differentiation markers MAPT, GAP43, and MYCN, showed an abnormal pattern. This added a further line of evidence that TAF13 is involved in transcription of genes of neuronal development.

Our results suggest that the p.(Met40Lys) variant leads to the neurological phenotype in the affected children through disruption of the interaction of TAF13 and TAF11, leading to transcriptional deregulation of various neural development. Causality of intellectual disability by the identified variant in TAF13 is supported by reports of similar phenotypes caused by mutations in other subunits of TFIID; TAF1, TAF2, and TBP. However, validation through identification of further variants in TAF13 in other similarly affected individuals, and of pathogenicity through further functional tests are necessary.

WS1-06

Identification of FRRS1L as a novel intellectual disability gene in humans and a central determinant of AMPA-receptor biogenesis

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We investigated three consanguineous families with children with intellectual disability. One Syrian and one Saudi Arabian family have two and one children, respectively, with profound intellectual disability, early-onset epilepsy, muscular hypotonia, and developmental regression with loss of acquired skills with onset at about one year of age. An Algerian family has three affected children that presented with moderate to severe intellectual disability, focal or generalized epilepsy with age of onset at 5 to 13 years, and neither muscular hypotonia nor regression.

Whole exome sequencing revealed three homozygous variants in FRRS1L (C9ORF4): two truncating variants in the Syrian and the Saudi Arabian families, c.584delT;p.(Val195Glufs*35) and c.961C>T;p.(Q321*), and a missense variant, c.463A>G;p.(Lys155Glu), in the Algerian family. The encoded FRRS1L protein is a constituent of the proteome of AMPA-type glutamate receptors (AMPARs). AMPAR are key elements of excitatory neurotransmission in the mammalian brain, and mediate fast excitatory postsynaptic current at glutamatergic synapses. SDS-PAGE separation followed by Western-probing showed that the truncating variants lead to a loss of protein expression, and that the missense variant lead to a co-expression pattern similar to that of absent protein, thus confirming that all three are bona fide loss-of-function variants.

Since the role of FRRS1L in receptor physiology has not yet been resolved, we performed multiepitope affinity purifications (ME-APs) with different antibodies of AMPAR components on specimens of rat brain. This revealed FRS1l (ortholog of FRRS1L) is assembled into AMPARs that are only found in the endoplasmic reticulum. These AMPAR with a FRS1l lack the core-subunits typical for AMPARs at the plasma membrane and the postsynaptic membrane. SDS-PAGE separation of distinct pools of total, surface, and intracellular membrane fractions followed by Western-probing showed that a further AMPA constituent, CPT1c, effectively retained FRS1l in intracellular membranes and that both proteins form stable ternary complexes with the glutamate receptor subunit GluA1. Our results indicate that glutamate receptor subunits (GluA tetramers) co-assemble with FRS1l-CPT1c complexes in early biogenesis and get the GluA pore ready for effective co-assembly with further core-subunits (TARPs and CNIH). However, at this step, FSR1l-CPT1c complexes dissociate from the GluA tetramers, and do not move with the AMPAR to the postsynaptic membrane. This seems to have an endurable effect on the AMPAR since virus-directed deletion of FRS1l in adult rats resulted in markedly reduced amplitudes of the excitatory postsynaptic currents (EPSCs) without affecting their time course in all types of synapse or neurons investigated.

In summary, our results characterize a new form of intellectual disability due defective AMPAR biogenesis caused by bi-allelic mutations in FRRS1L presenting with a recognizable phenotype.

WS2-CANCER GENETICS

WS2-01

Exome sequencing identifies a novel recessive subtype of colorectal adenomatous polyposis

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Background: In around 30% of families with colorectal adenomatous polyposis, a cancer predisposition syndrome, no germline mutation in the known tumor suppressor and DNA repair genes *APC</i>, </i>MUTYH</i>, </i>POLE</i>, </i>POLD1*, or *NTHL1* can be identified, although a hereditary etiology is likely.

Methods: To uncover new high-penetrant causative genes, exome sequencing of leukocyte DNA from 106 unrelated patients with unexplained, clinically confirmed adenomatous polyposis of the colorectum was performed. For data analysis and variant filtering an established bioinformatics pipeline including in-house tools was applied.

Results: We identified two unrelated patients with different biallelic truncating germline mutations in a DNA repair gene. In both patients, the mutations were validated to be in compound heterozygous state. Both pedigrees were consistent with an autosomal recessive mode of inheritance, underpinned by non-affected parents and genetic examinations of a healthy and an affected sibling. The clinical symptoms of these patients met the criteria of an attenuated adenomatous polyposis, including colorectal and duodenal adenomas. Additionally, one patient developed an astrocytoma and an affected sibling displayed a colorectal and a gastric carcinoma. Though commonly reported as somatic mutations in cancer, germline truncating mutations of the gene are found very rarely in large sets of controls and none has been identified in homozygous state. By transcriptional analysis, by functional analysis of the tumor tissue on genomic and protein level and by use of various in-silico tools we found further strong evidence for a loss-of-function effect. Thus, it is very likely that the identified alterations represent high-penetrant mutations causative for the phenotype. In addition, we detected a patient with a biallelic *PMS2* germline mutation. This patient comprised distinct clinical features of a Constitutive Mismatch Repair Deficiency Syndrome (CMMRD) beside an early-onset polyposis. However, her tumor spectrum had not raised suspicion on CMMRD.

Conclusions: This is the first study that identified biallelic loss-of-function germline mutations of that DNA repair gene in patients with a suspected hereditary tumor syndrome. According to our data, biallelic mutations of the gene represent a novel and rare recessive subtype of colorectal adenomatous polyposis. Consistent with previous data, unexplained tumor syndromes appear to show extreme genetic heterogeneity, and large patient cohorts are therefore warranted to identify recurrently mutated genes. Further cases are needed to explore the phenotypic spectrum of this novel condition.

WS2-02

Low-level APC mutational mosaicism is the underlying cause in a substantial fraction of unexplained colorectal adenomatous polyposis cases

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Background: In up to 50% of patients with colorectal adenomatous polyposis, no germline mutation in the known genes APC, causing familial adenomatous polyposis (FAP), MUTYH, causing MUTYH-associated polyposis (MAP), and POLE or POLD1, causing Polymerase-Proofreading-associated polyposis (PPAP) can be identified, although a hereditary etiology is likely. However, the impact of APC mutational mosaicism might be underestimated in routine diagnostics using Sanger sequencing of leukocyte DNA. Methods: To comprehensively screen for somatic low-level APC mosaicism, high-coverage next-generation sequencing of the APC gene was performed using DNA from leukocytes and a total of 53 colorectal tumors from 20 unrelated patients with unexplained sporadic adenomatous polyposis. APC mosaicism was assumed if the same loss-of-function APC mutation was present in ≥ two anatomically separated colorectal adenomas/carcinomas per patient. All mutations were validated using diverse methods (targeted next-generation sequencing, Sanger sequencing). Results: In 25% (5/20) of patients, somatic mosaicism of a pathogenic APC mutation was identified as underlying cause of the disease. In 2/5 cases, the mosaic level in leukocyte DNA was slightly below the sensitivity threshold of Sanger sequencing (around 10% mutated alleles); while in 3/5 cases, the allelic fraction was either very low (0.1-1%) or no mutations were detectable at all despite deep sequencing. In all of these three patients, the mutation was present in at least three colorectal adenomas, a finding which is strongly suggestive of mosaicism. Each tumor-derived DNA sample originated from a single independent adenoma. At least two adenomas with the same APC mutation were separated anatomically by a distance of more than 10 cm, the majority even grew in different colonic segments. In contrast, a systematic screening in NGS data of leukocyte-derived DNA from 80 unselected, sporadic polyposis patients and subsequent sequencing of the respective APC exons in adenoma DNA, detected low-level APC mosaicism in two cases (2.5%) only. The majority of all mosaic mutations were located outside the somatic Mutation Cluster Region of the gene. Conclusions: The present data indicate a high prevalence of pathogenic mosaic APC mutations below the detection thresholds of routine diagnostics in unexplained adenomatous polyposis, even if high-coverage sequencing of leukocyte DNA alone is taken into account. Using a multiple adenoma approach, the detection rate of mosaicism could be increased tenfold. This has important implications for routine work-up and genetic counselling. It also might be reasonable to carefully exclude APC mutational mosaicism prior to admittance of this patient group in studies that aim to identify new causative highly penetrant genes.

WS2-03

Co-occurrence of MYC amplification and TP53 mutations in human cancer

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Correct identification of the molecular components of signaling pathways is of utmost importance to understand the underlying biology of cancer and to improve treatment options. To this end several groups currently extensively explore The Cancer Genome Atlas (TCGA) database with various bioinformatics tools.

Here, we described that global, automated analyses may yield different results than targeted studies, which represents novel bioinformatics challenges in pathway analyses.

We illustrate this using the MYC oncogene, which is one of the most highly amplified oncogenes in a variety of human cancers and belongs to the small number of currently known cancer driver genes.

MYC amplifications have been suggested to affect the cell cycle/apoptosis pathway.

Leiserson et al. have recently performed a pan-cancer analysis of mutated networks in 3,281 samples from 12 cancer types from TCGA using a new algorithm, i.e. HotNet2, to find mutated subnetworks (Nat Genet [2015] 47:106-14). MYC was found to be the second most frequent gene after CCND1 affected by somatic copy number alterations (SCNAs). They then identified 16 significantly mutated subnetworks that comprised well-known cancer signaling pathways as well as subnetworks with less characterized roles in cancer. Surprisingly, MYC, one of the most frequently altered cancer driver genes, was not a member of any of the identified subnetworks.

This prompted us to conduct a targeted re-analysis of the TCGA database to investigate the contribution of MYC to pathways (Ulz et al. Nat Genet, in press). We first defined “focal amplification” by analyzing SCNAs of 20 previously described significantly amplified genes in the TCGA pan-cancer data set. We then searched for co-occurrence of mutations with focal MYC amplifications.

Strikingly, we observed highly significant co-occurrence of MYC amplicons with mutations in the TP53 gene in the TCGA BRCA data set as well as the pan-cancer data set. Most interestingly, MYC amplified cases with TP53 mutations had significantly increased MYC expression levels as compared to MYC amplified cases without TP53 mutations further supporting a link between these two somatic alterations.

In breast cancer, MYC amplification and TP53 mutations most commonly co-occurred in cases corresponding to the basal-like or triple-negative, i.e. ER-, PR-, and ERBB2-, subtype. However, the co-occurrence of MYC amplifications and TP53 mutations was also observed in other tumor entities..

Our analyses suggest that TP53 mutations are a frequent mechanism in MYC amplified tumors to disrupt the apoptosis pathway. This illustrates the need to complement global, automated pathway analyses with targeted studies to investigate in depth individual genetic alterations. Somatic alterations in MYC and TP53 may represent an exception from the rule that mutations in a single pathway are mutually exclusive. Furthermore, the combination of MYC and TP53 may define a subset of tumors in several cancer entities.

WS2-04

Integrative DNA-methylation and chromatin state analysis demonstrates that MYC-positive and negative Burkitt lymphoma share a common epigemomic profile

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Burkitt lymphoma (BL) is a mature, aggressive B-cell lymphoma that constitutes the most common lymphoma in childhood. The hallmark chromosomal aberration of BL is a translocation involving the *MYC*-oncogene and one of the immunoglobulin loci leading to MYC deregulation. It has been a matter of debate whether BL without *MYC*-translocations does exist. Recently, we described a subgroup of high-grade B-cell lymphomas without *MYC*-translocation resembling BL which are characterized by a specific pattern of chromosomal aberration on chromosome 11 consisting of a focal gain in 11q23.3 and a loss in 11q24 (Salaverria et al., 2014). Based on the similarities in morphology, immunophenotype and clinical presentation with BL, these lymphomas are called *MYC*-negative Burkitt-like lymphoma with 11q aberration (mnBLL). The aim of this present study was to compare the epigenomic landscape of mnBLL and BL.

In the framework of the ICGC MMML-Seq and the BLUEPRINT projects we analyzed the DNA methylation using the HumanMethylation 450k Bead Chip (Illumina) of 62 BL and 14 mnBLL and contrasted the findings to 32 diffuse-large B-cell lymphoma (DLBCL) and 36 follicular lymphoma (FL). Furthermore, we investigated whole-genome bisulfite sequencing (WGBS) data of 13 BL (Kretzmer et al., 2015) and one mnBLL to decipher differentially methylated regions (DMR). These are defined as 10 or more CpGs to be differentially methylated between those two lymphoma entities. Finally, histone modification maps and derived chromatin states were obtained by ChIP-Seq from one mnBLL and two BL cell lines which were generated according to IHEC standards in the BLUEPRINT project.

Comparison of the lymphoma entities confirms the previously shown DNA methylation differences between BL and DLBCL/FL. Strikingly, in a multigroup comparison the mnBLL cluster together with the BL. These results corroborate the morphologic and gene expression findings suggesting that mnBLL are indeed BL-like. Overall, in the array-analyses only 270 of 462,413 analyzed CpGs were differentially methylated between BL and mnBLL (σ/σ_{max}=0.4, $q<0.001$). Interestingly, these include the *MYC* gene being hypermethylated in mnBLL compared to BL. WGBS revealed 889 DMRs to harbor a minimal methylation difference of 0.25 between BL and mnBLL. Of these, 31 DMRs determined by WGBS overlapped with the differentially methylated CpGs between mnBLL and BL. Strikingly, 10 of these loci also showed a switch in the chromatin states in the mnBLL compared to BL cell lines.

In conclusion, we extend the molecular similarity between mnBLL and BL to the DNA-methylation profile. In turn, by integrating DNA methylation and chromatin state differences we identified such loci which might point to functional relevant genes linked to *MYC*- and 11q-associated Burkitt lymphomagenesis, respectively.

WS2-05

Cancer associated fibroblast-like state in primary skin fibroblasts with constitutional BRCA1 epimutation sheds new light on tumour suppressor deficiency-related changes in healthy tissue

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Constitutive epimutations of tumour suppressor genes are increasingly considered as cancer predisposing factors equally to sequence mutations. In light of the emerging role of the microenvironment for cancer predisposition, initiation and progression we aimed to characterize the consequences of a *BRCA1* epimutation in cells of mesenchymal origin. We performed a comprehensive molecular and cellular comparison of primary dermal fibroblasts taken from a monozygous twin pair discordant for recurrent cancers and *BRCA1* epimutation. Comparative transcriptome analysis identified differential expression of extracellular matrix-related genes and pro-tumourigenic growth factors, such as collagens and CXC chemokines. Moreover, genes known to be key markers of so called cancer-associated fibroblasts (CAFs), such as *ACTA2*, *FAP*, *PDPN* and *TNC* were upregulated. Further analyses detected CAF-typical cellular features including an elevated growth rate, enhanced migration, altered actin architecture and increased production of ketone bodies in *BRCA1*^{mosMe} fibroblasts compared to *BRCA1*^{wt} fibroblasts. In addition, conditioned medium of *BRCA1*^{mosMe} fibroblasts was more potent than conditioned medium of *BRCA1*^{wt} fibroblasts to promote cell proliferation in an epithelial and a cancer cell line. Our data demonstrate, that a CAF-like state is not an exclusive feature of tumour-associated tissue but also exists in healthy tissue with tumour suppressor deficiency. The naturally occurring phenomenon of isogenic twin fibroblasts differing in their *BRCA1* methylation status revealed to be a unique powerful tool for exploring tumour suppressor deficiency-related changes in healthy tissue reinforcing their significance for cancer predisposition.

WS2-06

Clinical Utility of Circulating Tumor DNA Monitoring: A report of more than 400 plasma-Seqs

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Background: The use of circulating tumor DNA (ctDNA) as a prognostic and/or predictive biomarker has been proven in numerous studies. Most studies have focused on point mutations although there is a clear need to move beyond these specific mutations and towards the full spectrum of genomic alterations, i.e. aneuploidy, amplifications, deletions, and translocations, since these aberrations represent some of the most clinically useful genomic targets in cancer (e.g. ERBB2, AR, KRAS amplifications).

Material and methods: We developed a whole-genome sequencing-based technique called “plasma-Seq” that enables the minimally invasive establishment of tumor-specific alterations in plasma on both a genome-wide and gene-specific level. We are able to establish copy number variations within 24h at affordable costs. With the addition of a targeted approach, we can detect specific driver mutations that might also be used as therapy targets or for monitoring purposes. A total of 427 plasma samples from patients with solid tumors including breast (n=134), colorectal (CRC, n=137), and prostate (n=126) cancer were analyzed.

Results: Tumor-specific somatic copy number alterations (SCNAs) were identified in 290/427 samples (67%). A general pattern observed was that the ctDNA allele frequency (AF) decreased in patients who responded well to a given therapy, a pattern which was not observed in non-responders. In addition to an increased ctDNA AF, patients who developed resistance frequently showed new SCNAs, explaining the emerging resistance, such as KRAS or MET amplifications in CRC patients under anti-EGFR therapy or AR amplifications in prostate cancer patients undergoing ADT. Moreover, we observed clonal shifts of prostate cancer cases that switched from hormone dependency to neuroedocrine tumors. In breast cancer patients, some plasma-Seq cases resulted in a reclassification when a HER2 amplification was observed in patients whose tumor had previously been classified as HER2-negative. To investigate the frequency of predictive changes, we used data from My Cancer Genome (www.mycancergenome.org) and tested the presence of established predictive biomarkers in our samples. Interestingly, some of these biomarkers, such as FLT3 or RET amplifications, emerged during the clinical course of therapy, thus exemplifying the need to frequently monitor tumor genomes

Conclusion: Our analyses highlight the various areas in which ctDNA demonstrates clinical potential and represent another step forward in transferring ctDNA genomics to the clinic. Plasma-Seq enables the early identification of resistance mechanisms before they become clinically obvious. Furthermore, novel therapy targets that have not been present in available tumor samples can be identified and the tumor evolution can be monitored.

WS3-COMPLEX GENETICS

WS3-01

Exome sequencing of European families densely affected with bipolar disorder reveals rare variants in synaptic genes contributing to disease etiology

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Bipolar disorder (BD) is a major psychiatric disorder affecting more than 1% of the world's population. The disease is characterized by recurrent episodes of manic and depressive symptoms and shows a high heritability of about 70%.

Molecular genetic candidate and genome-wide association studies have identified several susceptibility genes contributing to the etiology of BD. However, the disease driving pathways and regulatory networks remain largely unknown. Models of illness are most consistent with a polygenic contribution of common and rare variants to disease susceptibility. As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD (Lee et al., 2011), rare variants of high penetrance have been suggested to contribute to BD susceptibility. One way to evaluate this hypothesis is to investigate large multiply affected pedigrees, in which the existence of a genetic variant of strong effect inherited from a common ancestor may be more likely (Collins et al., 2013).

In the present study we investigated 226 individuals of 70 large multiply affected families of German and Spanish origin using whole exome sequencing (WES). In each family, two to five affected individuals with BD or recurrent major depression were selected for sequencing. WES was performed on the Illumina HiSeq2500 platform and the Varbank pipeline of the Cologne Center for Genomics was used for data analysis. All identified variants shared within each family were filtered for a minor allele frequency <0.1% and potentially damaging effects on protein function predicted by at least four of five different bioinformatics tools (Purcell et al., 2014).

So far, WES data of the first 49 families have been evaluated. We identified a total of 857 rare segregating variants implicating 799 different genes, of which 601 were brain-expressed. These genes were then ranked according to the Residual Variation Intolerance Score (RVIS, Petrovski et al., 2013) resulting in 213 genes that belonged to the 25% most "intolerant" genes in the genome (RVIS <25%). Pathway analysis of these genes showed a significant enrichment ($p<0.001$) for a total of 25 pathways including neuron differentiation and axon development. In addition, rare variants in 13 prioritized genes were found in at least two unrelated families. These comprise *NRXN2* which encodes a synaptic cell adhesion molecule connecting pre- and postsynaptic neurons and mediating synaptic signaling as well as *CDH22* which encodes a calcium-dependent cell adhesion protein that plays an important role in brain development.

Our preliminary results suggest that rare and highly-penetrant variants in synaptic and neurotransmitter signaling genes contribute to BD development. Further investigation of the remaining families and follow up analyses of the implicated genes in independent case/control samples are currently underway and will be presented at the upcoming conference.

WS3-02

Systematic insights into the biology of male-pattern baldness and its association with other human traits.

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Male-pattern baldness (MPB) is a highly heritable trait and the most common form of hair loss among men. It is characterized by an androgen-dependent, progressive loss of hair from the scalp that occurs in a distinct pattern. In recent years, research has identified 12 genome-wide significant risk loci for MPB and has already implicated several plausible candidate genes. However, a significant fraction of the overall heritable risk still awaits identification and conclusive data on how these genetic risk factors contribute to the key pathophysiological signs of MPB is still incomplete. A systematic identification of additional genetic risk factor that yields deeper insights into the genetic basis and the underlying biological mechanisms is therefore strongly warranted. Here, the enlargement of current GWAS samples is a promising strategy to augment identification of additional genetic risk factors. We therefore performed the largest meta-analysis on MPB to date, comprising 10,846 early-onset MPB cases and 11,672 controls from 8 independent GWAS samples. After imputation to the 1000Genomes reference panel, >8M SNPs were tested for association with MPB. Our analysis identified a total of 63 loci with genome-wide significance, 52 of which are novel. These include association signals near plausible candidate genes such as *IRF4*, *DKK2* and *FGF5* and *SRD5A2*. The latter encoding the known MPB drug target 5-alpha-reductase type II, which underlines the utility of the GWAS approach for drug target identification. A pathway-based analysis revealed enrichment of genes at MPB risk loci in several pathways including androgen and oestrogen receptor signalling, canonical and non-canonical WNT-signalling and pathways that regulate adipogenesis and epithelial-mesenchymal transition. Beside these novel insights into the biology of MPB, our data allow a systematic investigation for shared genetic determinants between MPB and other human traits. The comparison of MPB associated regions with published GWAS findings identified a total of 152 shared genetic determinants between MPB and other human traits such as hair and eye colour (N=1); height (N=5); bone mineral density (N=3); blood pressure (N=3); puberty onset (N=2), and different types of cancers. These findings strongly suggest that MPB may not be an isolated trait but share a substantial biological basis with several other human traits and disorders. In summary, our study identified 52 novel risk loci for MPB and yields systematic insights into the pathobiology of MPB and its associations with other human traits. It is hoped that our data will not only benefit the understanding of healthy hair biology and hair loss disorders but will also lead to a deeper understanding of associated traits and will place MPB within a wider medical and biological context.

WS3-03

Systematic investigation of a potential role of miRNAs in the pathogenesis of male pattern baldness

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Male pattern baldness (MBP) is the most common form of hair loss in men. The phenotype is highly heritable and is characterized by an androgen-dependent shortening of the hair follicle (HF) growth phase and an abnormal transformation of terminal into vellus hair, which may be caused by androgen-induced changes in gene expression. Paradoxically, these pathophysiological changes are restricted to HFs in the frontal (F) and vertex area of the scalp but do not affect occipital (O) HFs. The exact molecular mechanism that underlies these differences in androgen-sensitivity between HF subpopulations remains however

elusive. In recent years, microRNAs (miRNAs) have been implicated in the regulation of HF development and morphogenesis and have been reported to show differential expression in androgen-dependent and -independent tumours of the prostate, which renders their contribution to the differences in androgen-sensitivity between HF-subpopulations likely. To investigate for a potential role of miRNAs in the pathogenesis of MPB, we systematically analysed for a differential expression of miRNAs in HF samples from the F and O scalp area of 25 healthy male donors. Genome-wide miRNA profiling was performed using the Affymetrix miRNA4.0 chip. MiRNAs that were present in ≥20% of the samples were subsequently tested for differential expression between F and O HFs using the Wilcoxon Rank-Sum Test. The analysis revealed a total of 42 differentially expressed mature miRNAs ($P<0.05$). To gain insights into the biological pathways that may be regulated by these miRNAs, we performed a database research for validated and predicted target genes using miRWalk 2.0. Validated target genes and genes that had been predicted by the miRWalk algorithm and at least three implemented databases were then tested for their expression in human HF, using in-house data on HF-mRNA expression. A total of 104 validated and 956 predicted target genes showed expression in the human HF. These target genes were tested for enrichment in specific signalling pathways using the Ingenuity Pathway Analysis Software. Validated miRNA targets, were found to accumulate in mTOR and AKT signalling, which both have been implicated in cell proliferation of HF stem cells, which play a critical role in the maintenance of HF-homeostasis. Moreover we found an enrichment in prostate cancer signalling, which may reflect the androgen responsiveness of both the HF and the prostate tissue. For the predicted targets, we found an enrichment in the mTOR and IL-1- signalling. The latter has been shown to increases keratinocyte and vascular endothelial growth factor expression in vitro and to influence androgen-regulated gene expression in dermal papilla cells. In summary, our data support a role of miRNA in the pathogenesis of MPB. Further studies are now warranted to elucidate the underlying genes and biological pathways, which will eventually lead to an improved understanding of the pathobiology of MPB.

WS3-04

Identification of de novo variants in nonsyndromic cleft lip with/without cleft palate patients using whole exome sequencing

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations and has a multifactorial etiology. In the last years, a number of common variants have been identified as risk factors for nsCL/P, explaining about 24% of the genetic heritability. It has been suggested that some of the remaining genetic liability might be explained by rare *de novo* mutations, which is supported by the frequent observation of sporadic nsCL/P cases and a higher nsCL/P recurrence risk in offspring compared to parents.

We performed whole exome sequencing (WES) in 50 sporadic nsCL/P cases of Central European ancestry and their unaffected parents. Individuals were drawn from the Bonn cohort which provides comprehensive clinical and familial information. Exome capture was performed using SureSelect^{XT} Human All Exon V5 encompassing 50.4 Mb. Libraries were sequenced on a HiSeq2500 with 2x125 bp. Raw data were processed using the default BWA/GATK v.3.4 pipeline including VQSR and the genotype refinement workflow that is particularly suitable for the analysis of *de novo* variation. Annotation of the possible *de novo* events was done using ANNOVAR.

Mean target coverage was 116.40x (±14.12), and parental status was confirmed for all 50 trios using IBS computation. We identified 202 high-confidence *de novo* variants (genotype quality ≥ 20 in all three trio individuals) of which 37 were either missense or nonsense variants which did not map to regions of segmental duplications. In a first series of validations we Sanger-sequenced seven of these candidate *de novo* variants. Five of these, including the *de novo* status, were confirmed in the index patient. Two variants could not be confirmed in the index patients, suggesting false-positive calls. Correlating these findings with different quality measures revealed the quality-by-depth (QD) measure to be useful to detect false-positives. Further validations using QD ≥ 10 are currently ongoing.

The five confirmed *de novo* variants, located in different genes (*ATXN7*, *GLRA3*, *CSMD1*, *EIF3G*, *SEC14L3*), are not reported in any of the queried databases (ExAc, EVS and 1000 Genomes). For two variants, analyses of unaffected siblings revealed absence of the respective mutation. Although no functional effect in nsCL/P has yet been demonstrated for any of the genes, the *CSMD1* gene is a strong candidate as it maps to a potential recessive risk locus (Camargo et al. 2012) and has been identified as candidate gene for oro-pharyngeal squamous cell carcinomas (Toomes et al., 2003).

Our preliminary results show the presence of *de novo* mutations in nsCL/P patients. We are currently performing further confirmation and follow-up studies, results of which will be presented at the conference.

WS3-05

Nonsyndromic cleft lip and palate: Identification of a causal element at 13q31

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Introduction: Nonsyndromic cleft lip and cleft palate (nsCLP) is one of the most common birth defects, with both environmental and genetic factors contributing to disease risk. In a recent meta-analysis of data from genome-wide association studies (GWAS), we identified an nsCLP risk locus on chromosome 13q31 (Ludwig et al. 2012). Similar to numerous GWAS findings in other traits, the associated markers map to a non-coding region (here: ~210 kb upstream of the *SPRY2* gene). Therefore, the exact causal variants and their function in disease pathomechanism still have to be identified. In the present project we followed-up the GWAS finding at 13q31 by (i) identifying the putative causal SNP, (ii) functionally annotating the top associated region and (iii) understanding the molecular mechanism that explains the genetic association in vivo.

Methods: Using local imputation at 13q31 in the meta-analysis dataset, high-risk variants were identified. The strong association was confirmed by genotyping in an independent replication cohort. Database mining in functionally annotated datasets were used to assess potential regulatory functions. Two candidate regulatory regions were together cloned upstream of the minimal promoter E1b, which in combination drive the expression of GFP. The vector construct was injected into one-cell stage zebrafish embryos, and GFP expression pattern was monitored for five days after injection.

Results: Based on imputation, we identified variant rs1854110 to be the candidate SNP at the 13q31 locus. Interestingly, we found that this marker is located in a putative regulatory enhancer element relevant for craniofacial development. Preliminary results of our transient zebrafish reporter assay revealed that zebrafish embryos injected with the enhancer element show GFP expression in cephalic regions. This was not observed in embryos injected with a GFP plasmid lacking the enhancer.

Discussion: Our comprehensive genetic data and findings from enhancer search identified rs1854110 as functional causal variant at 13q31. First findings of *in vivo* studies in zebrafish confirm the presence of an enhancer element and show that it is active in cephalic regions at appropriate developmental time points. However, the exact nature of the GFP-positive cells requires further investigations using immunostaining. We also currently establish a dual luciferase assay in zebrafish embryos to test potential allele-specific effects of rs1854110 on GFP expression.

Our study is the first one that identified a specific regulatory element at a GWAS nsCLP risk locus. Ongoing studies, which include investigation of the target gene regulated by the enhancer, will provide deeper insights into the biological mechanism contributing to craniofacial development.

WS3-06

The Role of the CAD-risk gene Zc3hc1 in Atherosclerosis and Smooth Muscle Cell Proliferation and Migration

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Zc3hc1, the gene encoding for NIPA (Nuclear Interaction Partner of Anaplastic Lymphoma Kinase), has shown a strong association with coronary artery disease/ myocardial infarction (CAD/MI) in genome-wide

association studies (GWAS). The functional link between NIPA, a protein that plays a role in cell cycle control, and CAD/MI is so far unknown. Hence, we aim to investigate the role of NIPA in atherosclerosis, a condition leading to MI, using a knockout (KO) mouse model.

Zc3hc1-KO mice at the age of 10 weeks, backcrossed to the proatherogenic ApoE-KO-background, were given a high fat diet to induce atherosclerosis, or a control diet. After the duration of 8 weeks, Zc3hc1-KO/ ApoE-KO and Zc3hc1-WT/ ApoE-KO female mice were sacrificed, and the extent of atherosclerosis was determined in the aortic root as well as in the thoracic aorta. Plasma lipid parameters such as Total Cholesterol, HDL, LDL, and Triglycerides were also determined.

Our results demonstrate no difference between the female Zc3hc1-KO (n=8) and WT (n=9) mice after short term high fat diet (8 weeks) in lipid metabolism or plaque size, assessed in sections of the aortic root and in en-face preparation of the thoracic aorta. However, KO mice display a significantly lower amount of collagen (45% of plaque area), compared to controls (53%).

Thus, further investigation was focused on the in-vitro characterisation of migration and proliferation of primary aortic Smooth Muscle Cells (aSMC). aSMC from female Zc3hc1-KO and wildtype control mice (BL/6) were isolated and expanded. Zc3hc1-KO aSMC showed a significantly reduced rate of proliferation (0.6x compared to wildtype controls after 5 days, $p = 0.0124$). Additionally, preliminary results on migration indicate that Zc3hc1-KO aSMC have an initially lower migration rate compared to controls, but reach the same level within a few days.

These findings are very well in line with the observation of reduced collagen content in plaques of female Zc3hc1-KO mice.

Both proliferation and migration are crucial in the development of atherosclerotic plaques. Any changes in these cell functions might impact plaque stability. More stable plaques are less likely to rupture, thus lowering the risk of an ischemic event like myocardial infarction. Our data suggest a critical role of NIPA on proliferation and migration of aSMC, hence also on plaque stability, indicating a possible functional link between NIPA, a protein involved in cell cycle control, and Myocardial Infarction.

WS4-CLINICAL GENETICS I

WS4-01

The molecular cause of arhinia

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Arhinia is the complete or partial absence of the nose at birth and only a few cases have been described with arhinia or its syndromic form, known as Bosma syndrome, which is mainly characterized by severe nasal hypoplasia or arhinia accompanied by complete absence of the olfactory system, uni- or bilateral microphthalmia, and hypogonadotropic hypogonadism. So far, the genetic basis for this striking phenotype has not yet been identified. Using a trio-based whole-exome sequencing (WES) approach, we now identified de novo causative mutation in SMCHD1 in two index patients of German and Moroccan origin both presenting with the typical characteristics of Bosma syndrome including complete arhinia and microphthalmia. Interestingly, both missense mutations, p.Ser135Cys and p.Glu136Gly, affect neighbouring amino acids within the ATPase domain of the protein. Further mutation screening in patients with arhinia and Bosma syndrome identified two additional de novo mutations in SMCHD1, p.Ser135Asn and p.Asp420Val. All identified missense mutations are predicted to be damaging by different in silico analysis tools, are not present in current databases of normal genetic variation, and alter residues mainly localized in the N-terminal region containing its catalytic domain. Our ongoing functional studies indicate that these mutations influence the catalytic activity of the protein and induce alterations in the cellular response to DNA damage. Taken together, our data provide evidence that the identified, specific de novo missense mutations induce impairment in DNA damage responses and cause Bosma syndrome.

WS4-02

Sequencing the GRHL3 coding region reveals rare truncating mutations and the first common susceptibility variant for nonsyndromic cleft palate

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Orofacial clefts are common and occur either in isolation or as part of a syndrome. The two frequent forms are nonsyndromic cleft lip with/without cleft palate (nsCL/P) and nonsyndromic cleft palate (nsCPO). Both are considered multifactorial with a strong genetic background, but with very limited overlap regarding the genetic etiology. The most common syndromic form of orofacial clefts is Van der Woude syndrome (VWS). Mutations in the interferon regulatory factor 6 (IRF6) gene and more recently grainy head-like 3 (GRHL3) were identified in ~70% and ~5% of VWS patients respectively. VWS patients have either CL/P or CPO often accompanied with lower lip pits. As lip pits have an incomplete penetrance, VWS can sometimes mimic nonsyndromic clefting. Of note, CL/P and lip pits are less frequent in GRHL3 mutation carriers while CPO is more frequent compared to IRF6 mutation carriers. In this study, we evaluated patients with apparently nonsyndromic clefts for mutations in GRHL3 sequencing the complete coding region in 672 Europeans with nonsyndromic clefts (567 nsCL/P, 96 nsCPO). Most interestingly, among the nsCPO patients we identified a significantly higher minor allele frequency for rs41268753, located in the GRHL3 coding region (9,9%), compared to controls (4,9%) (P=1,24×10-2). This association was replicated in 3 nsCPO/control cohorts from Latvia, Yemen and the UK (Pcombined 2,63×10-5; ORallelic 2,5 (95% CI 1,6-3,7)), which reached genome-wide significance in combination with imputed data from a GWAS in European nsCPO triads (P=2,73 x 10-9). Rs41268753 encodes the missense variant p.Thr454Met. The in silico prediction program PolyPhen2 denotes it as “probably damaging” and SIFT as “deleterious”, and the PSIPRED workbench predicts a strongly increased protein binding capacity. The only published GWAS for nsCPO did not detect genome-wide significance for this or any other locus. Rs41268753 did not show an association with nsCL/P (P=0,833) in genome-wide imputed nsCL/P GWAS meta-analyses data. Thus, we have identified the first specific common genetic risk factor for nsCPO. Furthermore, with our sequencing approach we identified 4 novel truncating GRHL3 mutations (1 frameshift and 3 splice site), 2 of them de novo. In the familial cases, these also segregated such that collectively 7/8 mutation carriers had a CPO and 1/8 had symptoms suggestive of a submucous cleft palate. None showed the VWS typical lip pits. This finding of an autosomal-dominantly inherited form of clefting among nsCPO has implications for genetic counseling. Screening GRHL3 for mutations should be considered if an estimate on recurrence risks in nsCPO patients is needed. In summary, with both rare dominant mutations and a common risk variant in the coding region, we have identified an important contribution for GRHL3 in nsCPO.

WS4-03

Studying the genetic basis of idiopathic short stature using whole exome sequencing

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Shortness of stature is a common medical concern in childhood and has an incidence of 3 % in the general population. After excluding defects of the growth hormone pathway and recognizable syndromes the underlying cause remains unknown in approximately 70-80 % of individuals.

We now recruited and clinically characterized in detail a large study group of more than 500 families with idiopathic short stature and selected 200 individuals where common genetic causes or copy number variants were excluded. We first performed trio whole exome sequencing (WES) of affected individuals and their parents of normal height for 100 individuals (trio analysis). Variants were selected unbiased based on all possible modes of inheritance in agreement with the segregation in the families and their potential effect on protein function. To further evaluate candidate genes we performed WES in 100 further individuals (affected only analysis) with targeted follow-up of single variants in parents.

Surprisingly, in 21 individuals we found mutations in known short stature genes including *COL2A1*, *CUL7*, *PDE3A* and *KDM5A* (59% dominant, 14% recessive and 27% X-linked inheritance). The underlying diagnosis was missed clinically as characteristic clinical features of the syndromes were missing. In the remaining 89 trios we found potential protein affecting variants in 126 novel candidate genes in 62 of the affected individuals. Within our study, a second variant with a compatible inheritance was found for 4 of the 126 candidate genes in the 100 patients of the affected only analysis (1x dominant *de novo*, 1x recessive and 2x X-linked inheritance). Two further genes were also recently listed in another WES study for idiopathic short stature in agreement with our observed dominant *de novo* model of inheritance.

Currently we are analyzing the remaining 120 candidate genes for gene-level relevance. We investigate the constraint of each gene, its expression levels in chondrocytes from RNASeq, associated animal models, known short stature associated CNVs, functional GO terms and known protein interactions. Of these, 31 genes show evidence for causation in four or more of these categories and 73 in at least three, suggesting that they are involved in idiopathic short stature.

In conclusion, exome analyses of 200 patients with idiopathic short stature identified a known cause of shorted stature in 11% of cases. As the clinical spectrum of most known genetic defects is yet to be explored, an unbiased genetic analysis of patients with idiopathic short stature can help establish a diagnosis in these cases. Furthermore, we found 126 potential novel candidate genes in 62% of the individuals including 6 genes with independent mutations in two individuals each. Thus, our data strongly suggest that single gene defects may be a frequent cause for idiopathic short stature.

WS4-04

De novo mutations of TCF20 in individuals with intellectual disability and postnatal overgrowth

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Recently, germline mutations of the transcriptional co-regulator gene *TCF20* have been implicated in the aetiology of autism spectrum disorders (ASD, Babbs et al., 2014). However, the knowledge about the associated clinical picture of patients with mutations remained fragmentary. Previous studies showed that *TCF20* is strongly expressed in premigratory neural crest cells and the developing mouse brain. The protein which is composed of 1960 amino acids contains nuclear localisation signal domains, DNA- and chromatin-binding domains and PEST domains which mediate proteasomal destruction. In the present study, we analysed a cohort of individuals with intellectual disability of unknown aetiology and their unaffected parents by whole exome sequencing. In total, we identified de novo *TCF20* sequence variants in two out of 313 patients. Patient 1 carried a nonsense mutation (Gln319*) whereas patient 2 carried a frameshift mutation (Asp1280Ilefs*71) leading to a premature stop codon at position 1350. According to prediction algorithms, both detected variants are deleterious and cause nonsense-mediated decay. If alternatively the variants should lead to protein truncation, the resulting proteins would lack either all known functional domains (Gln319*) or most of them (Asp1280Ilefs*71). A comprehensive clinical characterisation of the two patients yielded mild intellectual disability, postnatal tall stature and macrocephaly, obesity, behavioural anomalies and muscular hypotonia as common clinical signs. In contrast, ASD was present in only one proband. Our study considerably expands the clinical picture of individuals with de novo nonsense and frameshift mutations of *TCF20* which includes features such as proportionate overgrowth and muscular hypotonia. Furthermore, intellectual disability / developmental delay seems to be fully penetrant amongst known individuals with de novo nonsense and frameshift mutations of *TCF20* while ASD is shown to be incompletely penetrant. The transcriptional co-regulator gene *TCF20* is hereby added to the growing number of genes implicated in the aetiology of both ASD and intellectual disability. Furthermore, such de novo mutations of *TCF20* may represent a novel differential diagnosis in the overgrowth syndrome spectrum.

WS4-05

Novel phenotypic- and functional aspects of heterozygous de novo mutations in GRIN2B

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N-methyl-D-aspartate receptors (NMDAR) are ligand-gated ion channels which mediate excitatory neurotransmission via cation influx through the postsynaptic membrane. NMDAR are obligatory heterotetramers composed of two Glycine-binding GluN1- (GRIN1) and usually two Glutamate-binding GluN2-subunits (GRIN2A, GRIN2B, GRIN2C and GRIN2D). Heterozygous mutations in GRIN2B have previously been reported in patients with intellectual disability (ID), autism and epilepsy. We identified 26 previously unreported patients with heterozygous GRIN2B mutations, including 20 novel mutations. Combined with 21 previously reported patients in the literature with heterozygous GRIN2B mutations, we are thus able to collectively review the genotype and phenotype of 47 patients and comprehensively delineate the phenotypic spectrum associated with a GRIN2B-encephalopathy. In addition to common symptoms like severe ID and epilepsy, we found novel phenotypic aspects comprising cortical visual impairment and movement disorders such as dystonia. Most strikingly, three patients presented with a seemingly pathognomonic phenotype on neuroimaging resembling diffuse polymicrogyria and enlarged basal ganglia linking NMDARs to a neuronal migration disorder in humans for the first time.

Recently, there was a report of a promising example of modern translational medicine in a patient with an early-onset epileptic encephalopathy due to a heterozygous gain-of-function mutation in GRIN2A. In vitro functional analysis and subsequent screening for a suitable FDA approved drug to block the NMDAR paved the way for an off-label drug therapy with memantine, a use-dependent partial trapping blocker of the NMDAR, which markedly reduced the patient's seizure burden. We investigated the functional effect of most GRIN2B mutations via two-electrode voltage-clamp in Xenopus laevis oocytes. Our analyses are the foundation to identify possibilities for a personalized drug therapy in patients with a GRIN2B-encephalopathy.

WS4-06

Genotype and phenotype in patients with Noonan syndrome and a RIT1 mutation

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Noonan syndrome (NS) is a common autosomal-dominantly inherited disorder characterized by distinct craniofacial dysmorphism, postnatal growth retardation, cardiac abnormalities, pectus deformities, and learning difficulties. Affected individuals are at risk of developing cancer. NS belongs to the RASopathy group of disorders as these genetic syndromes are caused by germline mutations in genes encoding components of the RAS-MAPK signaling pathway. *PTPN11*, *SOS1*, *RAF1* and the recently identified *RIT1* are the major causative genes for NS, and heterozygous gain-of-function mutations in these genes collectively result in prolonged signal flux through the RAS-MAPK pathway.

We sequenced *RIT1* in a cohort of 310 mutation-negative individuals with a RASopathy. Using a standardized form we recorded clinical features of *RIT1*-mutation positive patients from this cohort and additional cases ascertained prospectively. Clinical and genotype data from 36 individuals with *RIT1* mutation reported previously were reviewed.

Eleven different *RIT1* missense mutations, including the three novel amino acid substitutions, p.(Gly31Arg), p.(Ala77Thr) and p.(Phe82Ser), were identified in 33 subjects from 28 families. Codons 57, 83 and 95 represent mutation hotspots. The most common clinical findings were prenatal abnormalities (54%), feeding difficulties (48%), cardiovascular disease (97%), and lymphatic abnormalities (41%), while pectus anomalies (42%), short stature (31%), intellectual problems (27%), and ectodermal findings (39%) were less frequently observed. A neoplasia was present in 9% of *RIT1*-mutation positive individuals with three cases of malignancy. Types of neoplasia included acute lymphoblastic leukemia, giant cell lesions of the jaw, lipoma, and a gastrointestinal stromal and a neuroendocrine tumor, the latter two in the same individual.

We conclude that *RIT1* is frequently mutated in patients with a characteristic NS phenotype. The mutation spectrum in patients with NS significantly overlaps with that of somatic mutations found in tumors leaving open the question of an increased tumor risk in NS-affected subjects with *RIT1* germline mutation. By careful clinical evaluation in a patient cohort of unprecedented size and review of previously reported cases, we delineated in detail the clinical characteristics of *RIT1*-associated NS. Our data show that the *RIT1*-specific clinical picture is different from other gene-specific NS subgroups and comprise a combination of typical facial features, cardiovascular abnormalities, especially hypertrophic cardiomyopathy, and lymphatic abnormalities, while short stature, pectus anomaly, developmental delay and/or ectodermal abnormalities can be absent.

WS5-MONOGENIC DISEASE I

WS5-01

Ehlers–Danlos syndrome periodontal-type (type VIII) is caused by altered function of complement C1 subunits C1r or C1s

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Ehlers–Danlos syndrome (EDS) periodontal-type (type VIII) is an autosomal dominant connective-tissue disorder characterized by aggressive periodontitis and various joint and skin manifestations. In order to elucidate the genetic basis of this condition we studied a 5-generation Tyrolean family with periodontal-type EDS. Clinical features include aggressive periodontitis, an unusual gingival soft tissue abnormality with lack of attached and keratinized gingiva prior to loss of teeth, increased tendency to bruising, inguinal hernias, and organ ruptures. Two family members have autoimmune disorders (Crohn's disease, Sjögren syndrome). Collagen analysis in fibroblasts from two individuals indicated an abnormal proportion of collagens I:III:V. Linkage and exome analyses in the Tyrolean family and re-evaluation or testing of additional 14 independent families from North America and Europe (total >50 affected persons) revealed heterozygous (mostly) missense mutations in the complement *C1R* or *C1S* genes in all tested individuals. There were 15 different mutations that clustered at specific protein domains. Expression analysis of one mutation indicated a possible processing impairment; further functional analyses are pending. The mutations cause periodontal-type EDS through a dominant negative effect; *C1R* or *C1S* null alleles are asymptomatic in heterozygotes and associated with autoimmune diseases like systemic lupus erythematosus without periodontal destruction in homozygotes. Serine proteases C1r and C1s are essential for the activation of the classical complement pathway upon binding of C1q to appropriate targets. Our results confirm periodontal-type EDS as a distinct entity and provide a unique link between EDS and the complement system with as yet unknown functional properties. This connection may open new ways of understanding crosslinks between inflammatory diseases and connective tissue homeostasis.

WS5-02

Recurrent de novo mutations affecting residue Arg138 of pyrroline-5-carboxylate synthase cause a progeroid form of autosomal dominant cutis laxa.

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Several cutis laxa syndromes are known to show metabolic features. The progeroid forms of cutis laxa overlapping with De Barsy syndrome (DBS) are the most important entities in terms of phenotype severity and frequency. These conditions are due to biallelic mutations in the metabolic enzymes pyrroline-5-carboxylate reductase 1 (PYCR1) and pyrroline-5-carboxylate synthase (P5CS), respectively, that both operate in the mitochondrial proline cycle.

We report on eight unrelated individuals born to non-consanguineous families clinically diagnosed with DBS. Three distinct heterozygous mutations in *ALDH18A1* were identified leading to exchanges of the same highly conserved residue Arg138 in P5CS. A de novo origin was confirmed in all six probands for whom parental DNA was available. Using patient derived fibroblasts and heterologous expression we found that the P5CS-p.Arg138Trp protein was stable, able to interact with wild type P5CS molecules, but showed an altered sub-mitochondrial distribution. Native gel electrophoresis showed a reduced size of the endogenous P5CS p.Arg138Trp-containing protein complex versus wild type P5CS indicating an altered composition, conformation or modification of this complex. Furthermore, we found that the cells carrying the p.Arg138Trp substitution had a reduced P5CS enzymatic activity leading to lower proline accumulation in vitro (Fischer-Zirnsak et al. 2015, AJHG).

In summary, recurrent de novo mutations affecting P5CS cause a novel autosomal dominant form of cutis laxa with progeroid and metabolic features. Our data provide new insights into the etiology of cutis laxa diseases and have an important impact on diagnostics and genetic counseling.

WS5-03

Bi-allelic SCYL1 mutations underlie a syndrome characterized by recurrent episodes of acute liver failure, peripheral neuropathy, cerebellar vermis atrophy, and ataxia

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Hereditary cerebellar ataxias are heterogeneous disorders characterized by clinically variable gait disturbances, often accompanied by additional neurological symptoms and involvement of other organs. The molecular underpinnings for many of these disorders are widely unknown and still not all causatively implicated genes have been identified so far. In a preceding study, we uncovered the genetic defect underlying the *mdf* ("muscle deficient") mouse by identifying a recessive frame-shifting mutation in the murine *Scyl1* gene. The *mdf* mouse, initially considered as motor neuron disease model, is affected by a complex form of spinocerebellar neurodegeneration, characterized not only by neurogenic muscular atrophy,

but also by progressive gait ataxia, cerebellar vermis atrophy, Purkinje cell loss, and optic nerve atrophy. Whether a SCYL1-related disease exists in humans remained elusive at that time.

Here, we report on three human individuals from two unrelated families, who presented with recurrent episodes of acute liver failure in early infancy, peripheral neuropathy, cerebellar vermis atrophy, and ataxia. By whole exome-sequencing, we identified compound heterozygous mutations within the *SCYL1* gene in all affected individuals. All mutations identified were predictably disruptive, causing loss of SCYL1 at the protein level. Despite clear parallels in clinical phenotypes between mouse and human patients, recurrent hepatic failure represents a non-neurological clinical manifestation that has not been anticipated from the mouse study. SCYL1 belongs to the evolutionarily highly conserved SCY1-like family of catalytically inactive protein kinases and plays an important constituent of the coatomer (COPI)-coated vesicles mediated membrane trafficking machinery. We showed that in SCYL1-deficient human fibroblasts the Golgi apparatus is massively enlarged, substantiating the notion that SCYL1 maintains Golgi morphology by interacting with several key components of COPI coats. Intriguingly, SCYL1 shares a functional role in retrograde Golgi transport with the *NBAS* gene, which was recently implicated in a syndromic as well as a non-syndromic form of recurrent episodes of infantile liver failure, which suggests a common pathogenetic underpinning.

Our study demonstrates that the discovery of human disease related genes can be accelerated by studying naturally occurring mouse mutants and further argues for an unbiased genome-wide strategy in the molecular diagnosis of patients with rare inherited disorders.

WS5-04

Duplications and a reciprocal deletion of regulatory elements within the topological domain of NPR3 are associated with an overlapping phenotypic brachydactyly spectrum

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Identifying the role and impact of regulatory sequences is essential when placing gene expression in the context of human disorders. A biological context can be assigned to these sequences when they are tied to a well characterized phenotype. We previously mapped a locus for brachydactyly type A1 (BDA1) to 2.9 Mb at chromosome 5p13.3 in a single 7 generation Canadian family. As no mutations were found in the protein coding genes, we conducted deep sequencing of the 2.9 Mb targeted locus. We report a novel 9.5 kb intergenic duplication of a limb enhancer element 265 kb downstream of NPR3 which is associated with BDA1 in two unrelated families. Genomic analyses by high-density array CGH of 28 probands identified a reciprocal deletion in one case of brachydactyly A2. To determine whether this dosage sensitive region contains regulatory elements, we performed an in-vivo transgene LacZ reporter assay on the human 9.5 kb segment in mouse. Indeed the sequence exhibited enhancer activity through its transgene expression at the hindlimbs/pelvis/shoulders and forelimb/forepaw during embryonic development. To investigate the cis-regulatory architecture of the NPR3 locus we performed chromosome conformation capture followed by high-throughput sequencing (4C-seq) in embryonic mice. In wild type mice the duplicated/deleted fragment demonstrates a high interaction frequency with the promoter of Npr3. This could indicate the presence of important cis-regulators of NPR3 in the region. Since enhancers are known to exert their effects on distal target genes within their topological domain, we surveyed gene expression by RT-qPCR on two duplication-positive patient fibroblast cDNAs and could show significant increase in expression of NPR3 compared to controls. Currently CRISPR/Cas9 genome editing experiments are being conducted to generate transgenic mice with the corresponding duplications and deletions.

In summary, intergenic regions have been largely overlooked in mutation screening strategies despite evidence showing an abundance of noncoding transcriptional units and potential cis-regulatory sequences. In our study we show in three unrelated families that duplications and a reciprocal deletion of a limb enhancer element within the topological domain of NPR3 are associated with an overlapping phenotypic brachydactyly spectrum.

WS5-05

Recurrent Mutations in the Basic Domain of TWIST2 Cause Ablepharon Macrostomia and Barber-Say Syndromes

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Ablepharon macrostomia syndrome (AMS) and Barber-Say syndrome (BSS) are very rare congenital malformation syndromes. They are characterized by overlapping clinical features including abnormalities of eyelids and nose, large mouth, malformed ears, generalized hypertrichosis, redundant and dry skin, and genital abnormalities. To establish the genetic basis of AMS and BSS, we performed whole exome and candidate gene sequencing, and functional validations. We identified a recurrent *de novo* mutation in *TWIST2* in seven independent AMS-affected families, and different recurrent *de novo* mutations affecting the same amino acid in ten independent BSS-affected families. Moreover, a consistent genotype-phenotype correlation was observed, because the two syndromes differed based solely upon the nature of the substituting amino acid: a lysine at TWIST2 residue 75 resulted in AMS, whereas a glutamine or alanine was associated with BSS.

TWIST proteins are basic helix-loop-helix (bHLH) transcription factors that exhibit highly overlapping expression profiles during development and have the ability to either form homo- or heterodimers. They are known to directly interact with a large set of transcription factors and may behave either as transcription repressors or activators, depending on the cellular context. Recessive *TWIST2* mutations were previously found to cause Setleis syndrome, a focal facial dermal dysplasia syndrome that shares some similarities with AMS and BSS (Tukel et al.: Am J Hum Genet 2010). The gene *TWIST1*, that encodes a protein with strong sequence homology, is long known to be mutated in craniosynostosis disorders belonging to the phenotypic spectrum of Saethre-Chotzen syndrome (SCS).

All identified mutations in this study fell in the basic domain of TWIST2 and altered the DNA-binding pattern of Flag-TWIST2 in HeLa cells. Comparison of wild-type and mutant *TWIST2* expressed in zebrafish identified abnormal developmental phenotypes and widespread transcriptome changes. Our results suggest that autosomal-dominant *TWIST2* mutations cause AMS or BSS by inducing protean effects on the transcription factor's DNA binding.

Results were published recently (Marchegiani et al. Am J Hum Genet 2015; 97:99–110).

WS5-06

Mosaic Activating Mutations in FGFR1 Cause Encephalocraniocutaneous Lipomatosis

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Encephalocraniocutaneous lipomatosis (ECCL) is a sporadic condition characterized by ocular, cutaneous and central nervous system anomalies. Key clinical features include a well-demarcated hairless fatty nevus on the scalp, benign ocular tumors, and central nervous system lipomas. Seizures, spasticity and intellectual disability can be present, although affected individuals without seizures and with normal intellect have also been reported. Given the patchy and asymmetric nature of the malformations, ECCL has been hypothesized to be due to a post-zygotic, mosaic mutation. Despite phenotypic overlap with several other disorders associated with mutations in the RAS-MAPK and PI3K-AKT pathways, the molecular etiology of ECCL remains unknown.

Using whole exome sequencing of DNA from multiple affected tissues from five unrelated individuals with ECCL, we identified two mosaic mutations, c.1638C>A (p.N546K) and c.1966A>G (p.K656E) within the tyrosine kinase domain of FGFR1, in two affected individuals each. These two residues are the most commonly mutated residues in FGFR1 in human cancers, and are associated primarily with CNS tumors. Targeted resequencing of FGFR1 in multiple tissues from an independent cohort of individuals with ECCL identified one additional individual with a p.K656E mutation in FGFR1. Functional studies of ECCL fibroblast cell lines show increased levels of phosphorylated FGFR and phosphorylated FRS2, a direct substrate of FGFR1, as well as constitutive activation of RAS-MAPK signaling. In addition to identifying the molecular etiology of ECCL, our results support the emerging overlap between mosaic developmental disorders and tumorigenesis.

WS6-TECHNOLOGY AND MOLECULAR MECHANISMS

WS6-01

Effective identification of pathogenic regulatory variants in Mendelian disease

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Nowadays whole-exome sequencing has enabled coding variants to be comprehensively investigated in research and diagnostic settings, and whole-genome sequencing has the potential to do the same for non-coding variation. However, interpretation of non-coding variants still constitutes a major challenge in Mendelian disease and non-coding sequences, such as enhancer elements, have been poorly investigated. To address this gap, we have developed a whole genome analysis framework, Genomiser, which combines phenotypic and genotypic information along with a novel Regulatory Mendelian Mutation (ReMM) score for predicting the relevance of variation in the non-coding genome. The score comes along with a novel machine learning approach that can deal with highly unbalanced datasets. ReMM is applied to a new high-quality set of manually curated regulatory Mendelian disease-associated variants, and statistical significantly outperforms other state-of-the-art scoring methods. Genomiser additionally assesses allele frequency, inheritance, regulatory sequences, chromosomal topological domains, and phenotypic relevance to filter and rank non-coding along with coding variants. The software is efficiently designed so that the computational time is 10 minutes on a whole-genome with a standard laptop computer. Overall, Genomiser is able to identify causal regulatory variants as the top candidate in over 80% of simulated whole-genomes. This new comprehensive approach allows, for the first time, effective detection and discovery of regulatory variants in Mendelian disease and will drive the identification of new elements involved in genetic disorders forward.

WS6-02

Alternative Splicing in Germinal Center B-cell Derived Lymphomas: Analyses within the ICGC MMML-Seq Project

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Alternative splicing is a biological process of fundamental importance in most eukaryotes. It plays a pivotal role in cell differentiation and gene regulation. Alternative splicing has also been associated with a number of diseases. In this study, we investigated differential alternative splicing in germinal center B-cell (GCB) derived lymphomas using a novel robust compositional data analysis method developed in the context of this project. The method relies on direct evidence for splice events and is not restricted to known gene models. Our analysis comprises RNA sequencing data from follicular (FL; n=46), diffuse large B-cell (DLBCL; n=47) and Burkitt's lymphomas (BL; n=18) as well as normal germinal center B cell controls (n=5) obtained within the BMBF-funded German ICGC MMML-Seq network. Interestingly, all lymphoma entities share about 300 genes that are alternatively spliced compared to normal germinal center B-cells. This set includes cancer-associated genes like *MYO9B*, *CTTN*, *SMARCA5* and *TP53*. Furthermore, our data also reveal many alternative splicing events from and to exons that are not yet annotated.

For BL, we report more than 700 alternatively spliced genes and establish a significant enrichment of alternative transcripts in the apoptosis and B-cell receptor pathways. Genes with a BL-specific splice pattern include well known players of carcinogenesis such as *TERT*, *PRDM10* or *PPP3C*. In the case of *TCF3* we identify differential splicing in BL that affects the basic helix-loop-helix domain and appears to be mutually exclusive with mutations in *TCF3*'s interaction partner *ID3*.

Finally, we demonstrate that alternative differential splicing in lymphoma separates cancer entities well and is frequently independent of significant quantitative gene expression changes. Therefore, we conclude that the analysis of differential alternative splicing in lymphomas and other cancers could be of help to elucidate the mechanisms of carcinogenesis.

WS6-03

Identification of the NEK1 protein network in skeletal ciliopathies

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Several disorders have been associated with defects of a variety of proteins involved in cilia formation, maintenance and function. These ciliopathies can affect the intraflagellar transport, components of the cilia, the basal body, or the centrosome. Many of the associated phenotypes include brain malformations, polydactyly, kidney cysts and skeletal abnormalities. In particular, this phenotypic spectrum is present among patients with short rib-polydactyly syndromes (SRPS). This group constitutes the most frequent lethal autosomal recessive osteochondrodysplasias. We identified mutations in *NEK1* as the underlying cause of SRPS II and postulated a digenic diallelic inheritance in the *NEK1* and *DYNC2H1* genes. In recent years, different studies and various data sets have uncovered cilia associated genes. To define and expand the ciliary proteome, we now first compiled a set of known ciliary proteins and many putative cilia-associated proteins based on published data. To establish additional candidate ciliary proteins we then performed yeast two-hybrid screening of a mouse retinal cDNA library with NEK1 as a bait. Here we identified 82 NEK1 interacting proteins of which 66 have functionally not been associated with the primary cilium before. Among these 16 cilia / centrosome associated proteins were KIF3A, RAB11A and DYNC2LI1. We defined DYNC2LI1 as a highly interesting candidate, as *Dync2li1* knockout mice die before embryonic day 11.5 and display neural tube defects in addition to a variety of developmental patterning abnormalities. Furthermore, DYNC2LI1 is part of the dynein-2 complex, where mutations are associated with the SRPS phenotype spectrum. In immunofluorescence analysis in fibroblasts we identified a co-localization of NEK1 and DYNC2LI1 proteins at the basal body region of the primary cilium. Co-immunoprecipitation experiments with our custom made NEK1 antibody confirmed that NEK1 and DYNC2LI1 are part of the same complex. As a functional confirmation, we identified compound heterozygous mutations in *DYNC2LI1* in a patient with a SRPS-like phenotype. Immunofluorescence analysis of *DYNC2LI1* depleted cells revealed a significantly reduced cilia length proposed to affect cilia function. In addition, depletion of *DYNC2LI1* induced altered cilia morphology with broadened ciliary tips and accumulation of the IFT-B complex proteins (IFT57/88) confirming a retrograde IFT defect in these cells.

Based on our estimation of more than 400 cilia-associated genes we expect to identify mutations in other yet unknown genes in our remaining patients. Therefore, our results of the interactions between human proteins involved in centrioles, centrosomes, basal bodies and cilia might provide a global characterization of the functional roles of ciliary proteins in the manifestation of phenotypic features.

WS6-04

Missense mutations in RAB12 affect the transferrin receptor-signaling pathway

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In recent years, next generation sequencing (NGS) facilitated the identification of new genetic causes for multiple diseases. Important information about the pathogenicity of novel mutations can be obtained from studies investigating the cellular function of the mutated gene. By means of NGS, we found mutations in RAB12 in patients with musician´s dystonia (unpublished data). Four affected members of two families carried the same missense mutation in RAB12 (c.586A>G, p.I196V, rs143888944, minor allele frequency according to the Exome aggregation consortium<0.001). Further screening of 238 unrelated patients identified the p.I196V mutation in a third index patient as well as a novel missense mutation (c.38G>A, p.G13D) in another patient.

RAB12 encodes a small GTPase that regulates the lysosomal degradation of transmembrane proteins, e.g. the transferrin receptor (TfR) which is involved in the transferrin-independent c-Jun N-terminal kinase

(JNK) signaling cascade. Activation of (JNK) results in the ubiquitination of Mitofusin 2 (MFN2) and consequently in its proteasomal degradation. Reduced levels of MFN2 prevent fusion and thus result in increased fission of the mitochondrial network. A disrupted integrity, as well as impaired mitochondrial function is related to a number of movement disorders. This study aims to investigate whether the identified mutations in RAB12 affect the TfR signaling pathway.

We established SH-SY5Y cells and fibroblasts from healthy controls overexpressing RAB12 wildtype (WT), G13D, and I196V proteins by viral transfection. First, the GTPase activity of RAB12 was investigated and we detected elevated GTPase activity in both mutants (significant for G13D). Next, immunofluorescent staining for RAB12 in fibroblasts revealed colocalization of RAB12 with lysosomes and an altered subcellular localization of both mutant RAB12 forms. Further, to evaluate an effect of RAB12 mutations on TfR degradation and MFN2, protein levels were measured by western blotting. We observed reduced degradation of the TfR in both mutants in SH-SY5Y cells and fibroblasts. In addition, there was a reduction in MFN2 levels in both mutants (G13D>I196V) compared to WT RAB12 in SH-SY5Y cells. In contrast, protein levels of other mitochondrial proteins (GRP75, MTCO2) used as controls were unchanged. This prompted us to study the mitochondrial network of the transfected cell lines and we demonstrated an increase in the form factor, i.e. fragmentation of the mitochondrial network, in the mutants compared to WT RAB12 fibroblasts.

The results of this study demonstrate that ectopical expression of the two missense mutations in RAB12 led to an altered function of RAB12 in SH-SY5Y cells and fibroblasts. In conclusion, different steps in the TfR signaling pathway including TfR degradation, MFN2 abundance and ubiquitination, and mitochondrial morphology are affected in the mutant cell lines suggesting a pathogenic role of the identified mutations in RAB12.

WS6-05

Plastin 3, a human protective modifier is highly upregulated in iPSC-derived motoneurons in asymptomatic SMN1-deteted individuals and rescues spinal muscular atrophy in mice by restored endocytosis

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Spinal muscular atrophy (SMA) is a devastating motoneuron (MNs) disorder caused by functional loss of SMN1. Dramatic reduction of SMN levels as seen in type I SMA patients and mice cause additional multi-organ impairment as SMN is a housekeeping protein involved in snRNP biogenesis and splicing. The cellular mechanism causing primarily MN impairment is still unknown. Here we show that the identification of naturally occurring individuals fully protected from developing SMA despite carrying SMN1 deletion let us to identify the cellular mechanism underlying MN dysfunction in SMA.

Previously, we identified Plastin 3 (PLS3) as a strong candidate protective modifier using transcriptome differential expression analysis in SMA discordant families. PLS3 was highly upregulated in lymphoblastoid cell lines of asymptomatic but not SMA siblings at both, RNA and protein level. Instead fibroblasts from both SMA and asymptomatic siblings showed similar PLS3 expression, suggesting a cell-specific regulation. To investigate whether PLS3 is upregulated in MNs, we generated induced pluripotent stem cells (iPSCs) from fibroblasts of three asymptomatic and three SMA III-affected siblings and compared these to iPSCs from a SMA I patient and control individuals. After full characterization of pluripotency, small molecule neural precursor cells (smNPCs) were generated from iPSCs. MNs were differentiated from smNPCs and characterized for any possible changes including survival, gem counts, protein and RNA expression of SMN and PLS3. Most strikingly, PLS3 was highly upregulated only in MNs from asymptomatic siblings pinpointing a cell-specific regulation. Strong PLS3 accumulation in shaft and rim of growth cones in MN cultures from asymptomatic individuals implies an important role in NMJ formation and maintenance.

To address the PLS3 rescuing effect in SMA disorder, we generated PLS3 overexpressing (OE) mice, which were crossed into the severe Taiwanese SMA mouse model. While PLS3 OE improved NMJ function and motor abilities, survival elevated only mildly in comparison to SMA mice without PLS3 OE, due to multi-organ impairment. We therefore generated a milder SMA phenotype by applying low amounts of antisense oligonucleotides, which triggers *SMN2* exon 7 inclusion (SMN-ASOs). SMN-ASOs improved the multi-organ dysfunction in these severe SMA mice and increased the survival from 14 to 28 days. Most importantly, crossing the PLS3 transgene homozygously into these mice led to a robust increase of survival (>60% survived >250 days) and motor function improvement. Moreover, since PLS3 is an actin-binding and -bundling protein, our findings emphasise the essential role of impaired actin dynamics in SMA pathology.

Essentially, we found that endocytosis, a major process in neurotransmission at NMJ level is severely impaired and fully restored by PLS3 OE. These data provide strong evidence for PLS3 as protecting modifier in humans and SMA mice by restoring impaired endocytosis.

WS6-06

VarWatch – a registry of in-limbo genetic variants

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The development of 'next-generation sequencing' (NGS) technologies has allowed the generation of rich genetic data sets that promise to substantially improve our understanding of the molecular basis of human disease. However, fully harnessing the power of these technological advances in a diagnostic context still faces significant challenges. First and foremost, researchers and clinicians are likely to discover an increasing number of variants of possible but unconfirmed significance to a given clinical phenotype. Validation of these 'in-limbo variants' usually requires support from one or more independent reports e.g. in peer-reviewed scientific papers but this requirement appears difficult to fulfil especially for very rare diseases and/or mutations.

To facilitate the validation of in-limbo genetic variants, we developed VarWatch, a national variant 'watch list' that is intended to serve as a neutral broker in this regard. Unlike traditional databases, however, VarWatch will operate under a give-and-take principle. Users will be allowed to submit their queries in the form of candidate variants only with the understanding that these variants become part of the watch list themselves. Internally, VarWatch matches a query to its inventory and informs the user about the outcome. This way, the utility of the service is bound to increase with time while maintaining a high level of data quality. In the case of a match, the inquirer and all owners of the respective variant(s) in watch list will be alerted by email and put into contact for further clarification of causality.

Matching is a core functionality of VarWatch and will be implemented in two forms – identity-based and attribute-based matching. Identity-based matching simply elucidates whether the same variant is already present in a given dataset, say the watch list. Particularly for rare variants, however, the same mutation will not have been reported before, but information about the presence of similar variants in the dataset would be helpful. Therefore VarWatch employs a sophisticated algorithm to identify variants that are functionally, phenotypically or physically related to one another.

VarWatch is currently supported by the BMBF. It will become fully operational in 2017 at the latest and a beta test is envisaged for early 2016. Work proceeds in close collaboration with the developers of software platforms for genetic data analysis. This is because we aim at providing a service that integrates seamlessly into existing workflows. We are also pursuing the implementation of standards and technologies developed by the Global Alliance for Genomics and Health (GA4GH), an international consortium with the goal to share medical knowledge more efficiently and to translate it into practical medical care.

WS7-CLINICAL GENETICS II

WS7-01

Two years of preimplantation genetic diagnosis (PGD) in Germany: a single center experience from Lübeck

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The „Präimplantationsdiagnostikgesetz (PräimpG)" and the „Verordung zur Regelung der PID (PIDV)" regulate PGD in Germany for couples with a high risk for a severe genetic disease in offsprings or abortion. In February 2014 the PGD center Lübeck received the approval from the ministry of Schleswig-Holstein and the appropriate ethics committee (PID-Kommission Nord) was constituted. 147 couples contacted our center from March 2014 to December 2015. To minimize the travel expenses for the couples we established a two step assessment procedure. In the first step we evaluate the genetic reports with regard to the technical feasibility and presumptive indication for PGD. If this is the case, the couples come to Lübeck for genetic and psycho-social counselling and for clinical examinations concerning assisted reproduction. Then a second assessment takes place. 52 couples came after first assessment for counselling to Lübeck, 50 of these

passed the second assessment. 21 of these couples made an ethical application for PGD and received a positive evaluation of the PID-Kommission Nord until December 2015, up to date no request was rejected. 9 of these couples had balanced translocations, 12 had monogenic diseases within the family. In addition to severe childhood disorders these included also neurofibromatosis type 1 and Huntington's disease. The high costs for the PGD procedure itself and even for the application for ethical approval are daunting for many couples with legitimate interest in PGD. Here we give an overview of the reasons for requesting PGD as well as the courses of PGD and pregnancy.

WS7-02

Preimplantation genetic diagnosis for female carriers of myotonic dystrophy type 1

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Myotonic dystrophy type 1 is one of the major indications for preimplantation genetic diagnosis (PGD) for monogenic inherited disorders worldwide. Transmission of the instable extended CTG trinucleotide repeat within the 3´-untranslated region of the DMPK gene from the prospective carrier mother on average results in a further expansion by about 950 CTG units with congenital and severe clinical manifestation in her offspring (Martorell et al., 2007). While the prospective mother herself may be clinically healthy or only mildly affected, her further health and clinical course may be compromised by any pregnancy and delivery, but also by an assisted reproduction required for PGD. Furthermore, preliminary data suggest a reduced PGD success rate compared to PGD for other monogenic inherited disorders (Srebnik et al., 2014), demanding thorough pre-PGD assessment and counselling of each couple regarding their individual risks and chances to deliver a healthy child.

In the unique setting of the German embryo protection law we performed at our center in Regensburg polar body diagnosis (PBD) to 9 female DMPK carriers. Transfer of 38 embryos in 22 of 24 PBD treatment cycles (91,7% PBD cycles with embryo transfer) resulted in 4 clinical pregnancies (18,2% pregnancy rate/transfer cycle), 2 missed abortions and delivery of 2 children. However, 59 PBD cycles for the other 3 main indications in our lab (CFTR 31, FraX premutation 16, FraX full mutation 12 cycles) resulted in higher pregnancy rates per cycle with embryo transfer (ET) of 45,8% (CFTR), 23,1% (FraX premutation) and 55,5% (FraX full mutation) and birth/ongoing pregnancies per PBD cycle to ET of 41,6% (CFTR), 15,3% (FraX premutation) and 33,3% (FraX full mutation) respectively, confirming an important impact of the underlying genetic condition on PGD outcome.

Current functional data suggest RNA toxicity as a major pathomechanism underlying the multisystemic clinical manifestations in myotonic dystrophy type 1 including a diminished ovarian reserve. However, preliminary data from our 24 PBD treatment cycles for myotonic dystrophy type 1 did show a similar number of retrieved (9,7 vs. 10,8 overall cohort), mature (9,1 vs. 10,6) or fertilized oocytes (6,0 vs. 6,7) per PBD cycle at a median female age of 33,19 years (34,67 years overall cohort). We did, however, observe a reduced implantation rate of 13,1% vs. 17,5% per transferred embryo and birth rate of 8,3% vs. 20,9% per PBD cycle compared to our overall cohort, correlated with decreased Anti-Mueller-Hormon levels.

In an ongoing study we currently address potential predictive biomarkers for favourable PGD outcome in order to improve individual pre-PGD counselling of female carriers interested in PGD. Moreover, important PGD outcome parameters after PBD and trophectoderm biopsy for Myotonic dystrophy type 1 will be assessed and compared in order to identify PGD treatment conditions, most likely to result in livebirth with a minimal number of oocyte retrieval cycles.

WS7-03

Whole exome sequencing in 161 sudden infant death syndrome (SIDS) cases identifies potentially disease-associated variants in one third of our cohort

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Sudden infant death syndrome (SIDS) is defined as the sudden and unexpected death of an apparently healthy infant younger than one year of age. The cause of death remains unexplained after a thorough investigation, including performance of a complete autopsy, review of the circumstances of death and the clinical history. Although the number of SIDS cases has drastically decreased in the last decades, the prevalence of SIDS is still between 0.1-0.8 per 1000 live births. The occurrence of SIDS is described by a triple risk model, involving (i) a vulnerable infant, (ii) a critical developmental period, and (iii) exogenous risk

factors. Genetic studies in SIDS cohorts indicate that 10-20% of SIDS cases might be explained by inherited cardiac diseases (mainly ion channelopathies or cardiomyopathies) causing lethal arrhythmias in an otherwise normal heart. Undiagnosed metabolic diseases might contribute to the cause of death in another 1% of the SIDS infants.

Postmortem genetic testing by using next generation sequencing (NGS) approaches represents an efficient and rapid tool to investigate potential disease-causing mechanisms that remained undetected during conventional autopsy and may help to identify the cause of death in some of the SIDS infants. The aim of this study was to perform whole exome sequencing (WES) in 161 SIDS cases in order to identify potentially disease-causing variants in 250 cardiac, metabolic and additional SIDS-associated genes. Exome capture was performed with the SureSelect Human All Exon V5 + UTRs target enrichment system (Agilent) and sequencing was done on the Illumina HiSeq2500 platform. Mean coverage of the whole exome was 72.2x (range 44.04 -178.21) and all samples had at least 80% on-target reads at 20x coverage. The variants were reduced to 250 genes, which we considered as candidate genes for SIDS. This filtering step resulted in about 2000 variants per case. Variants were then selected according to a global minor allele frequency (MAF) of ≤ 1% based on public databases, associations with diseases in the Human Gene Mutation Database (HGMD®), and deleterious in silico predictions. In around 35% of our SIDS cases, putative pathogenic variants were detected. These included 78.2% missense, 8.9% nonsense, 6.4% frameshift, 3.8% splice site and 1.3% 5'UTR mutations.

To our knowledge, this is the first WES study in a large SIDS cohort with a specific focus on cardiac, metabolic and other SIDS-associated genes. WES is not substantially more expensive than more targeted approaches, such as gene panels, but provides an opportunity to identify new candidate genes for sudden infant death.

WS7-04

Targeted sequencing of 15 genes in a cohort of 216 patients with unexplained lissencephaly detects mutations in 31% of patients

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We have collected DNA samples on more than 988 children with lissencephaly (LIS) over ~ 30 years. Many have been tested for deletion 17p13.3 and mutations of LIS1 and DCX, and for other genes. Disease causing mutation are known in 539 patients (55%) but have not been found in 449 patients. We therefore designed a targeted sequencing panel of 15 genes including ACTB, ACTG1, DCX, LIS1, TUBA1A, TUBA8, TUBB2B, TUBB, TUBB3, TUBG1, KIF2A, KIF5C, DYNC1H1, RELN and VLDLR, using single molecule molecular inversion probes (smMIPs). We analyzed DNA samples from 216 of 449 patients with unexplained LIS ascertained between 1990 and 2015, and found mutations in 68 of 216 (31%) patients. LIS1 remains the most common causative gene (N=23, most not previously tested), followed by DYNC1H1 (N=20), the largest known LIS gene. 13 of 20 mutations in DYNC1H1 are novel, and include the first splice site mutation. Two patients presented with bilateral congenital cataracts, expanding the spectrum of DYNC1H1 associated malformations. We also report a novel recurrent mutation of TUBG1 (p.S259L) in three patients with posterior LIS and normal head size or mild postnatal microcephaly; most reported patients have had severe congenital microcephaly. We detected 5 mutations in ACTG1, all with mild features of Baraitser-Winter (cerebrofrontofacial) syndrome noted in retrospect. Finally, we identified one child with a homozygous truncating mutation of RELN with the severe diffuse LIS rather than the mild frontal predominant LIS seen in the few currently reported splicing and missense variants, a striking expansion of the RELN-associated phenotype. Despite deep sequencing of most known LIS genes, we found no mutations in 148 of 216 (69%) patients. This strongly suggests that several and possibly many additional LIS genes remain to be discovered. The majority of the unsolved patients presented with posterior predominant pachygyria with or without additional features suggestive for tubulinopathies, subcortical band heterotopia or mild anterior predominant pachygyria. Unsolved cases will be followed up by whole exome sequencing.

The total diagnostic yield in 755 LIS patients tested in the Dobyns lab over 30 years was 80%. We observe a strong genotype-phenotype correlation allowing reliable prediction of the most likely causative gene based on the recognizable MRI imaging pattern.

WS7-05

TCF12 mutations in coronal craniosynostosis – spectrum and frequency

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Premature synostosis of the coronal suture occurs as an isolated craniosynostosis or as main feature in craniosynostosis syndromes i.e. Saethre-Chotzen or Muenke syndrome. Mutations in *TWIST1* or *FGFR3*, respectively, are the underlying genetic causes. Recently, mutations in *TCF12* (MIM 600480) have been identified in individuals with coronal synostosis. The initial study by Sharma et al. (2013) detected heterozygous point mutations in 11% of individuals affected by coronal synostosis. Overall *TCF12* mutations constitute 4% among craniosynostosis cases with proven genetic cause.

In this study we resequenced *TCF12* in 121 cases affected by coronal craniosynostosis without a previous molecular genetic diagnosis. We detected 14 sequence variants in 13 families, 12 of which are novel, i.e. four nonsense (two of these have not been described previously), four missense, two splice site and three frameshift as well as one intragenic deletion. In addition, another *TCF12* nonsense variant was identified by targeted NGS analysis. Variants cluster in the 3' half of the gene affecting the activation domain 2 and the bHLH domain.

Unexpectedly, in one family (affected twin brothers) we identified two changes i.e. a *TCF12* missense variant on one allele and a larger deletion on the second allele detected by array CGH. The proximal deletion breakpoint is located in *TCF12* intron 5 as confirmed by sequencing and removes the C-terminal part of the protein including the bHLH domain. Analysis of the parents detected the missense variant in the father and the microdeletion in the mother confirming compound heterozygosity in the affected twins. Both parents were phenotypically not affected by craniosynostosis. There is phenotypic variability between the twins i.e. bilateral (twin 1) and unilateral (twin 2) coronal synostosis, respectively. Incomplete penetrance and clinical variability in familial cases is in concordance with previous reports.

TCF12 is a transcription factor of the basic helix-loop-helix (bHLH) E-protein family which forms heterodimers with TWIST1, a known player in craniosynostosis. Of note, mice only show a cranial phenotype i.e. severe coronal synostosis if doubly heterozygous for loss-of-function mutations in *Tcf12* and *Twist1*. Thus, the dosage of heterodimers is likely critical for suture development. The exact function of TCF12 and its interaction with TWIST1 during suture development is still elusive.

In summary, pathogenic *TCF12* variants are frequent in non-syndromic coronal craniosynostosis cases. We show a similar frequency (12%) as initially described by Sharma et al. (2013). Thus, sequence analysis of *TCF12* is recommended if the Muenke mutation (FGFR3, p.P250R) is excluded and *TWIST1* does not show any clinically relevant changes.

WS7-06

The Mendeliome in Clinical Practice: Results of a Pilot Study on 95 patients with undiagnosed congenital malformation syndromes

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In order to provide timeous and accurate diagnoses to patients with suspected genetic disorders, we initiated an innovative pilot study to explore the utility of an NGS gene panel as the first-line test for patients referred to us from various local, national, and international paediatric or genetic clinics with an unrecognized congenital malformation syndrome. Developed by Illumina, the TruSight One sequencing panel, which we call the "Mendeliome", encompasses 4.813 clinically relevant genes. All aspects of the Mendeliome, from testing to data analysis, are more rapid than other diagnostic modalities such as conventional Sanger sequencing or whole exome sequencing (WES), making it an attractive tool for fast diagnosis in the clinical setting. Data from a larger and systematic study on similar patient cohorts do not exist.

Here, we report on the first 95 patients enrolled in our Mendeliome pilot study and describe our diagnostic pipeline. We tested 29 trios (index with parents) and 66 index patients. Patients had a variety of phenotypes including many clinically unrecognized multiple congenital malformation syndromes, primordial dwarfism disorders, skeletal dysplasias, and syndromic forms of intellectual disability. In the majority there was no clear clinical diagnosis made prior to enrolment in the study and many patients had a history of extensive molecular testing. Our Mendeliome data were run on two distinct bioinformatics pipelines. For the analysis of variants, each patient dataset was assigned to at least four members of our Mendeliome "M-team", all of whom have different educational backgrounds. This multi-disciplinary approach enhanced our ability to find the causative variants. Thus far, we have made a definitive diagnosis in 43 of the 95 patients (solve rate of 45%) and have putative causative variants in 3 additional patients. Furthermore, we found 3 novel likely disease-associated genes (*PRMT7*, *RNF213*, *AP3B2*), and 5 novel phenotypes associated with variants in known genes (*BMP1*, *FOXG1*, *PMM2*, *RARS2*, *USP9X*). Further functional analyses of these novel variants are underway. Interestingly, in 3 cases, we were also able to diagnose microdeletion syndromes, which were subsequently validated using either array CGH or qPCR. The as yet "unsolved" patients are either currently being reviewed or have been enrolled in our WES/WGS research studies for further investigation.

Taken together, our initial results show that the Mendeliome is indeed an excellent first-line test for prompt diagnosis of unrecognized congenital syndromes, and that an efficient clinical-laboratory-bioinformatic pipeline as well as a multidisciplinary approach to variant classification, is essential to analyse the data effectively.

WS8-MONOGENIC DISEASE II

WS8-01

Mutations in HACE1 cause an autosomal-recessive neurodevelopmental disorder

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Intellectual disability (ID) is characterized by the impairment of general mental abilities associated with defects in adaptive function. Many patients with ID exhibit additional clinical features or symptoms, such as speech delay, motor abnormalities or epilepsy. ID affects about 2-3% of the general population and with recent advancements in genetic technology, the number of genes known to be involved in ID is increasing rapidly. Here, we describe two families in which eight individuals displayed a variable neurodevelopmental phenotype with ID, spasticity and abnormal gait. Because of the consanguinity in one family, and sibling recurrences in the other, an autosomal recessive inheritance was suspected. Autozygosity mapping followed by candidate gene sequencing and exome sequencing were used to identify loss of function mutations in HACE1. In the consanguineous family, a homozygous mutation p.R219* predicted a truncated HACE1 protein entirely lacking its catalytic domain. In the other family, compound heterozygosity for nonsense mutation p.R748* and a 20-nt insertion interrupting the catalytic HECT domain was present; Western analysis of patient cells revealed an absence of detectable HACE1 protein.

HACE1 encodes for an E3 ubiquitin ligase, which provides proteosomal degradation of its substrates by polyubiquitylation. Known target proteins of HACE1 are small Rab- and Rho GTPases, including RAC1. Previous studies showed that mice with constitutive active RAC1 show a similar symptoms as the patients. In addition to its E3 ligase activity, functional investigations have indicated an E3-independent role of HACE1 in repressing the transcriptional activity of retinoic acid receptors (RARs).

By ongoing functional investigations, we could detect increased levels of active RAC1 protein levels and upregulation of RARb expression in patient cells indicating alterations in E3-dependent as well as independent pathways of HACE1.

WS8-02

Functional characterization of a novel TREM2 coding variant linked to familial Alzheimer's disease.

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The triggering receptor expressed on myeloid cells-2 (TREM2) is an immune-modulatory receptor involved in the regulation of inflammatory processes. Recently, rare coding variants in TREM2, in particular p.R47H, were associated with increased risk of sporadic Alzheimer's disease (AD). However, contribution of rare TREM2 variants to the etiology of familial AD is less clear. We searched for rare TREM2 coding variants in AD families without mutation in known AD genes and negative for APOE4. We identified one family by whole exome sequencing (WES) carrying a novel rare TREM2 variant, which was present in two members affected by AD and absent in the healthy member. This variant was absent in 1000 German and 400

Spanish controls. Furthermore, one heterozygous carrier was identified in ExAC database. We performed molecular and cellular biological experiments in HEK293 cells overexpressing either wild type (wt) or mutant (mut) TREM2-constructs. While mRNA of wt-TREM2 and mut-TREM2 showed similar expression level, mut-TREM2 protein level was significantly lower than protein levels of wt-TREM2. The main TREM2 band migrated at a size of ~30kDa in the SDS PAGE and western immunoblot. Additional bands representing glycosylated forms were detected at higher molecular weights. TREM2 undergoes ectodomain shedding at the cell surface resulting in the generation of a 10 kDa. C-terminal fragment (CTF). Interestingly, mut-TREM2 variant migrated at 2–3 kDa. lower molecular weight than wt-TREM2. The difference in the apparent molecular mass of mut-TREM2 was also observed after inhibition of protein glycosylation, suggesting altered conformation. This shift in the protein migration was not detected in the CTF, suggesting that the conformational change seems to affect only the ectodomain. In a functional assay, we observed that mut-TREM2 is less sensitive to specific antibody induced activation than wt-TREM2 indicatiung loss-of function of mut-TREM2. The identification and characterization of novel variants linked to familial AD will provide additional insight into TREM2 function and its role in AD pathology.

WS8-03

SLC13A5 is the second gene associated with Kohlschütter-Tönz syndrome

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Kohlschütter-Tönz syndrome (KTZS, OMIM # 226750) is a rare neuroectodermal syndrome characterized by the combination of early onset epileptic encephalopathy, intellectual disability, and yellow teeth due to amelogenesis imperfecta (AI). To date 23 families with the clinical diagnosis have been described. In the majority of the affected individuals biallelic mutations in ROGDI were identified as disease causing but in several patients no ROGDI mutations were detected which led to the assumption of genetic heterogeneity. Through exome sequencing we identified biallelic SLC13A5 mutations in 9 patients from four families with the clinical diagnosis of KTZS. Biallelic mutations in SLC13A5 were recently described in patients with early infantile epileptic encephalopathy (EIEE25, OMIM # 615905), and some of the affected were reported to have tooth hypoplasia. Our patients all showed early onset epilepsy and variable dental discolouration representing AI of the hypoplastic type. Clinical features differ between ROGDI- and SLC13A5-related disease: most patients with SLC13A5 mutations show dental hypoplasia but dental discolouration may vary from severe to discrete. Transient postnatal hypercalcaemia and hyperphosphataemia requiring treatment was noted in three affected siblings from one family. We conclude that SLC13A5 is after ROGDI the second gene to consider in patients with the combination of early onset epileptic encephalopathy and the clinical presentation of amelogenesis imperfecta.

WS8-04

A novel multiple joint dislocation syndrome associated with a homozygous nonsense mutation in the EXOC6B gene: spondyloepimetaphyseal dysplasia with joint laxity type 2-like phenotype

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Recent technological advances in molecular genetic approaches have enabled the identification of disease genes for ultra rare syndromes even in small families. We performed homozygosity mapping and whole exome sequencing to unravel the genetic cause of a yet undescribed spondyloepimetaphyseal

dysplasia (SEMD) in two sons of a consanguineous couple. Both boys presented with SEMD, multiple joint dislocations at birth, severe joint laxity, scoliosis, gracile metacarpals and metatarsals, delayed bone age and poorly ossified carpal and tarsal bones. This condition has clinical overlap with autosomal dominantly inherited SEMD with joint laxity, leptodactylic type, caused by recurrent missense variants in the kinesin family member 22 gene (*KIF22*). By Sanger sequencing of *KIF22* in one of the brothers we could exclude a pathogenic variant in this gene.

Consanguinity of the parents suggested a pathogenic variant within a shared region of homozygosity (ROH). We performed single nucleotide polymorphism array analysis and identified ten shared ROHs. Whole exome sequencing (WES) was done in both affected brothers, through HiSeq2500 at a mean coverage of 68X. Visual examination of WES reads did not identify any rare variant in genes within the ROHs and/or which had previously been related to skeletal dysplasias, such as *KIF22*, *NIN*, *B3GALT6*, *MMP2* and *MAFB*. By bioinformatic analysis of WES data we found ten private or rare (minor allele frequency <0.5%) homozygous variants, which were absent in the homozygous state in the ExAC browser. All variants were validated by Sanger sequencing; the parents of the siblings carried each in the heterozygous state. No X-chromosomal variant passed the filter suggesting that X-linked inheritance is rather unlikely.

Of the ten identified homozygous variants, the only nonsense variant [c.906T>A/p.(Tyr302*)] in *EXOC6B* turned out to be the most likely cause for the disease. *EXOC6B* encodes a component of the exocyst complex required for tethering secretory vesicles to the plasma membrane. As transport of vesicles from the Golgi apparatus to the plasma membrane occurs through kinesin motor proteins along microtubule tracks, the function of EXOC6B is linked to KIF22 suggesting a common pathogenic mechanism in skeletal dysplasias with joint laxity and dislocations.

WS8-05

Whole-exome sequencing identifies mutations of TBC1D1 encoding a Rab-GTPase-activating protein in patients with congenital anomalies of the kidneys and urinary tract (CAKUT)

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are genetically highly heterogeneous leaving most cases unclear after mutational analysis of the around 30 causative genes known so far. Assuming that phenotypes frequently showing dominant inheritance, such as CAKUT, can be caused by de novo mutations, de novo analysis of whole exome sequencing data was done on two patient-parent-trios to identify novel CAKUT genes. In one case, we detected a heterozygous de novo frameshift variant in TBC1D1 encoding a Rab-GTPase-activating protein regulating glucose transporter GLUT4 translocation. Sequence analysis of 100 further CAKUT cases yielded three novel or rare inherited heterozygous TBC1D1 missense variants predicted to be pathogenic. TBC1D1 mutations affected Ser237-phosphorylation or protein stability and thereby act as hypomorphs. Tbc1d1 shows widespread expression in the developing murine urogenital system. A mild CAKUT spectrum phenotype, including anomalies observed in patients carrying TBC1D1 mutations, was found in kidneys of some Tbc1d1-/- mice. Significantly reduced Glut4 levels were detected in kidneys of Tbc1d1-/- mice and the dysplastic kidney of a TBC1D1 mutation carrier versus controls. TBC1D1 and SLC2A4 encoding GLUT4 were highly expressed in human fetal kidney. The patient with the truncating TBC1D1 mutation showed evidence for insulin resistance. These data demonstrate heterozygous deactivating TBC1D1 mutations in CAKUT patients with a similar renal and ureteral phenotype, and provide evidence that TBC1D1 mutations may contribute to CAKUT pathogenesis, possibly via a role in glucose homeostasis.

WS8-06

A paternally inherited mutation in the imprinted IGF2 gene: First description in a multigenerational family with impaired growth and craniofacial dysmorphism

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Mutations in the genes IGF1 and IGF1R are a well-known cause of intrauterine and postnatal growth restriction in humans. We report for the first time on a mutation in IGF2 (c.191C>A; p.Ser64Ter), which was detected via exome sequencing and traced in four generations of a large family. Affected family members had prenatal onset of short stature with relative macrocephaly and craniofacial dysmorphism resembling Silver-Russell syndrome. Interestingly, the phenotype only occured if the IGF2 mutation was paternally transmitted. This finding is consistent with the maternal imprinting status of IGF2.

The severe growth restriction in affected family members underlines the important role of IGF2 in prenatal growth. Reduced IGF2 levels persist postnatally, although IGF2 expression increases by a larger proportion of biallelic expression in the liver. Our results underline a major impact of IGF2 in prental growth, whereas IGF1 predominantly regulates postnatal growth (Begemann M, Zirn B et al. Paternally Inherited IGF2 Mutation and Growth Restriction. N Engl J Med. 373:349-56, 2015).

WS9-EPIGENETICS

WS9-01

DNA methylation patterns of cortical neurons and glia distinguish humans from chimpanzees – implications for human brain evolution

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The increasing complexity of social behavior along the ascending scale of primates, peaking in human spoken language, is accompanied by an impressive expansion of the human brain, particularly the prefrontal cortex. It is generally considered that the organization of behavior and social interactions depend on input of virtually all cortical structures to the prefrontal cortex. Hence, prefrontal cortex appears to be one of the most interesting structures of the human brain, at least from an evolutionary perspective. But not only size but also function, in particular the interplay of neurons and glia cells, are suspected to distinguish the human brain from great apes and other primates. It is plausible to assume that proper brain function is controlled by a coordinated and well balanced transcriptional landscape, orchestrated by the underlying genetic and epigenetic backbone.

Among epigenetic regulatory mechanisms, which are chromatin manipulating biochemical modifications of the otherwise unchanged DNA sequence, DNA methylation is surely a key modification, occurring mainly at CpG-sites. Reduced Representation Bisulfite Sequencing (RRBS) is a NGS-based technique interrogating methylated CpG-sites on a genome wide scale.

We extracted neurons and glia from 3 human and 3 chimpanzee cortices, using a highly efficient method that allowed us to isolate highly pure fractions of the very cell types, and generated methylation profiles using RRBS sequencing. To our best knowledge, this was done for the first time. The rationale of our study was to survey the RRBS-signals for genome-wide species specific neuron and glia cell methylation, assuming that human specific epigenetic regulation is essential for human specific cognitive abilities.

Bioinformatic analyses revealed 3,553 and 2,782 promoter regions that were differentially methylated between neurons and glia in humans and chimpanzees, respectively. A direct comparison between humans and chimpanzees yielded a setup of gene promoters distinctive for each species and potentially helping to explain their apparently different cortex architectures.

Given the impact of DNA methylation on gene expression, we suggest that human specific neuron/glia-interplay is at least in part a consequence of sophisticated epigenetic control mechanisms and affect the concerted expression of genes implicated in human cognitive abilities.

WS9-02

Paternal age effects on sperm DNA methylation and its impact on the next generation

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Children of old fathers bear an increased risk for neurodevelopmental disorders such as schizophrenia and autism spectrum disorder (ASD). Accumulating experimental evidence links changes in the sperm epigenome with advanced paternal age. Here we investigated the correlation of sperm DNA methylation of ten candidate genes (*FOXK1, KCNA7, DMPK, PDE4C, GET4, TNXK, NKX2-5, NCOR2, DRD4, TBKBP1* and the repeat LINE1) with paternal age and its impact on the epigenome of the resulting offspring. Methylation levels were quantified by bisulfite pyrosequencing in sperm of 164 donors (undergoing IVF/ICSI) and fetal cord blood (FCB) of their offspring (in FCB 5 of the selected genes were studied). Sperm DNA methylation of all studied genes was negatively correlated with paternal age. For *FOXK1* and *KCNA7* the correlation with paternal age was significant in both sperm ($p < 0.001$) and cord blood ($p < 0.01$ and < 0.05, respectively). In sperm, the age effect was replicated in an independent cohort (188 donors). In contrast to pyrosequencing, deep bisulfite sequencing (DBS) allows one to study DNA methylation at the single-molecule level. Using DBS we observed that the number of fully demethylated sperm alleles increases significantly with advanced age. For DBS analysis we selected cord blood samples with an informative SNP to distinguish between paternal and maternal alleles. Consistent with sperm results, FCB methylation of the paternal *FOXK1* allele was negatively correlated with paternal age, whereas methylation of the maternal allele was not influenced by maternal age. The DBS results were confirmed by allele-specific bisulfite pyrosequencing of FCBs. Because *FOXK1* duplication has been associated with autism in the literature, we studied blood methylation of *FOXK1* in 47 ASD children and 28 sex and age matched controls. Variation in *FOXK1* promoter methylation was significantly larger in ASD patients than controls. Collectively our study shows that age-associated DNA methylation changes in sperm can be transmitted to the next generation and, thus, may contribute to the increased disease risk in offspring of older fathers.

WS9-03

The unmethylated allele of oppositely imprinted (i.e. MEST and MEG3) genes is highly susceptible to epimutations during early development and may contribute an additional layer of complexity to phenotypic variation

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Genomic imprinting is characterized by a parent-specific gene activity. Imprinted genes play an important role for early growth and metabolism. Most imprinted genes are regulated by differentially methylated regions (DMRs). Here we studied the methylation profile of two oppositely imprinted genes at single allele resolution by two independent deep bisulfite sequencing (DBS) techniques, relying on different library preparation and sequencing protocols. To distinguish the parental origin, heterozygous individuals were selected by genotyping an informative SNP. Using Roche (GS Junior) next generation sequencing (NGS) technology we studied the maternally methylated (maternally imprinted) MEST promotor and the paternally methylated (paternally imprinted) MEG3 intergenic (IG) DMR in fetal cord blood (FCB), adult blood and visceral adipose tissue. Epimutations were defined as alleles containing >50% aberrantly (de)methylated CpG sites, showing the wrong methylation imprint. An unusually high epimutation rate (ER) was observed for the normally unmethylated paternal MEST and the maternal MEG3 (IG-DMR) allele, compared to the normally methylated maternal MEST and paternal MEG3 alleles. Since both genes are oppositely imprinted, the unmethylated

allele accumulates significantly more (p<0.001) epimutations during development than the methylated allele, regardless of its parental origin. DBS with Illumina (MiSeq) technology and increased amplicon length confirmed this finding (p<0.001). ERs generated by the two NGS approaches for the same individuals correlated with each other (MEG3 IG-DMR p=0.024; MEST p=0.012). The DLK1-MEG3 gene cluster contains two imprinting control regions, the previously mentioned germline IG-DMR and the MEG3-DMR, which is established after fertilization. The IG-DMR functions as upstream regulator of the secondary DMR in the MEG3 promoter. In contrast to the IG-DMR with an average ER of 25.9% for the unmethylated allele, the promotor MEG3-DMR showed a much lower ER of 5.6% in FCB. Nevertheless, the ER for the methylated allele was again lower (1.0%). Our results argue that once imprinting of the MEG3-DMR is established, the IG-DMR may be become redundant and its methylation is no longer strictly maintained. In sperm samples, the ER for MEST (0.9%) and MEG3 IG-DMR (0.2%) is very low, consistent with an important role of the appropriate germ-cell DMRs for early development. The observed relaxation of maintenance of an unmethylated state on the normally unmethylated parental copy of imprinted genes after fertilization leads to mosaic loss of imprinting in different tissues of the body and is highly variable between individuals. We propose that variation in methylation imprint maintenance plays an important role in normal and pathological phenotypic variation. The unmethylated copies of imprinted genes can be considered as metastable epialleles, which can generate enormous phenotypic variation in the absence of genetic variation.

WS9-04

Epigenetic dynamics of monocyte to macrophage differentiation

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Macrophages are part of the innate immune system. They are derived from monocytes that left the blood circulation and migrated to various tissues throughout the body. During this process, monocytes do not divide, but undergo profound transcriptional, biochemical and structural changes that allow macrophages to exert their defence functions such as phagocytosis.

In order to investigate whether epigenetic changes play a role in macrophage differentiation, we analysed this process within the German Epigenome Programme (DEEP). Human monocytes were isolated by leukapheresis and elutriation and differentiated *in vitro* into naïve macrophages by addition of MCSF for 5 days in serum-free medium. Based on high throughput sequencing, we analysed the methylome, transcriptome (small and large RNAs), six histone marks (H3K27ac, H3K4me1, H3K4me3, H3K36me3, H3K9me3 and H3K27me3) and the nucleosome occupancy profile of both cell types in two biological replicates.

We found that ~30% of long transcripts and 46 miRNAs were up- or downregulated. While this was reflected in changes in histone marks, there was no correlation with DNA methylation changes at the transcription start sites. Methylome comparisons with the help of BSmooth (minimum numbers of CpG=4, minimum methylation difference=0.3) allowed us to identify 114 differentially methylated regions (DMRs; size range 85-1697 bp), which map far away from transcription start sites. Almost all of these regions were rapidly demethylated after oxidation by TET enzymes, became nucleosome-free and gained histone marks indicative of active enhancers. Addition of the TET inhibitor (2S)-Octyl-alpha-hydroxyglutarate prevented demethylation of these regions and the formation of cell protrusions, but not the appearance of macrophage specific surface markers. Further *in silico* analyses (Transfac biobase and GREAT) revealed that the DMRs are enriched for binding sites of pioneer transcription factors such as AP-1, RFX1 and KLF4, which open chromatin, and preferentially associated with genes involved in cellular component organization, response to growth factor stimulus, immune response, Fc-gamma receptor signalling and phagocytosis. We predicted target genes for about 2/3 of the DMRs, and half of these seem to affect their expression. These data suggest that demethylation and activation of the identified enhancers during macrophage differentiation is associated with immediate gene expression changes as well as the creation of poised states required for a prompt and coordinated response to future stimuli.

WS9-05

Epigenetic dysregulation in the developing Down syndrome brain

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With an incidence of approximately 1 in 700 to 1 in 1000 live births, Down Syndrome (DS) or trisomy 21 is the most common genetic disorder and the leading genetic cause of intellectual disability (ID). Although it has been known since 1959 that DS is caused by an extra copy of chromosome 21, the mechanisms by which trisomy 21 disrupts development is still not well understood. Chromosome 21 carries several genes, i.e. DNA (cytosine-5-)-methyltransferase 3-like (DNMT3L), whose dosage imbalance might affect epigenetic regulation of numerous genes on other chromosomes. Epigenetic dysregulation due to the extra chromosome 21 during brain development may provide an important contribution to the highly variable cognitive impairment observed in Down Syndrome. We used Illumina Human Methylation BeadChips to analyze DNA methylation profiles of fetal and adult frontal cortices, and fetal temporal cortices. Using 450K arrays, 1.85% of analyzed CpG sites were significantly hypermethylated and 0.31% hypomethylated in fetal Down syndrome (DS) cortex throughout the genome. The vast majority of differentially methylated promoters and genes was hypermethylated in DS and located outside chromosome 21, including the protocadherin gamma (PCDHG) cluster on chromosome 5q31, which is crucial for neural circuit formation in the developing brain. The differentially methylated regions were identified via a genome-wide screen and subsequently validated using bisulfite pyrosequencing in fetal frontal, temporal, and occipital Down syndrome cortices, in addition to liver. Furthermore, adult Down syndrome brain and blood DNA, and isolated NeuN positive neuron cells, exhibited a hypermethylation across the studied protocadherin genes indicating constitutive soma-wide changes. Targeted RNA sequencing revealed that several genes of PCDHG subfamilies A and B are transcriptionally downregulated in fetal DS cortex. Decreased PCDHG expression is expected to reduce dendrite arborization and growth in cortical neurons. The DNMT3L gene on chromosome 21q22.4 is known to stimulate de novo methylation by DNMT3A and DNMT3B. To test whether an extra DNMT3L copy is associated with genome-wide methylation changes, we analyzed whole genome bisulfite sequencing data in human embryonic stem cells (hESCs) with disrupted DNMTs. Hypomethylated CpG islands in DNMT3A (FDR-adjusted $p = 0.02$), DNMT3B ($p = 0.02$), and double knockout ($p = 0.03$) hESCs were significantly enriched with CpGs that are hypermethylated in DS fetal brains. Since constitutive hypermethylation of PCDHG and other genes affects multiple tissues, including blood, it may provide useful biomarkers for DS brain development.

WS9-06

3D nuclear topography of active and inactive regulatory sequences studied with super-resolution fluorescence microscopy

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The coordinated expression of genes requires the coordinated action of transcription regulatory elements (TREs) including non-transcribing sequences such as promoters, enhancers, insulators, silencers and locus control regions. Genome targets with active TREs show an increased sensitivity to DNAse I digestion, called DNAse I hypersensitive sites (DHS+) with an average size between 100-1000 bp. Genome wide maps of DHS+ sites have been published for diverse human cell types. Many such DHS+ targets in one cell type are not DNase I sensitive in another cell type (DHS-), indicating that regulatory sequences are not actively used there. In the present study we investigated for the first time the 3D nuclear topography of active and inactive regulatory sequences. We demonstrate significant differences between the 3D topography of active and inactive targets. We have recently proposed a model for a functionally defined nuclear organization based on two co-aligned three-dimensional networks: an active and an inactive nuclear compartment (ANC and INC) (Cremer et al., 2015. FEBS Letters 589, 2931–2943). Experimental evidence for this model shows that chromosome territories (CTs) are built up from chromatin domain clusters (CDCs), which form still higher networks pervading the nuclear space. Whereas the compacted chromatin core of CDCs, called the INC, is

enriched in repressive histone marks, a peripheral peripheral layer of low density chromatin, called the perichromatin region (PR) is enriched in epigenetic marks for transcriptionally competent chromatin and represents the nuclear domain, where transcription, splicing, chromatin replication and DNA repair occur. The PR lines a contiguous channel system, the interchromatin compartment (IC), which starts at nuclear pores, permeates the nuclear space between the higher order chromatin network and serves a role in nuclear import and export functions. The IC carries nuclear bodies and splicing speckles and interacts with the PR. Accordingly, the PR together with the IC is called the ANC. In line with the ANC-INC model we demonstrate that active regulatory sequences are exposed at the outer periphery of CDCs with loops penetrating into the IC. By contrast, inactive regulatory sequences are more embedded within the interior of CDCs, although still excluded from the most compact core.

POSTER

P-NOMINIERTE ABSTRACTS FÜR DEN GFH-POSTERPREIS 2016

P-Nomin.-001

Single molecule real time sequencing allows complete reconstruction of the MUC1 VNTR and exact positioning of causative mutations in ADTKD

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Autosomal dominantly inherited tubulointerstitial kidney disease (ADTKD) is a collective term for a heterogeneous group of slowly progressive tubulointerstitial nephropathies with onset in childhood to young adulthood and shared clinical findings (e.g. gradual decline of kidney function, hyperuricemia, hypouricosuria). ADTKD results in end stage renal disease (ESRD) from the third decade of life to late adulthood. Disease causing variants were identified in four different genes: UMOD, HNF1β, REN, and recently MUC1. The disease subtypes can only be reliably distinguished by genetic testing. The high complexity of the large coding variable number of tandem repeat () region within exon 2 of the MUC1 gene renders current standard and NGS-sequencing methods useless. Therefore, the identification of pathogenic variants within the VNTR is extremely difficult and there is a huge backlog of potential ADTKD-MUC1 cases for genetic testing. To date only one hotspot mutation, the insertion of a cytosine into a homopolymer stretch of seven cytosines in only a single copy (normal size of the VNTR is 20 to 120 copies) of the canonical 60mer repeat of the MUC1 VNTR, has been published. Detection was entirely based on a method associated with a low reproducibility called SNaPshot minisequencing. This method is also restricted to the specific insertion. We developed an alternative method and that would allow complete physical reconstruction of both parental MUC1 VNTR alleles. We were able to amplify the complete MUC1 VNTR of both parental alleles by longrange PCR for the first time. PCR amplicons were subsequently used for single molecule, real time (SMRT) sequencing with the PacBio RS II system which can generate single sequencing reads (up to 30 kb) spanning the complete VNTR structure. In contrast to the labour intensive SNaPshot method the new approach enables the investigation of large sample numbers. With SMRT analyses we could confirm the insertion of a cytosine in the 7- homopolymer described hotspot mutation in six independent large families. We also were able to precisely determine the position within the VNTR where the insertion C occurred, which is impossible with a SNaPshot protocol. By apply the SMR- technique, we provide a novel powerful tool to analyse MUC1 VNTR assembly in total, a prerequisite to identify further ADTKD-causing missense mutations or larger structural changes within the repeat region. Out of the limited number of families analyzed so far we unfortunately could not identify new point mutations or larger structural variations within the VNTR, but we could add 8 previously unknown normal variants to the 14 existing different variants located in the canonical repeat unit which highlights the potential of the new approach. Analyses of large ADTKD cohorts (familial and sporadic cases) in the future will be necessary to fully exploit the potential of our method.

P-Nomin.-002

Deep bisulfite sequencing for quantification of constitutive epimutations in tumor suppressor genes

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The most prominent genetic cause for inherited breast and ovarian cancer are mutations in the BRCA1 and BRCA2 genes. However, BRCA1/BRCA2 germline mutations explain less than 25% of all familial breast cancers, even for women diagnosed before the age of 40 years. Constitutive epimutations in tumor-suppressor genes, in particular BRCA1 and RAD51C have been found in a percentage of BRCA1/BRCA2-mutation-negative patients with a high risk for hereditary cancer. To rapidly quantify the number of epimutations in different tumor suppressor genes, we developed a NGS-based deep bisulfite sequencing (DBS) protocol targeting multiple amplicons up to 550 bp length. Individual alleles (DNA molecules) of multiple tumor suppressor genes (BRCA1, BRCA2, ATM, PTEN, RAD51C, MLH1, TP53, MLH1, ESR1, and RB1) can be analyzed in both qualitative and quantitative manner. Since it is well known that tumor suppressor genes are transcriptionally silenced by promoter methylation, alleles with >50% methylated CpGs are considered as functionally relevant epimutations. Inclusion of a SNP in the target sequence allows one to distinguish parental alleles. Using Illumina MiSeq technology, up to 500 samples can be analyzed in parallel in a single run with read counts of 3,000-50,000 reads per sample and gene. We propose that quantification of constitutive epimutations of tumor suppressor genes in normal body cells can be developed as a biomarker for cancer risk prediction. In a pilot experiment, we have analyzed the epimutation rates in blood DNA samples of 48 BRCA1/BRCA2-mutation-negative women with early-onset breast cancer and 48 age-matched healthy controls. We found that constitutive epimutations rates largely differ between genes, being highest (0,411 ± 0,2371 %) for BRCA1 and lowest (0,0083 ± 0,0049 %) for PTEN, and between individuals, for example ranging from from 0.1% to 1.1% for RAD51C. Although we did not find a significant difference between the patient and control groups, we identified individuals in both cohorts, displaying unusually high numbers of epimutations, for example 0.9% RAD51C epimutations in control no. 6. Larger cohorts and prospective studies are needed to determine whether constitutive epimutation screening can identify patients with high risk for developing cancer.

P-Nomin.-003

Novel coronary artery disease loci identified by studying the pleiotropic effects of coxibs

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Background: Variants conferring the risk of a disease may advise drug discoveries and predict potential side effects. In this work, we reversed this approach to identify novel coronary artery disease (CAD) genes. For this, we studied molecular targets of a drug class with known side effects relating to coronary risk, cyclo-oxygenase-2 inhibitors (coxibs). We looked up the gene coding the molecular targets for coronary artery disease (CAD) in a genome-wide association study (GWAS).

Methods and Results: A Drug Gene Interaction Database search identified 47 gene products to be altered under coxib treatment. We traced association signals in 200-kb regions surrounding these genes in the CARDIoGRAMplusC4D 1000G GWAS meta-analysis on 60,801 CAD cases and 123,504 controls. We identified 5 loci with significant association after Bonferroni correction for the number of genes tested, i.e. P<2×10-4. To evaluate how many genes show association by chance, we re-ran the pipeline for drugs not reported to cause coronary side effects. Studying genes affected by epilepsy, ataxia and acne drugs (n=56), we did not find any comparable association in the CAD GWAS meta-analysis (P=0.02 versus number of signals in coxib-related genes). We then went on to seek for replication of the association signals in the four coxib-related genes. Studying further 24,012 CAD cases and 79,039 controls, all five lead SNPs showed the

same direction of effect, displaying robust association signals in a meta-analysis in MMP9 (rs7270354, P=6.76×10-8), BCAR1 (rs4888383, P =7.99×10-8), VEGFA1 (rs6905288, P=7.45×10-7), CACNA1E (rs556321, 8.27×10−6) and CYP3A4 (rs2572000, P=6.29×10-5).

Conclusion: Pleiotropic or off-target effects may affect the safety spectrum of drugs and thus need consideration in genomic investigations aimed at predicting the benefit-risk ratio of pharmacological treatment. Here a focused search directed at such targets of a drug known to increase coronary risk identified 5 novel genomic loci displaying association with CAD risk.

P-Nomin.-004

Better don't miss it: Case series of 5 patients with germline and/or somatic mutations in the PI3K/AKT signaling pathway and consequential therapeutic or prognostic relevance

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Germline and somatic mutations in genes encoding members of the PI3K/AKT signaling pathway are causative for several overlapping phenotypes. Current literature highlights the significance of somatic mutations, which have long been underestimated. The PI3K/AKT signaling pathway also plays a key role in both syndromic and non-syndromic tumor development and progression.

We describe a case series of 5 patients with disease-causing mutations in the PI3K/AKT signaling pathway: 3 of them, all males, have activating *PIK3CA*-mutations. All the boys have had extensive previous diagnostics, including arrayCGH and trio exome analysis from EDTA blood samples, which had not led to any diagnoses. The application of an ultra-deep next generation sequencing somatic tumor panel of DNA derived from saliva or buccal swabs revealed the respective low-grade somatic mutations. The other two patients were girls; the first had an *AKT3 de novo* germline mutation. The second had a combination of a germline *PTEN*-mutation and another somatic second-hit *PTEN*-mutation, enabling the diagnosis of a very rare subtype of *PTEN* hamartoma tumor syndrome (SOLAMEN syndrome). In both girls, the molecular genetic findings opened personalized treatment options. In all cases, specific screening recommendations could be given.

There are two lessons that need to be learned from our case series and review of the latest literature: First, Sanger sequencing can no longer be considered the "gold standard" method for the detection of genetic syndromes, especially when there is an indication for mosaicism and/or if there are various overlapping phenotypes. Second, the molecular genetic analysis from EDTA blood is not very sensitive in detecting somatic mosaicism. As the application of next-generation sequencing-based methods have shown that mosaicism occurs much more frequently than previously assumed (even in cases with no clinical signs of mosaicism), it must be questioned whether EDTA blood should still be considered the "standard material". Other material(s) might be more appropriate – for example, saliva or buccal swabs.

P-Nomin.-005

Exome sequencing of 81 individuals from 27 multiply affected families implicates the contribution of rare non-synonymous variants to bipolar disorder

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Bipolar disorder (BD) is a severe disorder of mood with a lifetime prevalence of about 1% and a high heritability of about 70%. The disease is characterized by recurrent episodes of mania and depression.

Models of illness are most consistent with a polygenic contribution of both common and rare variants to disease susceptibility. As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD (Lee et al., 2011), rare variants of high penetrance have been suggested to contribute to BD susceptibility. One way to evaluate this hypothesis is to investigate large pedigrees densely affected with BD, in which the existence of a genetic variant of high penetrance inherited from a common ancestor may be more likely (Collins et al., 2013).

In the present study we investigated the role of rare variants in BD by conducting whole-exome sequencing of 81 individuals from 27 large multiply affected families of Spanish and German origin. In each family we selected 3 genetically distant affected individuals with BD and performed exome sequencing on the Illumina HiSeq 2500 platform. For variant calling and data analysis, the VARBANK pipeline of the Cologne Center for Genomics was used. In our analysis we focused on rare non-synonymous variants with a minor allele frequency < 0.1% that were shared among all three affected individuals and that were predicted to be potentially/probably damaging by at least 3 of 5 applied bioinformatics tools (Purcell et al., 2014). Segregating variants in genes affected in at least two independent families were validated using Sanger sequencing.

Among all 27 families we identified a total of 404 rare non-synonymous and potentially damaging variants spanning 393 genes. 8 of these genes harbored rare segregating variants in at least two independent families. These include the RGS12 gene which is known to play an important role in promoting and maintaining neuronal differentiation. In addition, recent exome sequencing studies have reported the occurrence of rare de novo missense mutations in the RGS12 gene in two independent schizophrenia patients (Guipponi et al., 2014) making this gene a highly promising candidate for follow-up analyses. Pathway analysis of all identified genes with a Residual Variation Intolerance Score < 10 % revealed a significant enrichment for 18 pathways after correction for multiple testing including neuron projection, synaptic membrane and axon guidance. We are currently following-up the most promising candidate genes by performing re-sequencing in large independent samples of unrelated BD patients and controls. The results of these analyses will be presented.

AJ. Forstner and SB. Fischer contributed equally to this work.

P-Nomin.-006

Genome-wide methylation analysis of retrocopy associated CpG islands and their genomic environment

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Gene duplication by retrotransposition, i.e. the reverse transcription of a mRNA and integration of the cDNA into the genome, is an important mechanism in evolution. Based on whole genome bisulfite sequencing of monocyte DNA, we have investigated the methylation state of all CpG islands (CGIs) associated with a retrocopy (n=1,319), their genomic environment as well as the CGIs associated with the ancestral genes. Approximately 10 % of retrocopies are associated with a CGI. Whereas almost all CGIs of the human genome are unmethylated, 68 % of the CGIs associated with a retrocopy are methylated. In retrocopies resulting from multiple retrotranspositions of the same ancestral gene, the methylation state of the CGI often differs. There is a strong positive correlation between the methylation state of the CGI/retrocopy and their genomic environment, suggesting that the methylation state of the integration site determined the methylation state of the CGI/retrocopy, or that methylation of the retrocopy by a host defence mechanism has spread into the adjacent regions. Only a minor fraction of CGI/retrocopies (n=195) has intermediate methylation levels. Among these, the previously reported CGI/retrocopy in intron 2 of the *RB1* gene (*PPP1R26P1*) as well as the CGI associated with the retrocopy *RPS2P32* identified in this study carry a maternal methylation imprint. In conclusion, these findings shed light on the evolutionary dynamics and constraints of DNA methylation.

P-Nomin.-007

Novel and known heterozygous SPTAN1 mutations in four individuals broaden the phenotypic spectrum from epilepsy and intellectual disability to PEHO syndrome

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In 2010, de novo mutations in the *SPTAN1* gene have been reported in two unrelated Japanese infants with early infantile epileptic encephalopathy. Both had early onset of intractable seizures associated with hypsarrhythmia, progressive microcephaly, profound intellectual disability (ID) with lack of visual attention and speech development. Brain MRI showed diffuse hypomyelination and brain atrophy affecting the cortex, corpus callosum, brainstem, and cerebellum. To date, seven individuals with four different in-frame mutations [p.Gln2202del (1x), p.Glu2207del (2x), p.Asp2303_Leu2305dup (3x) and p.Arg2308_Met2309dup (1x)] have been described. *SPTAN1* encodes alpha-II spectrin which is an important structural protein of the cytoskeleton. The formation of heterodimers with beta-spectrins is essential for stabilizing membrane proteins and activating receptors and transporters. The mutations in *SPTAN1* affect the last two of 20 spectrin repeats, which mediate the alpha/beta-heterodimer formation. Expression of SPTAN1 mutant proteins demonstrated altered stability of alpha/beta-spectrin heterodimers and aggregation of alpha/beta-spectrins.

By trio whole exome sequencing we identified the *de novo* c.6908_6916dup (p.Asp2303_Leu2305dup) mutation in *SPTAN1* in a female patient with the clinical diagnosis of PEHO (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy) syndrome. We Sanger-sequenced *SPTAN1* exons 44 - 53 encoding spectrin repeats involved in alpha/beta-heterodimer formation in a cohort of 34 individuals with PEHO syndrome and identified the same mutation in a 6-year-old boy. The phenotype of both patients fits PEHO syndrome as they showed hypotonia, early onset of seizures, no psychomotor development, progressive atrophy of the cerebrum, brainstem and cerebellum and hypomyelination. Both had postnatal microcephaly, hypsarrhythmia, thick dorsum of hands and feet, and facial dysmorphism. Optic atrophy was only found in the boy. Targeted next-generation sequencing in a cohort of 337 individuals with early onset developmental epileptogenic disorders and epileptic encephalopathies identified two additional patients. The third one who presented with spasms, tonic and myoclonic seizures, hypsarrhythmia, microcephaly, profound ID, simplified gyral pattern, thin corpus callosum and pontocerebellar hypoplasia was found to carry the *de novo* p.Arg2308_Met2309dup *SPTAN1* mutation. A fourth 19-year-old individual harbored the novel in-frame deletion c.6908_6916del (p.Asp2303_Leu2305del) (parents need to be tested). He was initially diagnosed

with generalized tonic-clonic seizures occurring during fever at age 17 months and had moderate ID, a language disorder and no MRI abnormalities. Taken together, *de novo* in-frame mutations in *SPTAN1* are associated with a wide clinical spectrum from moderate ID and seizures to severe epileptic encephalopathy with progressive microcephaly and cerebral and cerebellar hypoplasia/atrophy.

P-Nomin.-008

Prediction of complex pedigree structures from NGS data

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Next generation sequencing (NGS) technology considerably changed the way we screen for pathogenic mutations in rare Mendelian disorders.

However, the identification of the disease-causing mutation amongst thousands of variants of partly unknown relevance is still challenging and efficient techniques that reduce the genomic search space play a decisive role.

Usually segregation- or linkage analysis are used to prioritize candidates, however, these approaches require correct information about the degree of relationship among the sequenced samples.

For quality assurance an automated control of pedigree structures and sample assignment is therefore highly desirable in order to detect label mix-ups that might otherwise corrupt downstream analysis.

We developed an algorithm based on likelihood ratios that discriminates between different classes of relationship for an arbitrary number of genotyped samples.

By identifying the most likeliest class we are able to reconstruct entire pedigrees iteratively, even for highly consanguineous families.

We tested our approach on exome data of different sequencing studies and achieved high precision for all pedigree predictions.

By analyzing the precision for varying degrees of relatedness or inbreeding we could show that a prediction is robust down to magnitudes of a few hundred genotypes.

P-Nomin.-009

Next generation sequencing in infantile cholestatic disorders

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Infantile cholestasis may result from a wide variety of genetic and non-genetic conditions. The differential diagnosis is especially challenging due to their similar clinical presentation including biochemicalparametersandoften non-specific liver histology. Rapid identification of an underlying genetic disorder is essential in order to initiate optimal treatment algorithms and discriminate patients who might benefit from liver transplantation in the long term, but also to avoid unnecessary and potentially harmful invasive diagnostic procedures.

Currently, more than 90 genes have been associated with monogenic forms of infantile cholestasis, thus preventing routine genetic workup by Sanger sequencing. In addition many multisystemic disorders such as mitochondrial or storage diseases and ciliopathies may primarily manifest as hepatic disease in infancy with or without cholestasis.

Here we demonstrate a next generation sequencing approach to discover the underlying cause in clinically well characterized patients. A multi gene panel was established, including 93 genes associated with inherited cholestatic disease in infancy and childhood, and validated retrospectively by re-evaluation of 15 former patients, in whom pathogenic mutations had previously been identified by conventional Sanger sequencing. We then prospectively assessed 12 children with this multigene panel for infantile cholestasis and identified seven novel mutations and nine known pathogenic mutations in 9 of these. The underlying disorders included for example one case of PFIC Type 2 (ABCB11: p.Gly628Trpfs*3, c.611+1G>A), one case of Niemann-Pick Disease type C (NPC1: p.Glu391Lys, p.Arg116*), one case of atypical PFIC (LBR: p.Arg372Cys, ABCB11: p.Val1112Phe) and one case of autosomal recessive polycystic kidney disease (ARPKD) (PKHD1: p.Thr777Met, p.Tyr2260Cys). We will demonstrate and discuss exemplary the clinical course of infants with identified inherited forms of cholestasis in the context of their genetic, histopathological

and electron microscopic findings. Our preliminary data confirm the power of this multigene panel approach for infantile cholestasis and highlight the critical impact of integrating clinical, histopathological and genetic data during the process of multi gene panel testing to ultimately pinpoint rare genetic diagnoses.

P-Nomin.-010

Clinical and genetic evaluation of hereditary spastic paraplegia.

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Hereditary spastic paraplegias (HSPs) comprise a clinically and genetically heterogeneous group of neurodegenerative disorders with progressive degeneration of the corticospinal tract. Patients with complicated forms show additional clinical findings, e.g. thin corpus callosum, cognitive impairment orperipheral neuropathy overlapping with a wide spectrum of other underlying genetic conditions.

Currently more than 80 loci have been assigned with causal mutations identified in almost 70 HSP genes, some of them also associated with other important neurodegenerative disorders including hereditary forms of neuropathy and amyotrophic lateral sclerosis (ALS). Genetic testing of HSP patients by conventional Sanger sequencing in the diagnostic setting so far was restricted to the genes, most frequently affected. We here report our results of genetic testing for HSP by linkage analysis and Sanger sequencing with MLPA over the last 15 years, and more recently by next generation multigene panel sequencing (NGS). A subgroup of 20 patients received extensive neurologic workup in our outpatient clinic for movement disorders (cohort 1).

In the overall cohort of 278 patients with suspected pure and complicated HSP causal mutationswere identified in SPG4 (n=52), SPG3a (n=6), SPG5 (n=2), SPG7 (n=1), SPG11 (n=4),SPG20 (n=1) and SPG31 (n=2) with conventional sanger sequencing and in SPG4 (n=3) with NGS. To further increase the diagnostic yield we had previously validated a gene panel for massive parallel sequencing of 31 HSP genes (core panel) and 43 additional genes (step 2 panel). For a cohort of 12 HSP patients with known mutationsall expected HSP causing mutations were reliable detected including nonsense (n=2), missense (n=7) and frameshift (n=1) mutations, one 5bp deletion and one 9bp duplication.

The expected increased diagnostic yield of NGS in combination with an extensive clinical evaluation will further support the functional assessment of underlying pathomechanisms and the development of novel gene specific treatment options. Mignarri et al. described 2014 the administration of cholesterol-lowering drugs in patients with SPG5a resulting from CYP7B1 mutations. Likewise, Stromilloet al. demonstrated 2011 first evidence for structural and metabolic brain damage in Spatacsin-associated SPG11, which appears to correlate with the severity of the disease. Over the last 7 years Spatacsin has not only emerged as the major gene affected in patients with autosomal recessive complicated HSP with cognitive impairment. More recently it has also been shown to account for a substantial proportion of patients with autosomal recessive inherited forms of juvenile ALS (Orlacchio et al., 2010) as well as axonal Charcot–Marie–Tooth disease (Montecchiani et al., 2015). In an ongoing interdisciplinary study we currently address functional mechanisms underlying Spatacsin dysfunction in SPG11 and metabolic markers associated with disease progression as potential treatment targets.

P-Nomin.-011

The ambiguity of syndromic and non-syndromic hearing loss

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Excluding environmental contributions, 50% of all hearing loss (HL) is attributed to a genetic etiology, which is further divided into non-syndromic (70%) and syndromic (30%) categories. Syndromic hearing loss (SHL) is associated with additional clinical features. There are at least 600 medical diagnoses associated with HL according to the London Medical Database V.1.0.31, including Pendred and Usher syndromes. Pendred syndrome is distinguished by congenital HL onset, temporal bone-associated abnormalities and goitre onset during puberty. Usher syndrome can be grouped into three clinical subtypes based on age of onset and severity of HL, retinitis pigmentosa and vestibular features. Many NSHL genes are recognized as causing autosomal recessive syndromes such as Usher and Pendred syndromes. While some of the mutations in these genes have been associated with Usher or Pendred syndromes, there is an acute need

for transparency of mutational consequence on the phenotype. We present a summary of six cases, wherein two and four probands presented mutations in Usher-associated genes and SLC26A4 for Pendred syndrome, respectively. All probands currently show exclusive NSHL, despite several having underlying syndrome-associated mutations.

We tested probands of Turkish, German and Iranian ethnicities with normal hearing parents. Next generation sequencing using the TruSight Exome/TruSight One panels (Illumina) was performed using the MiSeq and NextSeq 500 desktop sequencers. These panels include the targeted genomic enrichment of approximately 100 HL genes.

Data analysis in two siblings disclosed compound heterozygous mutations in GPR98 (c.6518A>G, p.D2173G and c.17759T>G, p.L5920R). Another case had a homozygous mutation in USH1G (c.310A>G, p.M104V), as well as additional compound heterozygous USH2A (c.12889T>C, p.S4297P and c.11864G>A p.W3955*) mutations. GPR98, USH1G and USH2A are known Usher genes. Two independent German cases had Pendred-associated compound heterozygous mutations in SLC26A4 (c.1001+1G>A and c.1334T>G, p.L445W (proband 1), c.412G>T, p.V138F and 1341+1G>C (proband 2)). Two independent consanguineous Iranian cases showed homozygous mutations in SLC26A4 (c.1198delT, p.C400Vfs*32 and c.2121_2129delGTTCTTTGA, p.F708_D710del). Contrary to the predicted outcome of these syndrome-associated mutations, all probands did not present clinical data suggestive of SHL. To date, we detected a positive solve rate of 27% in 6 out of 22 tested cases that included SLC26A4 and Usher genes in agreement with what is published in the literature. Interestingly, USH1G has been recently linked to NSHL, highlighting the broad clinical outcome of mutations in these genes. However, as the presence of additional clinical features may appear many years after HL onset, it is nearly impossible to discriminate syndromes in young children. This reinforces the importance of clinical surveillance for monitoring and early treatment of later developing syndromic features.

P-Nomin.-012

Sjögren Larsson Syndrome: The Molecular Mechanism of Disease-Causing Mutations of Fatty Aldehyde Dehydrogenase

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The Sjögren Larsson Syndrome is a severe autosomal recessive metabolic disease characterized by connatal ichthyosis, progressive spasticity, and intellectual disability. It is caused by mutations in the ALDH3A2 gene coding for fatty aldehyde dehydrogenase (FALDH). This membrane-bound enzyme is responsible for detoxifying long chain fatty aldehydes into fatty acids: dysfunction of FALDH causes altered lipid metabolism in patients' cells.

We have recently established the crystal structure of this enzyme, allowing us to investigate the reaction mechanism and the unique substrate specificity of FALDH as well as the molecular mechanisms of mutations causing Sjögren Larsson Syndrome. By combining structural observations with computational modelling approaches it was possible to divide all known ALDH3A2 missense mutations into different groups according to their most probable mechanistic pathogenic effects. Many mutations do not directly affect the active site itself but are predicted to change dimerization properties and folding stability of FALDH. One distinct set of mutations changes the chemical properties of a gatekeeper helix, a unique structural feature of FALDH which we identified on the basis of the solved structure. The gatekeeper helix is implicated in lipid bilayer interaction and enables efficient binding and turn-over of long chain aliphatic aldehyde substrates. ALDH3A2-mutation p.K447E, as an example, disrupts the correct positioning of this gatekeeper helix along the substrate funnel entrance.

Many other folding and dimerization mutations also impact on the correct three-dimensional arrangement of FALDH. Consequently stabilizing its protein fold with small molecule chaperones potentially represents a promising strategy to counteract the mutations detrimental effects. In a high through-put screening of 10 000 compounds from a chemical diversity collection we identified a set of pharmacological chaperone candidates that showed effective binding to FALDH and exhibited beneficial effects on both, protein fold stability as well as enzymatic activity. Thus impaired function of FALDH in Sjögren Larsson Syndrome patients may be potentially rescued by suitable small molecules, thereby opening prospects for future drug design strategies.

P-Nomin.-013

Exome Sequencing in extended pedigrees with rare subphenotypes of schizophrenia identifies new candidate genes

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Schizophrenia (SCZ) is a common multifactorial disorder with a lifetime risk of ~1%. However, only few of these patients are diagnosed with cataphasia (speech disorder) or periodic catatonia (movement disorder). To the authors' knowledge, this is the first study performing exome sequencing in patients with these infrequent subphenotypes. Given the rarity of individual mutations and the overall abundance of neutral variations in the genome, confirming a definite association with the illness is a difficult task. In addition to de novo emergence and identifying several mutations in the same gene, investigating familial segregation plays an important role in these research attempts.

In the present study, we included three multiple affected families. Four genetically distant individuals of each family were exome sequenced on an Illumina HiSeq 2500. For the data analyses three different algorithms were used: The Varbank pipeline v2.14 of the Cologne Center for Genomics (http://varbank.ccg.uni-koeln.de), the CLC bio Biomedical Genomics Workbench 2.5.1, and BWA GATK (Best Practices Pipeline). In our downstream analyses we included only those variants that were: (i) predicted to be deleterious (Combined Annotation Dependent Depletion score ≥ 15; http://cadd.gs.washington.edu/), (ii) rare in publically available databases (minor allele frequency ≤ 0.1% in the 1000 Genomes Project and the Exome Variant Server), (iii) confirmed by Sanger sequencing, and (iv) co-segregating within the respective family.

The initial results of the analysis revealed 35 variants in 35 genes. We are currently genotyping all identified variants in 1,000 German population-based controls and will exclude variants with a minor allele frequency ≥0.1%. In addition, we performed gene-based tests (as implemented in VEGAS) in the largest genome-wide association study of SCZ to date (containing 35,000 patients and 100,000 controls).

To validate the candidate genes identified in the exome sequencing and assess their generalizability, we will re-sequence these genes in large samples of phenotypically well-characterized unrelated patients with SCZ and controls. For the re-sequencing, we will prioritize those genes with an additional association with common variants (set-based tests) and those that are brain-expressed. So far, our most promising candidate genes are ZNF426, NOS3, and MECP2. In the literature, at least one de novo mutation was reported in a patient with SCZ for these genes. The analyses are ongoing and will be presented at the upcoming conference.

P-Nomin.-014

Targeted next-generation sequencing in routine diagnostics of childhood neurodegenerative disorders

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Childhood neurodegenerative disorders underlying cases of developmental delay and regression represent a genetically heterogeneous group. Although numerous causative genes have been identified, in many cases a specific genetic diagnosis remains elusive, even after extensive molecular testing. Next-generation sequencing (NGS) has proven to be a powerful tool in identifying disease-associated variants in many neurological diseases. The genetic data yielded by NGS has made a significant impact on the clinical diagnostic process, influencing management and increasingly even treatment of diseases, while also contributing to the discovery of molecular pathomechanisms. A definitive genetic diagnosis is also the prerequisite for prenatal or preimplantation decisions affecting families.

20 children with developmental delay and a neurodegenerative clinical course, seen at the genetic counselling unit of our institute, were analyzed with targeted exon enrichment and NGS using the so-called MPIMG-1-Test providing panel diagnostics for over 1200 brain related and pediatric recessive disease genes. For enrichment a TruSeq Custom Enrichment Kit (Illumina Inc., San Diego, USA) was used. 2x300 basepair paired-end sequencing (Illumina MiSeq Reagent Kit v3) was carried out on an Illumina MiSeq system. Read alignment was performed with SOAP version 2.2. A modified version of the Medical Resequencing Analysis Pipeline (MERAP, Hu et al., 2014) was used to check all detected variants against standard public databases. For segregation testing of all likely disease-causing variants and determination of copy number variants PCR, Sanger sequencing and MLPA-assays were performed according to standard protocols.

Confirmed disease-causing mutations have been identified in 10 out of the 20 patients. We found 2 families with homozygous or compound heterozygous mutations in genes responsible for rare forms of neurodegeneration with brain iron accumulation (PLA2G6, FA2H) presenting with non-specific symptoms such as dystonia, nystagmus, seizures and dysphagia and without typical radiological signs. Mutations in genes associated with progressive movement disorders (GAN, ALS2, ATP6AP2, AARS, SPG7) were identified in 5 patients with gait abnormality and 3 patients with a severe regressive course had an organelle related neurodegenerative disorder (NDUFV1, PEX1, SGSH). Pathogenicity of the variants was supported by prediction tools (SIFT, PolyPhen2, MutationTaster) and conservation scores. All variants were confirmed by Sanger sequencing.

Our findings so far support the clinical utility of targeted NGS in providing families with a definitive diagnosis, and the basis for future prenatal decisions, in a group of pediatric disorders with a rapid and severe course. Subsequent functional characterization of gene products via patient-specific iPSC models may lead to a better understanding of the molecular mechanisms and open up possibilities to develop therapeutic strategies.

P-Nomin.-015

Exome sequencing of 48 bipolar disorder patients with rapid cycling provides evidence for an involvement of previously described risk genes in disease development

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Bipolar disorder (BD) is a severe neuropsychiatric disorder characterized by recurrent episodes of mania and depression. It is a common disorder with a lifetime prevalence of about 1% in the general population and has a high heritability of about 70%. In addition, BD is classified as one of the top ten leading causes of the global burden of disease by the World Health Organization, given its early onset and often chronic course. Recent genome-wide association studies identified the first susceptibility genes contributing to BD. However, as the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD (Lee et al. 2011) rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In the present study we focused on BD patients with rapid cycling (RC). RC is a course specifier of BD (Kupka et al. 2003) defined as having at least four recurrent episodes of acute illness within one year. About one third of lifetime BD patients meet criteria for RC (Lee et al. 2010) suggesting that BD patients with RC might represent a more defined etiological subgroup.

Since RC showed strong evidence for familiarity (Saunders et al. 2007), we hypothesized that, in addition to common genetic variation, rare variants of high penetrance might contribute to the development of RC in BD patients. We selected 48 unrelated BD patients with RC of German origin and performed exome sequencing using the Illumina HiSeq2500 platform. For data analysis, the VARBANK pipeline of the Cologne Center for Genomics was used. We filtered for rare (minor allele frequency <0.1%), heterozygous and non-synonymous variants that were predicted to be possibly damaging or disease causing by at least 4 of 5 applied prediction tools. The identified variants were validated by Sanger Sequencing.

After the filtering process, the 48 BD patients with RC showed a total of 3,467 rare variants in 2,801 different genes. Further analysis revealed that 433 genes carried mutations in at least two different patients. To gain further evidence that the observed variants had a disruptive effect on the gene product, we applied the Residual Variation Intolerance Score (RVIS). Of the 433 overlapping genes, 155 were among the 25% most intolerant genes (RVIS <25%).

Five of these genes harbored rare potentially damaging variants in at least four or more independent BD patients, 22 genes were identified in three and 128 genes in two BD patients, respectively. Interestingly, these genes include the previously reported genome-wide significant BD risk genes *ANK3* and *SYNE1*, as well as *MLL2* which belongs to the genome-wide significant 3p21.1 locus (Chen et al. 2013). In all these three genes rare highly damaging missense variants were identified. Our results provide further evidence for the importance of these genes in BD development. Functional evaluation and pathway analyses are currently underway and will be presented.

P-Nomin.-016

MicroRNA miR-371a-3p – a novel biomarker for monitoring testicular germ cell tumors

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Testicular cancer represents the most frequent malignancy among men aged 20 - 40 years. There is great need for more serum biomarkers of testicular germ cell tumors (GCTs) because clinical management is largely based on marker monitoring but only 60% of patients express the classical markers AFP and beta HCG. This is particularly disadvantageous in classical seminoma, where only less than 20% of the patients have a beta HCG increase. Recently, microRNAs (miRs) have been suggested as a novel class of serum biomarkers for a variety of diseases. We measured serum levels of the microRNA miR-371a-3p in serum of GCT patients with the aim of establishing a new biomarker.

Serum samples of 84 patients with GCT (51 seminoma, 33 non-seminoma; 67 clinical stage (CS) 1 and 17 higher CS) were examined for miR-371a-3p measurements before and after treatment. 58 healthy men were used as controls. Levels of miR-371a-3p were measured by quantitative polymerase chain reaction with quantification in relation to miR-93 as internal standard. Measurements were correlated with clinical data and statistical analyses were performed.

In CS1 patients over 90% (exception: teratoma) had higher preoperative miR-371a-3p serum levels than controls ($p<0.001$) and miR levels decreased to normal range after orchiectomy. The velocity of decay of miR-371a-3p after elimination of the source of circulating molecules has been shown in six GCT patients CS1. In fact, there is a very rapid decline to lower than 5% of the initial value within 24 h after orchiectomy. After another 1–5 days, clearance of miR-371a-3p has been completed.

Metastasized patients had higher mean levels than stage 1 patients ($p<0.01$) and miR levels decrease only during chemotherapy. But after cure, miR-371a-3p expression remained low during follow-up. In these patients non-seminoma have higher mean levels than seminoma.

Our results show that the expression levels of miR-371a-3p in serum appear to be a useful biomarker for patients with testicular tumors because this marker is also expressed in seminoma and it is apparently superior to the classical markers.

P-Nomin.-017

Late onset of imprinted UBE3A expression in neurons derived from Angelman syndrome iPSCs with a defined mutation in the UBE3A gene

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Genomic imprinting is an epigenetic process resulting in parent-of-origin specific gene expression, which is regulated by differentially methylated region, which is established in one of the parental germ lines. Mutations that occur in the active copy of imprinted genes or failure during the process of establishment,

maintenance and erasure of differential DNA methylation lead to the development of imprinting disorders, such as Angelman syndrome. The symptoms of Angelman syndrome, like the absence of speech, ataxic gait and seizures, are caused by the absence of a functional UBE3A protein in the brain. In order to create a human neuronal model system for Angelman syndrome, we reprogrammed dermal fibroblasts from a patient with Angelman syndrome, caused by a defined three-base pair deletion in the maternal UBE3A allele into induced pluripotent stem cells (iPSCs), establishing the line AS_Δ3. AS_Δ3 iPSCs were characterized in vitro by determination of marker gene and protein expression by quantitative real-time PCR, immunofluorescence, FACS analysis and presence of a stable, normal karyotype was confirmed. In addition, teratoma formation of AS_Δ3 iPSCs proved their pluripotency in vivo. By deep bisulfite amplicon sequencing, we showed the exceptional epigenetic stability of the PWS-SRO, which is the differentially methylated region regulating imprinted UBE3A expression in the brain. Based on this analysis, we classified differentially methylated regions of IGF2/H19 (ICR 1), KCNQ1OT1 (ICR 2) and NESPAS as semi-stable and CpG85 of the RB1 gene and the IG-DMR of the DLK1/MEG3 locus as unstable DMRs in iPSCs. Since both parental alleles are present in AS_Δ3 iPSCs and distinguishable by the mutation in exon 4 of the maternal UBE3A copy, the onset of the silencing of the UBE3A paternal expression could be followed during neuronal differentiation. We observed upregulation of SNHG14 expression, the long non-coding RNA that silences paternal UBE3A expression specifically in the brain, at the stage of neuronal stem cells (day 14), but only a delayed onset of paternal UBE3A expression silencing at day 28 of differentiation. This indicates that imprinting of UBE3A expression takes place rather late during neuronal differentiation. Neural differentiation together with excision of the reprogramming vector without affecting pluripotency makes the newly generated iPSCs a versatile in vitro model for Angelman syndrome. They will complement the existing Angelman syndrome iPSCs carrying large chromosomal deletions. This iPSC model will enable the dissection of molecular pathways which depend specifically on the UBE3A function, contributing to a better understanding of the patho-mechanisms in Angelman syndrome.

P-Nomin.-018

Pathogenic NOTCH3 variants as a rare cause of AD

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Alzheimer's disease (AD) is the most common cause of neurodegenerative dementia. Genetic factors are important contributors to the susceptibility of the disease. Although most AD cases are sporadic, in rare cases familial aggregation has been described following a monogenic pattern of inheritance. Three genes carrying mutations have been described in these familial cases, i.e. APP, PSEN1, and PSEN2. These three genes account for approximately 30-50% of familial AD cases, leaving the rest unexplained. Development of new sequencing technologies, e.g. whole exome sequencing (WES), has provided scientist with tools to screen for rare variants (minor allele frequency < 1%) in families in which classic linkage analysis approach is not possible. Using WES, we have identified a rare single nucleotide polymorphism (SNP) in the NOTCH3 gene, in two affected members of a family clinically diagnosed with AD. The identified mutation has been described as a causative for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, OMIM #125310). Within the spectrum of symptoms observed in CADASIL, patients may develop a vascular dementia. NOTCH3 has been proposed as a candidate risk gene for AD because the gene product of NOTCH3 is involved in molecular pathways including APP, PSEN1, and PSEN2. To expand this finding in NOTCH3 to sporadic AD, we screened 651 AD patients of German or Spanish origin using Sanger sequencing or WES. Three additional variants considered pathogenic for CADASIL were identified, each one in an independent AD patients. One additional unknown variant was found within a hotspot region for pathogenic CADASIL variants, as reported by The Human Gene Mutation Database (HGMD®). None of these rare variants could be found in 1378 matched controls of German or Spanish background. The frequency of these variants was investigated in public available databases, i.e.

Exome Aggregation Consortium (ExAC) and Exome Variant Server (EVS). Three of the five identified variants were absent in both databases. Interestingly, two of the presently identified variants were previously found in AD patients. In conclusion, our data suggest that CADASIL mutations may mimic AD phenotype. Along these lines, AD neuropathology has been already described in CADASIL diagnosed patients. These observations may be explained by additional, yet unknown, modifying genetic factors which in term modulate expression of CADASIL mutations favoring amyloid pathology. In addition, as the era of precision medicine will soon become a reality, being able to confidently differentiate between closely related diseases is fast becoming a key priority. Hence, our finding may also have clinical relevance because therapeutic approaches from one disease may have negative effects when used in patients with different pathophysiological background.

P-BASIC MECHANISMS AND EPIGENETIC

P-BasEpi-019

Gender dimorphic expression of genes associated with autism spectrum disorders

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Neurodevelopmental disorders such as autism, attention deficit and hyperactivity syndrome as well as language problems and learning difficulties have a higher prevalence in male individuals compared to females.

Autism is characterized by impairments in social interaction, communication and restricted and repetitive behavior. Boys are significantly more frequently affected than girls; the ratio of affected boys compared to girls is 4:1 for autism spectrum disorders and 11:1 for Asperger syndrome.

In this study we aim to elucidate the reason for this gender-related difference by investigating the expression of genes associated with autism spectrum disorders in the brain of male and female mice to identify sex-dependent differences. Mutations in these genes may have different consequences on brain development if mRNA levels differ between male and female individuals.

We analyzed the expression of several well-known autism-related genes including the Shank gene family in five different brain regions (cortex, hippocampus, striatum, cerebellum, thalamus) at different developmental stages (E15, E17, P1, P7, P12 and adult) in male and female mice. By carrying out qPCR, we identified a gender dimorphic expression of Shank1 and Shank3, but not in Shank2. Due to the fact that early brain development is strongly influenced by sex hormones (estrogen, testosterone), we investigated the influence of these hormones on Shank gene expression in human neuroblastoma cells (SHSY5Y) and primary mouse hippocampal neurons.

A better understanding of gender differences in the brain that determine the vulnerability for neuropsychiatric disorders like autism paves the way to discover putative protective factors.

P-BasEpi-020

A maternal deletion upstream of the imprint control region 2 in 11p15 causes loss of methylation and familial Beckwith-Wiedemann syndrome with giant omphaloceles: suggestive evidence for transcription dependent imprint establishment

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We report on a familial case of Beckwith-Wiedemann syndrome (BWS), in which five pregnancies were complicated by a giant omphalocele. The first two pregnancies were terminated due to a poor prognosis. The third was a spontaneous abortion. A girl was born after the fourth pregnancy, but died shortly after birth. The fifth pregnancy started with dizygotic twins, of which the first twin died in utero (13th week of gestation). The pregnancy of the remaining twin was terminated later (24th week of gestation) again due to poor prognosis.

A molecular genetic analysis in the twins by MS-MLPA for chromosome 11p15 showed a hypomethylation of the ICR2 (*KCNQ1OT1*-differentially methylated region) in both cases, thus confirming the initial clinical diagnosis of BWS and pointing to an imprinting defect. MS-MLPA in the mother was normal. Further investigations by SNP array analyses revealed two deletions on chromosome 11. The proximal

deletion was 114 kb in size and affected genes in close proximity to the ICR2, including the 5' part of the imprinted *KCNQ1* gene, but not the ICR2 itself, which lies in intron 10 of *KCNQ1*. The second deletion spans 200 kb and is located about one Mb further telomeric. The mother harbours both deletions on her paternal allele and passed them on to both twins.

To characterise the breakpoints of the deletions in more detail and to investigate whether their presence was indicative of a more complex structural rearrangement, whole genome sequencing was conducted. The results confirmed the two deletions and showed that the region in-between was inverted.

So far, it is not clear, if this complex rearrangement leads to a disruption, removal or relocation of regulatory elements and thus causes the methylation defect in both twins. The most likely hypothesis is that the lack of expression of *KCNQ1* during oocyte growth leads to a failure in the imprint setting and the loss of methylation at the ICR2.

P-BasEpi-021

Mapping the accessible genome of human T-cell subsets by ATAC-sequencing

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Chromatin dynamics play an important role in gene regulation, cellular development and differentiation. Previous studies have already analyzed the chromatin state of numerous lymphocyte subsets, including CD4+, CD8+ and regulatory T-cells. However, concerning the specific chromatin state of Vδ1 and Vδ2 gd T-cells less data is available. Vδ1 gd T-cells are highly abundant in the gut mucosa and are thought to play an important role in the immune response in particular towards lipid antigens. Current studies imply also an important role of gd T-cells in the immune response towards malignant tumor cells as well as a contribution to allergies and asthma.

To characterize the chromatin states in Vδ1 and Vδ2 gd T-cells and to compare it to other peripheral T-cell subsets, we applied ATAC-seq (*Assay for Transposase Accessible Chromatin using sequencing*) to small numbers of cells from different T-cell subsets (CD4, CD8, regulatory T-cells, Vδ1 and Vδ2 gd T-cells) isolated from healthy individuals. Generated sequencing reads were used to determine accessible DNA regions of the chromatin as well as nucleosome positioning and transcription factor binding sites. Our preliminary results with different T-cell subsets reveal enrichment of sequencing peaks around housekeeping genes such as *GAPDH*, *ACTB* and respective marker genes like *CD4* in CD4+ T-cells, *CD8A* and *CD8B* in CD8+ T-cells and *FOXP3* in regulatory T-cells only.

The detailed analysis of ATAC-seq-based epigenomic profiles will help us to understand the comprehensive process of development and specification in functionally diverse human T-cell subsets in healthy and also in various pathophysiological conditions.

P-BasEpi-022

Genome-wide screen for synthetic lethal interactions revealed potential therapeutic targets for CENPA overexpressing tumors

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Overexpression and mis-localization of the evolutionarily conserved centromeric histone H3 variant CENPA is observed in various cancers including colorectal cancer and is associated with poor prognosis. We aimed to identify novel therapeutic targets for CENPA overexpressing (CENPA-OE) tumors by identifying synthetic lethal (SL) interaction partners. We used budding yeast as a model to perform a genome-wide screen for gene mutations/deletions that are SL in a strain overexpressing Cse4, the yeast ortholog of CENPA. Five alleles of cdc7 and dbf4 were amongst the top eight hits as the most significant SL interaction partners. Cdc7 is an evolutionary conserved, Dbf4 dependent kinase (DDK), essential for DNA replication. We validated the SL interaction of cdc7 and dbf4 with Cse4 overexpression using growth assays. Biochemical analysis showed that stability of excess Cse4 is increased in a cdc7-7 strain. Consistent with this, ubiquitination of Cse4 is reduced in a cdc7 mutant. ChIP and cell biology experiments showed enhanced mis-localization of Cse4 to non-centromeric chromatin in a cdc7-7 strain. Furthermore, in vitro kinase assays showed that DDK phosphorylates Cse4. These studies identified potential therapeutic targets for CENPA-OE tumors and revealed a novel role of the DNA replication factors DDK in regulating Cse4 levels and preventing it from mis-localizing to non-centromeric chromatin. Interestingly, inhibitors of Cdc7 are

currently being used for cancer treatment in clinical trials. Our results suggest that Cdc7 inhibitors may be especially effective in the specific treatment of CENPA-OE tumors.

P-BasEpi-023

NGS-RNAseq analyses of Silver Russell and Beckwith Wiedemann syndrome cases describe functional interactions of human imprinted genes and common syndrome associated dysregulations

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Beckwith Wiedemann- (BWS) and Silver Russell-syndrome (SRS) are two complex growth disorders caused by dysregulation of imprinted genes. While BWS-patients present with pre- and postnatal overgrowth and an increased risk to develop embryonal tumors, SRS-patients show intrauterine and postnatal growth restrictions. The majority of BWS and SRS associated molecular defects are epigenetic alterations in two clusters of imprinted genes on chromosome 11 (IC1 with *H19* and *IGF2*, IC2 with *KCNQ1OT1/KCNQ1* and *CDKN1C*). Yet approximately 10% of SRS cases have epigenetic defects affecting imprinted genes (including the *MEST* gene) on chromosome 7 and present with phenotypes nearly indistinguishable from that associated with chromosome 11 defects. This suggests a regulatory connection between the different imprinted genes or gene clusters. In mice an imprinted gene network (IGN), describing the interactions between different imprinted genes, has been predicted. Based on comparative RNAseq analyses of primary fibroblasts from BWS and SRS cases that have a loss of imprinting of *H19* and *IGF2* (BWS) or maternal upds of chromosomes 11 or 7 (SRS), respectively, we aim to uncover interactions in a human IGN. The NGS based analyses enabled us to detect gene dosage alterations in other imprinted and non-imprinted genes throughout the genome. The most likely transfactors mediating the transcriptional regulation of IGN genes are long non-coding RNAs like *H19*. The comparison of the expression profiles of the BWS and SRS fibroblasts with epigenetic defects on chromosome 11 help to uncover the interactions that are affected by the loss (BWS) or gain (SRS) of *H19* expression. Knock-down analyses and consecutive monitoring of the transcriptional consequences with qRT-PCR help to address the hierarchical structure of the IGN. In addition, we compare the RNAseq data of the two SRS cases. Despite the different chromosomes affected, both SRS cases show increased *H19*- and decreased *IGF2* expression. Our preliminary analyses show common dysregulation patterns for IGN genes throughout the genome in SRS cases with different epigenetic alterations, suggesting that these patterns are associated with the clinical phenotype.

P-BasEpi-024

Characterization of the expression of the imprinted Kcnk9-gene in specific brain regions of mice

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Kcnk9/KCNK9 is a maternally expressed imprinted gene whose mutations are responsible for the maternally inherited Birk-Barel mental retardation dysmorphism syndrome. It encodes a member of the superfamily of K+-channel with two pore-forming domains and is involved in the modulation of the resting membrane potential and excitability of neuronal cells. So far, only homozygous Kcnk9 knockout mice with inactivation of both parental alleles were phenotypically characterized. These mice displayed cognitive deficits as well as a reduction of K+ leak current by 50% and a reduced number of action potentials after current injection in cerebellar granule neurons. In the light of maternal-specific imprinted expression of Kcnk9/KCNK9 and the maternal inheritance of the Birk-Barel mental retardation dysmorphism, a through phenotypic analysis of heterozygous Kcnk9 knockout mice with inactivation of only the maternally inherited or only the paternally inherited Kcnk9 allele is also warranted.

In this study, we set out to characterize the parental allele-specific expression of Kcnk9 in various regions of the mouse brain. The second aim is to comparatively analyze several parameters of brain function in homozygous Kcnk9 knockout mice and heterozygous Kcnk9 knockout mice with inactivation of only the expressed maternal Kcnk9 allele.

Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) method was performed for different brain areas from several developmental stages of (C57BL/6xCast/Ei) F1 hybrid mice. Exclusive expression from the maternal Kcnk9 allele was detected in the dentate gyrus, hippocampus, mesencephalon, medulla oblongata, thalamus and pons. Biallelic expression with, however, a strong bias

towards the maternal Kcnk9 allele (94-99% of the transcripts) was observed in the olfactory bulbs, cortex, cerebellum, striatum and olfactory tubercles. QUASEP data was confirmed using the more sensitive real-time RT-qPCR with allele-specific PCR-primers that allows the detection of very low numbers of paternal transcripts. Counter-intuitively, Kcnk9 expression analysis in heterozygous Kcnk9-knockout mice with maternally inherited knockout allele showed a very low level of expression from the paternal allele in all investigated brain areas which may serve as a possible compensatory mechanism for loss of the maternal allele. A significant higher expression of the paternal allele of heterozygous Kcnk9 knockout mice was detected in cerebellum, hippocampus, thalamus and the olfactory bulbs in comparison to wt-hybrids.Furthermore, we investigated the expression levels of genes within the same imprinting cluster as kcnk9 and Ago2 was found to be significantly differentially expressed in hypothalamus, olfactory bulbs, olfactory tubercles and pons.

Our findings shall further elucidate the role of Kcnk9/KCNK9 in brain physiology and pathophysiology and open new avenues for treatment of cognitive dysfunctions in Birk-Barel syndrome.

P-BasEpi-025

Preovulatory aging affects maturation, fertilization and developmental competence of mouse oocytes

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Preovulatory aging of oocytes is caused by delayed ovulation and leads to impaired embryonic development in mice. In a previous study, we observed a decrease in mRNA expression levels of the maternal effect genes Brg1, Tet3 and Zfp57 upon aging. Brg1 and Tet3 are required for embryonic genome activation (EGA) at the 2-cell stage. Zfp57 is involved in the maintenance of genomic imprints during epigenetic reprogramming in the early embryo. We therefore investigated the effect of preovulatory aging on EGA and DNA-methylation in preimplantation embryos.

The gonadotropin releasing hormone (GnRH) antagonist Cetrorelix was used to postpone ovulation in female C57Bl/6J mice. After preovulatory aging the number of oocytes retrieved per mouse was significantly lower than in control mice, indicating lower maturation rates after aging. Females were mated with C57Bl/6JxCBA hybrid males to avoid inbred depression, and the 2-cell embryos were flushed from the oviduct. The 2-cell rate declined significantly after preovulatory aging, suggesting that also fertilization is impaired by aging. For analysis of EGA, embryos were incubated in BrUTP. BrUTP is converted to Bromouracil (BrU), incorporated into nascent RNA, and can be detected by immunohistochemistry. The Anti-BrU fluorescence intensity of 60 control and 38 preovulatory-aged embryos was compared and a 2.85-fold increase in intensity was detected after aging, pointing to a precocious activation of the genome.

A possible effect of preovulatory aging on genomic imprinting was investigated by analyzing DNA-methylation levels of Igf2r, Snrpn (both maternally methylated), H19 (paternally methylated) and Oct4 (unmethylated control gene) using deep amplicon bisulfite sequencing. To distinguish between the parental allels, C57Bl/6J females were mated with CAST/Ei males. Their offspring contained heterozygous single nucleotide polymorphism (SNPs) in all investigated loci. Single 8-cell embryos were analyzed, because at this stage the reprogramming of the parental genomes is completed. We did not find significant differences in methylation levels for the analyzed loci between 15 control and 14 preovulatory-aged embryos. This demonstrates stable postzygotic maintenance of the DNA-methylation levels after preovulatory aging. Overall, preovulatory aging affects oocyte maturation, fertilization rate and EGA, but not the DNA-methylation of the investigated loci, indicating individual control and regulation of different processes in the embryo.

P-BasEpi-026

The impact of ART on genome-wide oxidation of 5-methylcytosine and the transcriptome during early mouse development

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The use of assisted reproductive technologies (ART) has been increasing over the past three decades for treating infertility problems. Nevertheless, a negative impact of ART on methylation reprogramming is heavily discussed. Methylation reprogramming directly after fertilization manifests as genome-wide DNA demethylation associated with the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC)

in the pronuclei of mouse zygotes. To investigate the possible impact of ART particularly on this process and the transcriptome in general, we subjected pronuclear stage mouse embryos obtained upon spontaneous ovulation or superovulation through hormone stimulation representing ART to various epigenetic analyses. Immunofluorescence staining with antibodies against 5mC and 5hmC showed that pronuclear stage embryos obtained by superovulation have an increased incidence of abnormal methylation and hydroxymethylation patterns compared to their spontaneously ovulated counterparts. Additionally, a single-cell RT-qPCR of the Tet1, Tet2 and Tet3 genes that encode 5mC oxidases converting 5mC to 5hmC revealed no significant expression differences between pronuclear stage embryos from spontaneously and superovulated matings suggesting that the detected aberrant methylation patterns are not caused by differences in TET-mediated active demethylation mechanisms, but rather by replication dependent passive processes. Furthermore, a whole-transcriptome RNA-Seq analysis of pronuclear stage embryos from spontaneously and superovulated matings demonstrated altered expression of genes including imprinted and epigenetic regulator genes. Overall, our data further support a negative impact of ovarian stimulation on epigenetic reprogramming during gametogenesis and early embryogenesis.

P-BasEpi-027

Pelota regulates epidermal barrier acquisition by modulating BMP and PI3K/AKT signaling pathways.

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The depletion of evolutionarily conserved Pelota protein (PELO) causes impaired differentiation of embryonic and spermatogonial stem cells. In this study, we show that temporal deletion of PELO prior to epidermal barrier acquisition leads to neonatal lethality due to perturbations in permeability barrier formation. Further analysis indicated that this phenotype is a result of failed processing of profilaggrin into filaggrin monomers, which promotes the formation of a protective epidermal layer. Molecular analyses revealed that PELO negatively regulates the activities of BMP and PI3K/AKT signaling pathways in the epidermis. To address whether elevated activities of BMP and PI3K/AKT signaling pathways were the cause for the perturbed epidermal barrier in Pelo-deficient pups, we made use of organotypic cultures of skin explants from control and mutant embryos at E15.5. Inhibition of PI3K/AKT signaling did not significantly affect the BMP activity. However, inhibition of BMP signaling caused a significant attenuation of PI3K/AKT activity in mutant skin and, more interestingly, the restoration of profilaggrin processing and normal epidermal barrier function. Therefore, increased activity of PI3K/AKT signaling pathway in Pelo-deficient skin might conflict with the dephosphorylation of profilaggrin and thereby affects its proper processing into filaggrin monomers and ultimately the epidermal barrier acquisition.

P-BasEpi-028

Genomic signature of radiation-induced DNA-sequence alterations in human gingiva fibroblasts

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Ionizing radiation can induce genomic lesions such as DNA double strand breaks. Incomplete or faulty repair of such lesions can result in mutations, which have the potential to influence cellular functions that may alter the fate of the affected cells, organ systems and even their offspring. As such, radiation-induced sequence alterations may contribute to anincreased risk of the affected individuals to develop cancer and possibly hereditary disorders.. Exposure to low doses of ionizing radiation can induce mutations that occur in a stochastic manner. However, higher doses, exceeding 1.5 Gy, as often observed in radiation accidents, can also have deterministic effects. In part such effects could be the result of specific characteristics of the repair mechanisms involved. Since the genomic consequences of a radiation insult are only fragmentarily understood, we were interested to identify radiation-specific signatures in the genomes of primary human gingiva fibroblasts exposed to acute doses of ionizing radiation (x-y Gy) in combination with various repair intervals to be able to investigate alterations modulated by either or both parameters. To this end we harvested the exposed cells and subjected their genomic DNA to exome- as well as whole genome

sequence analysis. When comparing treated and untreated cells we found that the frequency of radiation-induced SNPs per megabase differed distinctly and characteristically between chromosomes and their subregions. Moreover, we observed that certain chromosomal regions are seemingly more prone to the accumulation of SNPs than others. These observations may relate to structural and/or functional differences that might mediate a certain bias in DNA repair mechanisms towards specific chromosomes and/or chromosomal regions.

P-BasEpi-029

Genome-wide epigenetic profiling of Non-Small Cell Lung Carcinomas for the prediction of chemotherapy resistance (EPITREAT)

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Non-small cell lung cancer (NSCLC) is a complex disease that, due to its heterogeneity and poor prognosis, raises many challenges to diagnosis, prognosis and treatment. DNA methylation is an important epigenetic mechanism regulating developmental processes and, with a deregulation leads to cancer. The modification is covalent and repetitive in regard to specific genomic regions, which makes it a suitable marker for epigenetic profiling and stratification of cancers.

Here we performed MeDIP-Seq experiments (Methylated DNA immunoprecipitation followed by next generation sequencing) of 25 primary lung tissue materials and xenograft-derived NSCLC models. MeDIP-Seq data were validated by targeted bisulfite sequencing with the Methyl-Seq approach. The Xenograft mouse models were treated with seven different chemotherapies and tumors were classified as responders and non-responders according to the tumor volumes. Applying an extended MEDIPS software package, we identified distinct epigenetic alterations which are now followed up with bisulfite pyrosequencings in an independent patient's cohort to evaluate their utility as epigenetic biomarkers. Furthermore, pathway analyses revealed a close relationship to the oxidative stress response and mitogen-activated protein kinase pathways for carboplatin resistant xenografts. Our previous studies had implicated the bromodomain containing protein BRD4 as central regulator of both pathways. We therefore performed combination treatments (carboplatin and BRD4 inhibitor JQ1) of resistant xenograft models and found a synergistic effect. This suggests that JQ1 may sensitize carboplatin- resistant cancers.

Taken together, we provide genome-wide DNA methylation maps of NSCLC xenografts, their paired lung tissues and therapy response information of seven chemotherapies which is used for the identification of epigenetic biomarkers. Furthermore, we explored epigenetic mechanismis of therapy resistance and potential intervention to overcome resistance.

P-BasEpi-030

Identification of regulatory interactions in the human hair follicle: Expression profiling and target gene identification for nine candidate microRNAs

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The human hair follicle is a complex mini organ that is capable of life long cycling through phases of active growth, regression and rest. Each phase is characterized by a tightly regulated activation and orchestration of specific signaling cascades that control the relevant cellular processes. However, our knowledge on the underlying molecular mechanisms is still incomplete. In recent years, microRNAs (miRNAs) have gained considerable attention in the analysis of regulatory mechanisms for numerous cellular and developmental processes. Regarding hair biology, several miRNAs have been reported to play critical roles in the control of hair follicle cycling, keratinocyte differentiation/proliferation and melanogenesis. But the current knowledge on the expression and function of these miRNAs is largely based on murine data or in vitro experiments and little is known about their function in the human hair follicle. To obtain a better understanding of the role of miRNAs in human hair biology we sought to systematically investigate the expression of nine previously reported hair-relevant miRNAs and their correlation with mRNA-expression levels in the human hair follicle. MiRNA expression was analyzed in hair follicle samples from 25 healthy male donors using the Affymetrix miRNA 4.0 Array. Corresponding mRNA expression levels were

determined using the Illumina HT12v4 array. Our analysis confirmed expression of seven of the nine candidate miRNAs (miR-24, miR-31, miR-106a, miR-125b, miR-205, miR-214, miR-221). To identify potential miRNA target genes, we subsequently tested for a correlation of miRNA and mRNA expression levels. The analysis revealed significant miRNA/mRNA correlation for four of the seven miRNAs (miR-24, miR-31, miR-106a, miR-221) and identified 49; 109; 41, and two mRNA target genes, respectively. Among them were several genes with known functions in hair biology (e.g. *SOX9*, *KRT15*) and genes whose described function matched the reported role of their regulating miRNA. Among them, the miR-24 target *ITGB1*, which regulates keratinocyte differentiation and *RXRA*, a target of miR-31, which has been reported to regulate anagen initiation. Interestingly, the majority of identified target genes had not yet been implicated in hair biology such as *SESN1* or *TAX1BP3*. We subsequently tested for a pathway enrichment of these target genes using Ingenuity Pathway Analysis and PANTHER. MiRNA target genes were enriched in signaling cascades such as WNT or integrin signaling. In summary, our analysis confirmed the expression of seven candidate miRNAs in the human hair follicle. The systematic correlation of miRNA and mRNA expression levels revealed numerous miRNA target genes and pathways thereby yielding novel insight into regulatory interactions in the human hair follicle. This knowledge will eventually contribute to a deeper understanding of healthy hair biology and the pathobiology of human hair loss disorders.

P-BasEpi-031

An in vitro model for retinoblastoma using human embryonic stem cells

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Retinoblastoma is the most common childhood eye tumor. Most children can be cured with current therapeutic strategies, but many suffer from severe late effects. About half of the patients with retinoblastoma are heterozygous for an inactivating variant of the retinoblastoma gene *RB1*. The impact of specific *RB1* mutations on initiation and progression of retinoblastoma is not well characterized yet. Recent analyses determined the cell-of-origin of retinoblastoma to be a photoreceptor cone cell in the early developing retina. However, mechanistic insight into the development of retinoblastoma is difficult to gain because of the lack of a valid animal model and the inaccessibility to developing human retina.

We aim to create a model for retinoblastoma using human embryonic stem cells (hESCs) that includes their differentiation into neural retina and the introduction of *RB1* mutations by the CRISPR/Cas9 system. Up to now, we have achieved differentiation of hESCs into 3D organoids expressing the neural retina-specific markers *PAX6*, *RX* and *VSX2*, as could be shown by quantitative RT-PCR and immunostaining. In addition, we have introduced random mutations in *RB1* exon 3 by using CRISPR/Cas9 with high efficiency. Next steps will be the isolation of clonal hESC lines and their comparative differentiation to follow cell fate during neural retina differentiation of these genetically engineered hESCs. First results on these analyses will be presented.

This *in vitro* model for retinoblastoma will enable future studies on the effects of specific *RB1* mutations on susceptibility for oncogenic transformation, tumor initiation and progression.

P-BasEpi-032

Methylation analysis in primary human fibroblasts following X-ray irradiation

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Radiation therapy is one of the oldest, most highly effective forms of cancer treatment. Aside from the intended neoplastic targets, nearby normal cells can be affected by ionizing radiation leading to a variety of genetic changes. Recent studies suggest that irradiation can also lead to epigenetic alterations, especially to changes in DNA methylation patterns. Global methylation and hydroxymethylation levels are reduced in cancer cells. Therefore, we hypothesized that irradiation of normal cells might directly lead to loss of methylation/hydroxymethylation contributing to genome instability and tumorigenesis in later stages.

We used multiple approaches to analyze global and locus-specific methylation patterns in cultured normal human fibroblasts following various doses of X-ray irradiation. In contrast to cancer cells, the cell lines used here are diploid and not affected in repair mechanisms or cell cycle checkpoints. Methylation was mainly investigated within a single replication cycle (~24 h) to ensure that direct effects of ionizing radiation were analyzed and to exclude methylation changes associated with replication. Doses from 1 to 4 Gray (Gy) were chosen since therapeutic low dose fractions of 1.8 to 2 Gy are used during routine cancer therapy.

Fluorometric ELISA-based assays assessed global DNA methylation (5-mC) or hydroxymethylation (5-hmC) changes after irradiation with X-rays. These assays quantify global DNA methylation/ hydroxymethylation by specifically measuring levels of 5-mC or 5-hmC. For nearly all radiation doses and analyzed time points, we observed a slight trend towards hypomethylation as well as reduced hydroxymethylation after irradiation. However, results were not statistically significant.

As a further approach to assess global DNA methylation, we used a PCR based method which amplifies multiple repetitive elements (Alpha-Sat, ALU, LINE-1) from bisulfite-treated DNA that is followed by pyrosequencing. None of the three analyzed repetitive elements showed significant changes after radiation exposure in mass cultures and single cells.

For methylation profiling at gene- and locus-specific level, the Illumina HumanMethylation450K platform was used. No significant differentially methylated sites were detected comparing irradiated cells and non-irradiated cells.

Our results suggest that X-ray irradiation induces no dramatic effect on DNA methylation patterns in normal human fibroblasts directly upon exposure.

P-BasEpi-033

Epigenetic determinants of resilience – implications of positive environmental factors during pregnancy

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Resilience is the ability to adapt successfully to adverse experiences in order to maintain normal physiological and psychological functions. Studies in humans and rodent models indicate that development of stress-resistance already starts in utero. Prenatal stress is a well known risk factor for psychiatric disorders or behavioral changes later in life, whereas the effects of positive conditions during pregnancy are largely unclear. The aim of our study is to gain insights into the role of positive environmental factors early in life for the development of resilience. To this end, mouse dams were kept under standard housing conditions or under positive, stimulating environmental conditions. For each prenatal group, the resulting offspring was randomized in three postnatal groups (stress in the form of maternal separation, control, and continued positive environment). In early adulthood of the animals DNA methylation of 7 candidate genes (Maoa, Htr1a, Crhr1, Nr3c1, Fkbp5, Bdnf, Nos1) was analyzed in 5 different brain-regions (hypothalamus, frontal lobe, striatum, hippocampus, amygdala, raphe nuclei) using bisulfite pyrosequencing. Data analysis revealed 57 significant differences for the studied genes and brain regions, depending on the prenatal and/or postnatal environments the animals had experienced. Overall prenatal conditions had a bigger impact on brain methylation (n=39 significant differences between groups) than postnatal treatment (n=18). In addition, there was a clear sex difference. Female offspring appeared to be more sensitive to a positive prenatal environment (n=24 between group differences) than males (n=15). Vice versa, male offspring exhibited more methylation changes (n=19) due to postnatal treatment, compared to females (n=12). The genes sensitive to prenatal or postnatal environment also differed between sexes. For example, Bdnf methylation was mainly affected in female striatum, whereas NGFI-A and Nos1 001 were epigenetically regulated in all male brain regions apart of raphe nuclei. Collectively, our study shows significant effects of positive prenatal conditions and different postnatal environments on DNA methylation of genes associated with stress-response. The observed interrelations are highly complex, involving different brain regions and genes in males and females. Our results argue more in favor of the mismatch hypothesis, postulating that opposing prenatal and postnatal conditions may be more harmful than additive negative conditions.

P-BasEpi-034

Leukocyte telomere length is related to appendicular lean mass: cross-sectional data from the Berlin Aging Study II.

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Background: Age-related loss of muscle mass is an increasing problem in in our aging society, affecting physical ability. Telomere length has been recognized as a marker of biological age on the population level.

Objective: Here we evaluated the rarely examined relationship between lean mass and relative leukocyte telomere length (rLTL) in 1,398 participants of the Berlin Aging Study II (mean age 68.2±3.7 years, 49.6% men).

Methods: The determination of rLTL was carried out by real time PCR. Lean mass was estimated by dual X-ray absorptiometry and examined as leg lean mass (LLM), appendicular lean mass (ALM), and ALM corrected for body mass index (ALM_{BMI}).

Results: Highly significant correlations ($p<0.001$) of rLTL and ALM ($r=0.248$), ALM_{BMI} ($r=0.254$), and LLM ($r=0.263$) were found. Associations remained significant in linear models adjusted for age, gender, BMI, low-grade inflammation, life style factors and morbidities: ALM ($\beta=0.844$, $p=0.009$), ALMBMI ($\beta=0.032$, $p=0.011$), and LLM ($\beta=0.967$, $p<0.001$). Shorter rLTL, advanced age, female sex, sedentary lifestyle and elevated CRP level were associated with lower lean mass.

Conclusion: Short telomeres were associated with low lean mass. Our results indicate that rLTL may be related to loss of lean mass. To confirm the association between telomere attrition and loss of LLM and ALM_{BMI}, which are highly relevant for physical ability, further research should examine this subject in a longitudinal context.

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P-BasEpi-035

The effects of paternal body mass index (BMI) on sperm DNA methylation

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Background

The prevalence of metabolic disorders, in particular obesity has dramatically increased worldwide. Genetic variants explain only a minor part of this obesity epidemics induced by physical inactivity and over nutrition. Epidemiological studies in humans and animal models of diet-induced obesity indicate that epigenetic changes associated with adverse parental and/or intrauterine factors may contribute to the missing heritability of metabolic disorders. Possible adverse paternal effects are likely transmitted by the sperm to the next generation. To prove this hypothesis, we have systematically analysed the effects of paternal obesity on the sperm epigenome and its implications for the next generation.

Results

Using bisulphite pyrosequencing, we quantified DNA methylation of cis-regulatory regions of eight paternally expressed imprinted genes (PEG1, PEG3, PEG4, PEG5, PEG9, PEG10, NESPAS, and IGF2), two maternally expressed imprinted genes (MEG3 and H19), and the obesity related gene HIF3A in sperm samples of 188 donors from a fertility center. Sperm DNA methylation of PEG1, PEG3, PEG5, IGF2 and HIF3A showed a significant or trend correlation with paternal BMI. We tried confirming our results in an independent cohort (n=55) of sperm samples where fetal cord blood DNA (offspring) is also available. PEG3 maintained a negative correlation with paternal BMI in both the cohorts. We are currently studying PEG3 DNA methylation in the corresponding fetal cord blood DNA. Additionally, using deep bisulphite sequencing (DBS), we performed single allele analysis of four candidate genes (PEG1, HIF3A, H19 and NESPAS). Epimutations were defined as alleles showing >50% aberrantly (de)methylated CpG sites. Here, we observed a higher epimutation rate in the high BMI (28-40) group when compared to the low BMI (19-24) group across all the four studied genes. NESPAS and H19 exhibited the highest ER in sperm of obese individuals with a borderline significant trend of 0.05 and 0.06, respectively.

Conclusions

Our results suggest that male obesity is associated with modifications of the sperm DNA methylome, which may affect the epigenome (in fetal cord blood) of the next generation.

P-BasEpi-036

hSNM1B/Apollo editing by the CRISPR-Cas9 system

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The nuclease hSNM1B/Apollo is involved in the cellular response to DNA-damage induced by DNA interstrand crosslinking (ICL) agents and ionizing radiation. We were recently able to show that hSNM1B/Apollo interacts physically with FANCP/SLX4, a protein that coordinates the activity of various structure-specific nucleases in response to ICL induced DNA-damage, for example SLX1, XPF-ERCC1 and MUS81-EME1. *FANCP/XLS4* is one of 19 genes known to be associated with the recessively inherited disorder Fanconi Anemia (FA), and the physical interaction of its gene product with hSNM1B/Apollo links the latter to the FA/BRCA-pathway of ICL damage repair.

However, no *hSNM1B/Apollo* mutations have been found in FA patients so far and a mouse model deficient for *hSNM1B/Apollo* is not available, limiting functional studies on hSNM1B/Apollo. Here we have generated *hSNM1B/Apollo* deficient U2OS cell lines employing the recently discovered RNA-guided CRISPR-Cas9 system following the “double nicking strategy”.

We will present data on several independently isolated cell clones carrying different *hSNM1B/Apollo mutations*. This will include the detailed characterization of the elicited mutations, results from colony survival assays (Mitomycin C and ionizing radiation), and immunofluorescence experiments evaluating the ability to form nuclear DNA repair foci.

P-BasEpi-037

Maternal effect mutations in NLRP genes: a further non-Mendelian mode of inheritance

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The NLRP gene family contains 14 genes and forms a subgroup within the CATERPILLER protein family. They are involved in inflammatory processes and apoptosis. Recent reports indicate that they are also associated with recurrent reproductive wastage. For mutations in the NLRP2, NLRP5 and NLRP7 genes it has been shown that they represent maternal effect mutations, meaning that women are at risk for reproductive failure or for offspring with aberrant methylation leading to Imprinting Disorders (ID). In particular, NLRP7 mutations are responsible for recurrent reproductive wastage, and recent reports revealed that variants in this gene are associated with aberrant methylation at multiple imprinted loci (multi-locus methylation disturbances, MLID).

We report on the identification of heterozygosity for a frame-shift mutation in NLRP7 in a woman with recurrent reproductive wastage. Family history was empty. Three pregnancies in total failed within two years, one of which terminated in the 6th week. In the other two pregnancies elevated βHCG and ovarian cysts were observed. The first pregnancy was terminated in the 22 gw because of severe preeclampsia, the second in the 24 gw due to preterm labor. Both children died shortly after birth. Methylation specific tests revealed multi-locus methylation disturbances (MLID) at different loci on Chromosome 7, 11 and 20. Methylation profiles did not only vary in the fetuses but also between different tissues tested. This underlines the complex regulatory mechanism of imprinting. Since the pathologic examinations of both fetuses did not exhibit unambiguous assignments of the alteration to a clinical phenotype it is difficult to predict whether a future child will be affected by a specific imprinting disorder. In addition, an association between NLRP7 mutations and increased risk of aneuploidies has been suggested. This assumption is supported by the finding of X0 mosaicism in more than 50% of amniotic cells (45,X[18]/46,XY[14]).

Based on our findings and cases from the literature we delineate a pathogenic role of the NLPR7 mutation in our family leading to reproductive wastage and representing a maternal effect mutation contributing to MLID.

P-BasEpi-038

Epigenetic pre-aging in germinal center derived B cell lympho

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Accurate DNA-methylation marks are mandatory for regular cell differentiation and for establishing tissue specific gene expression patterns. Age-related changes in the DNA-methylation pattern of disease related genes have been linked to the onset of diseases characteristic for middle or high age including cancer. Furthermore it recently turned out that age-related changes in the DNA-methylation of a specific minor set of CpG loci are sufficient to determine the chronological age of the donor with high accuracy (Horvath, 2013).

Since alterations in the DNA-methylation pattern are a hallmark of cancer we wondered whether epimutations identified in disparate lymphoma entities affect the epigenetic clock of the lymphoma cells. Furthermore we aimed at identifying entity specific differences in the “re-setting” of the epigenetic clock.

To adress these questions we performed DNA-methylation profiling of 46 Burkitt lymphoma samples, 12 follicular lymphoma samples, 7 diffuse large B cell lymphoma samples available from the ICGC MMML-Seq consortium (Kretzmer et al., 2015) and 173 peripheral blood samples of healthy individuals available from

the SAME project and current publications of our group using the HumanMethylation450 BeadChip (Kolarova et al., 2015; Friemel et al., 2014). Subsequently, we determined the epigenetic age using the "Online Age Calculator" accessible at https://labs.genetics.ucla.edu/horvath/dnamage and compared the outcome with the corresponding chronological age of the donors.

While all samples collected from healthy donors showed high accordancy of the epigenetic age and the chronological age (Pearson correlation coefficient up to 0.97), our results indicate "epigenetic pre-aging" (age acceleration) in particular in Burkitt lymphoma while follicular lymphoma are less affected.

In conclusion we found a significant epigenetic "pre-aging" in Burkitt lymphoma samples as compared to other lymphoma entities and normal blood samples. Currently we are focusing on the effect of MYC, which is highly expressed in particular in Burkitt lymphomas, on the acceleration of epigenetic aging.

P-BasEpi-039

MicroRNA profiling: Increased expression of miR-147 and miR-518e in progressive supranuclear palsy (PSP)

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Progressive supranuclear palsy (PSP) is a lethal neurodegenerative sporadic disorder characterized by axial rigidity, postural instability, vertical gaze palsy, frontal dysexecutive syndrome, dysarthria, and dysphagia. Genetic, environmental and possibly epigenetic factors such as DNA methylation, histone acetylation, and modification of microRNA expression contribute to disease. In order to improve our understanding of potential epigenetic effects in the pathophysiology of PSP, we performed an extensive study of microRNA (miR) expression in brains of patients (n=20) and controls (n=20).

miRs are non-coding small (commonly 22 nucleotides) RNAs that regulate gene expression at the posttranscriptional level. They derive from primary transcripts (pri-miRs) that are processed into approximately 70 nucleotide stem loop precursor miRs (pre-miRs). Cleavage of the pre-miRs by cytoplasmic ribonuclease Dicer results in mature miRs. The mature miRs silence target genes by either interfering with their translation or increasing their degradation. Each miR regulates multiple target genes.

We used post mortem frontal lobe brain tissue for miRNA profiling. Extracted RNA was analyzed on TaqMan array cards. Dysregulation of miRNAs was validated by NCode first strand synthesis kit and SYBR-green qRT-PCR. Expression of selected potential targets of dysregulated miRs was analyzed by qRT-PCR.

First screening showed up-regulation of *miR-147*, *miR-518e* and down-regulation of *miR-525-3p* and *miR-504*. Up-regulation of *miR-147* and *miR-518e*, but not down-regulation of *miR-525-3p* and *miR-504* was confirmed by SYBR-green qRT-PCR. The target genes of these two miRs were repressed, including *NF1*, *ACLY*, *ALG12* (*miR-147*) and *CPEB1*, *JAZF1*, *RAP1B* (*miR-518e*).

Dysregulation of *miR-147* and *miR-518e* might be mediated by yet unknown environmental influences and appears to increase disease risk by repressing target genes. The latter are involved in various cellular/neuronal functions and might thus be potential targets for therapeutic interventions.

P-BasEpi-040

Establishing a Murine Xenograft-Model for long-term Analysis of factors inducing Human Leukemia: Pitfalls and successes

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Background:. Myelodysplastic syndromes (MDS) are difficult to culture long-term. Here, we present a tedious approach to reach the final goal to establish a xenograft transplantation model, transplanting human hematopoietic stem cells (HSC) with different independent lentivirally-mediated MDS-related modifications into immunodeficient mice.

Methods: 3 different modifications were chosen: 1) RPS14-haploinsufficient HSCs via shRNA knockdown, 2) TP53-deficient HSCs via shRNA knockdown and 3) HSCs, with different lentivirally introduced TP53 hotspot mutations (R248W, R175H, R273H, R249S).

Before transduction, HSCs cells were cultivated for 72 h (long protocol), 24 h (medium protocol) or 2 h (short protocol) with cytokines. Two days after transduction and shortly before transplantation (tx), the

successfully transduced cells were sorted (optionally) for the internal marker GFP via fluorescence-activated cell sorting (FACS). In 12 different transplantations, HSCs were intrafemorally transplanted into sublethally irradiated immunodeficient NSG and/or NSGS mice. Peripheral blood (PB) samples from mice were taken on a regular basis to monitor engraftment success (CD45+ and GFP+ cells) via FACS analysis starting at 4 to 8 weeks after transplantation and afterwards every 4 weeks. Bone marrow was taken for final evaluation of engraftment as well as for karyotyping.

Results:. Experiments with modification 1) (n=3) and the short or long protocol in NSG mice showed engraftment rates of CD45+ cells of up to 76 %, however, mostly GFP- cells engrafted.

In experiments with modification 2) (n=5) also the long protocol showed good engraftment up to 70 %, however, the amount of GFP+ cells was low (< 8,25 % of the CD45+ cells and decreasing). The short protocol gave poor to medium engraftment results in general and varying amounts of GFP+ cells (up to 27 %, but quickly decreasing in the CD45+ fraction).

Experiments with modification 3) (n=4) were performed in NSGS mice with the medium protocol showing good general engraftment (up to 72 %) of CD45+ cells. Also, a relatively high amount of GFP+ cells could be detected (up to 35 % in the first experiment and 4 % in the fourth), however, these cells showed to be mainly T-cells and the mice quickly died of graft-versus-host-disease (GVHD).

In all experiments engraftment was detected between week 8 and 12 after tx. Cytogenetic analyses of the bone marrow showed human cells with normal karyotypes.

Conclusions: In summary, engraftment of CD34+ cells in NSG and NSGS mice is very promising and could be used to analyze the induction of CIN. However, the analysis of transduced CD34+ cells with different vectors is limited as yet and the choice of an appropriate transduction protocol of CD34+ cells prior tx as well as an optimal mouse strains is seen as a critical step for success. In our hands, NSG mice seemed to be more robust towards GVHD compared to NSGS mice, but here strategies to minimize GVHD could be combined for optimal results.

P-BasEpi-041

MiR-449 family expression is induced by histone acetylation in hepatocellular carcinoma

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Introduction

Research over the past decade has led to the development of histone deacetylase inhibitors as anticancer agents. HDAC inhibitors increase the acetylation of histones resulting in altered expression of genes involved in oncogenic processes leading to antitumor effects.

Previously, we analyzed how the HDAC inhibitor trichostatin A (TSA) influences the expression of miRNAs in HCC cell lines. The tumor suppressive microRNA-449a (miR-449a), which is coded in a cluster with miR-449b and miR-449c, was strongly induced by increased histone acetylation. The microRNA-449 familiy (miR-449 family) has been mapped to the second intron of CDC20B on chromosome 5 and shares a common promoter with its host gene CDC20B (Yang et al., 2009). Our aim is to determine whether induced miRNA expression is directly influenced by altered histone acetylation at specific histone marks.

Methods

HCC cell lines Huh7, HepG2, HLE, and HLF were treated with 100 ng/ml TSA for 1 h or 24 h. Expression was analyzed by qRT-PCR for miR-449a, b and c and the host gene CDC20B. Chromatin immunoprecipitation was performed 1000 bp upstream of the CDC20B promoter using antibodies against acetylation marks H3K9ac, H4K5ac, H4K8ac, H4K12ac, and H4K16ac.

Results

TSA treatment increased the expression of the miR-449 family and its host gene CDC20B. Using chromatin immunoprecipitation after 1 h TSA treatment, we observed an increase of all tested acetylation marks at the promoter of CDC20B/miR-449 family. Interestingly, H4K5 and H4K8 showed the strongest enrichment in acetylation. It appears that these histone acetylation marks serve as an early marker of altered histone acetylation due to TSA in HCC.

Conclusion

Increased acetylation induced by TSA resulting in a more loosely chromatin formation ends up in an overexpression of genes and microRNAs involved in proliferation and apoptosis and thereby in tumor formation. In HCC cell lines, TSA treatment reactivates expression of the tumor suppressive miR-449 family and its host gene CDC20B. By chromatin immunoprecipitations we detected a direct regulation of induced histone acetylation of specific acetylation marks, which is followed by increased miR-449 and CDC20B expression.

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P-BasEpi-042

Analysis of gene expression in the APP/PS1KI mouse model for familial Alzheimer's Disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by intracellular hyperphosphorylated tau aggregates as well as early intraneuronal amyloid-β (Aβ) accumulation and extracellular deposition of Aβ peptides. As a consequence, dendritic and synaptic alterations as well as an inflammatory response in the affected brain are observed. Even though the neuropathological characteristics of this disorder are long known, the underlying molecular mechanisms are still not fully understood. The investigation of gene expression alterations in transgenic AD mouse models, using next-generation sequencing (NGS) technology, can yield insights as to the molecular mechanisms involved. Here, we performed a whole-brain transcriptome analysis, using the well-characterized APP/PS1KI mouse model for AD. These mice show working memory deficits at 6 months of age, a significant loss of neurons in various areas of the brain, such as the CA1 region of the hippocampus. Moreover they display a severe amyloid pathology. Using a NGS RNA-sequencing approach, we identified differentially expressed genes (DEGs) between 6-month-old APP/PS1KI and wild type or PS1KI . Compared to wild type mice, we found 250 DEGs in APP/PS1KI mice. Compared to PS1KI control mice 186 DEGs could be observed. The majority of DEGs were upregulated in APP/PS1KI mice and found to be either part of inflammation-associated pathways or involved in lysosomal activation pathways. This is likely the consequence of the robust intraneuronal accumulation of Aβ in this mouse model.

P-CANCER GENETICS

P-CancG-043

Relevance of SORBS2 in the process of cellular senescence

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Background:

Escape from senescence is a prerequisite for carcinogenesis. In a cell culture model for cervical carcinogenesis loss of genes located on chromosome 4q35→qter were found to be associated with escape from senescence. One of the identified and validated genes is SORBS2, which was down-regulated in cervical cancers as compared to precancers. Of interest is the novel finding that SORBS2 can induce senescence in primary human fibroblasts and keratinocytes, but not in "human papillomavirus" - immortalized cell lines. The relevance of this gene in the process of cellular senescence warrants further investigation.

Materials and Methods:

Primary human keratinocytes (n=10) and fibroblasts (n=10) were isolated from different foreskin donors. Assessment of the endogenous gene expression was done by qRT-PCR and by immunocytochemical staining at different culture passages. Cells undergoing senescence were identified by Senescence-associated beta-galactosidase as well as p16 staining. To assess the effect of SORBS2 on the life span of primary cells knock-down experiments were performed.

Results:

The endogenous expression of SORBS2 increases dramatically in primary keratinocyte and fibroblast cultures from different donors at near senescent passages. In parallel, increased p16 expression and

increased perinuclear beta-galactosidase staining pattern of enlarged and flattened cells were observed. Immunocytochemical analyses revealed that protein expression of SORBS2 is in line with the RNA data. The knock-down experiments are still on-going.

Conclusions:

In conclusion, our results provide further support that SORBS2 may contribute to senescence. We anticipate but have not yet proven that a knock-down of SORBS2 will result in a prolonged life-span of keratinocytes and fibroblasts in vitro. This would be a strong indication for a physiological role of this protein in the senescence process.

P-CancG-044

Analysis of potential splice site mutations in the BRCA2 gene and comparative assessment of detection methods

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Introduction: Since their discovery in 1994 and 1995, respectively, the breast cancer (BC) and ovarian cancer (OC) high risk genes *BRCA1* and *BRCA2* have been intensively studied by many research groups. Today, genetic testing is routinely offered to high risk families to assess risks to develop BC and/or OC. Nevertheless, even after 20 years of research/diagnostics on the *BRCA* genes, the classification of variants remains challenging for diagnostic laboratories. This is due to the fact, that many variants remain ambiguous regarding their functional impact and are extremely rare or even private in most cases. Thus, further investigation of those variants of unknown significance (VUS) of which many potentially affect the pre-mRNA splicing process is necessary.

Methods: Total RNA isolated from peripheral blood leukocytes was obtained from *BRCA2* mutation carriers. Analysis of 49 selected *BRCA2* variants was performed by transcript analysis (RT-PCR, agarose gel electrophoresis, Sanger sequencing, TapeStation analysis and capillary electrophoresis). The validity of *in silico* prediction programs MaxEntScan (MES) and HumanSplicingFinder (HSF) was evaluated based on the results obtained from *in vitro* analysis of these 49 variants. A comparison of three *in vitro* assays comprising agarose gel electrophoresis followed by Sanger sequencing, TapeStation analysis and capillary electrophoresis was performed.

Results: The analysis comprised of 29 intronic variants and 20 exonic variants revealed 10 intronic and 10 exonic variants with observed splicing effect. *In silico* predictions for intronic variants were in line with the *in vitro* results. However three exonic variants escaped the *in silico* prediction programs MES and HSF. Comparison of the three employed detection methods revealed different advantages and disadvantages in sensitivity and accuracy of each method. This study revealed agarose gel electrophoresis as a pragmatic method in detecting major splicing effects and CE as the most sensitive method.

Discussion: In this study four variants: c.8487G>T, c.426-6_438del, c.793+2T>G and c.8332-2A>G, with observed splicing effect were identified, which were not described in the literature yet. Classification of *BRCA2* variants which may influence the splicing process remains a challenging task during standard diagnostic procedures since prediction programs are not reliable and the *in vitro* splicing assays available differ in specificity and sensitivity. Results presented here showed a combined usage of agarose gel electrophoresis followed by Sanger sequencing and capillary electrophoresis assays, increase the number of variants, which can be classified. Nevertheless, for some variants without clear-cut results elaborate quantitative measurements including qPCR analysis or RNA-Seq are required for definite classifications.

P-CancG-045

Clinical and Prognostic Relevance of a 12-Gene Signature Associated with Genomic Instability in Breast Cancer

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To avoid over- or undertreatment and to reduce toxic effects of chemotherapy for breast cancer patients, it is of high impact to make an accurate diagnosis and estimation of prognosis. The 12-gene expression signature was previously identified from the expression profiles of 7,657 genes in 48 primary breast carcinomas by Habermann et al. (2009) and could be used to discriminate genomically stable from unstable

breast carcinomas independently from established clinicopathologic factors. The objective of this study was to validate the 12-gene signature's prognostic relevance at the protein level by immunohistochemistry and evaluate the association with clinical parameters.

The protein expression was semiquantitatively determined by immunohistochemical staining of a tissue microarray compromising 405 clinical formalin-fixed and paraffin embedded (FFPE) tissue samples (carcinoma, DCIS, benign neoplasia and normal tissue) from 245 patients. The samples were scored by a senior pathologist according to the percentage of the positively stained cells (score 0: < 1 %, score 1: ≤ 20 %, score 2: ≤ 50 %, score 3: > 50 % positive cells). Subsequently, statistical analyses were performed to evaluate associations with clinical parameters and patients' survival.

A total of eight target proteins was successfully evaluated by immunohistochemistry (AURKA, CDKN2A, FOXA1, NXF1, ACADSB, PSMD10, KIAA0882, and cMYB). After dichotomization of the expression, initial statistical analyses showed that patients with tumors of moderate and high PSMD10 expression had a significantly shorter median overall survival period (5.4 years vs. 10.3 years, $p = 0.013$) and a higher Hazard Ratio (HR = 1.71, 95 % CI 1.1 - 2.6, $p = 0.014$) compared to patients with no or low PSMD10 expression. Fisher's Exact Tests revealed significant associations between four markers (CDKN2A, FOXA1, ACADSB, and KIAA0882) and the clinical parameters grading, ER-status, PR-status, and molecular classification ($p < 0.05$).

In conclusion, eight out of twelve targets could be evaluated in 405 FFPE tissue samples. Significant associations between the targets' expressions and clinical parameters could be shown, whereas PSMD10 expression correlated with a worse prognosis. Further functional validation is warranted to confirm the clinical and prognostic relevance of the 12-gene signature.

P-CancG-046

Influence of decreased ZEB1 expression on apoptosis and DNA damage response in T-Cells and its role during the pathogenesis of Sézary syndrome.

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Sézary syndrome is a rare leukemic form of cutaneous T-cell lymphoma. During the last decades many studies revealed through comparative genomic hybridization (CGH) and next generation sequencing (NGS) analysis numerous genomic alterations, which are often associated with complex rearrangements. We hypothesize a major defect in DNA damage repair mechanisms to be responsible for this chromosomal instability. The zinc finger, homeodomain transcription factor ZEB1 was recently found to be involved in DNA damage response through Checkpoint kinase 1 (CHK 1). ZEB1 is known to be frequently deleted in Sézary syndrome. We analyzed the influence of this deletion on cell physiology, focusing particularly on DNA damage response.

To examine the functional consequences of reduced ZEB1 expression, CD4 T-cells from healthy donors and the T-cell lymphoma cell lines Jurkat and HuT 78 were treated with ZEB1 specific siRNAs. Knockdown efficiencies were verified by RT-PCR and Western Blot experiments. Annexin V/ 7 AAD double-staining was used for apoptosis detection. Furthermore a ZEB1 deficient Jurkat cell line was established through transduction of ZEB1 specific lentiviral shRNA constructs. DNA damage was induced by Etoposid, Camptothecin and UV-C light. The amount of DNA damage was analyzed through γ-H2A.X staining.

We could show that siRNA mediated ZEB1 knockdown does not lead to spontaneous apoptosis in healthy T-cells nor in the examined T-cell lines HuT 78 and Jurkat. It has also no influence on chemoresistance against Camptothecin and Etoposide.

Interestingly we were able to show an increased DNA damage rate after treatment with UV-C light, Camptothecin and Etoposide in Jurkat cells that were transduced with ZEB1 specific shRNAs compared to scrambled and non-transduced controls.

Jurkat cells with reduced ZEB1 expression were prone to DNA-damaging stimulants. Therefore the frequent ZEB1 deletion in Sézary syndrome might be involved in the accumulation of genetic alterations in those cells. These results provide a promising basis for further investigations on the underlying mechanisms.

P-CancG-047

Protein expression pattern discern sporadic from FAP-associated colon carcinomas at various stages of carcinogenesis

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Background: Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease with a germline mutation of the APC gene. In spite of this specific genetic alteration early diagnosis in young patients without polyposis onset and lack of family history can be difficult and finally letal. Thus, additional sensitive diagnostics are required. We aimed at identifying and validating a protein expression signature in macroscopically unaffected colon mucosa that allows identifying genetic carriers of the FAP-syndrome.

Material and Methods: Protein profiling by 2-D gel electrophoresis was performed on samples obtained from 15 different patients (FAP, n=8; sporadic colorectal cancer, n=7). Analysis was performed for normal mucosa, adenoma, and carcinoma while comparing FAP-associated tissue with the sporadic counterpart. Analysis aimed at identifying proteins that were expressed in FAP tissue but not in the corresponding sporadic tissue, comparing particularly FAP associated normal mucosa versus sporadic normal mucosa. Target validation was performed by Western and by immunohistochemistry on clinical samples (n=189), respectively.

Results: A total of 47 proteins were present in all macroscopically unaffected FAP mucosa specimens but absent in sporadic normal mucosa. Comparing FAP polyps with sporadic colonic polyps revealed 49 polypeptides being present in FAP samples but absent in all sporadic polyps. Comparing three FAP carcinomas with seven sporadic colorectal carcinomas yielded 66 proteins with absence/ presence expression pattern. CSTF2T and ACTB were validated by Western Blot and immunohistochemistry in unaffected colon mucosa of FAP patients.

Conclusion: The data obtained demonstrate specific differences of FAP and sporadic colorectal disease on the protein expression level and could help to identify patients with FAP disease already in macroscopically “normal” colorectal mucosa.

P-CancG-048

MALDI-imaging identifies overexpression of Thymosin beta-4 (TYB4) as a marker for aneuploid colorectal cancer

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Background: DNA aneuploidy has been identified as a prognostic factor for epithelial malignancies. In this study, we compared diploid and aneuploid colon cancer tissues against normal mucosa of the colon by means of matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS).

Material and Methods: DNA image cytometry determined the ploidy status of tissue samples that were subsequently subjected to MALDI-IMS. After obtaining protein profiles through direct analysis of tissue sections, a discovery and a validation set were used to predict ploidy and disease status by applying proteomic classification algorithms [Supervised Neural Network (SNN) and Receiver Operating Characteristic (ROC)]. Clinical target validation was performed by immunohistochemistry using tissue microarrays (TMA) comprising healthy controls as well as diploid and aneuploid colorectal carcinomas.

Results: SNN algorithm categorized 99% of normal mucosa and 90% of colon carcinoma as well as 99% of diploid and 94% of aneuploid colon cancers correctly. Validation of both comparisons showed a correct classification of normal mucosa in 92%, tumors in 96%, and diploid and aneuploid colon cancers in 92% and 78%, respectively. Five peaks (m/z 2,396 and 4,977 for the diploid vs. aneuploid comparison and m/z 3,375, 6,663, 8,581 for the normal mucosa vs. carcinoma comparison) reached significance in both SNN and ROC analysis. Among these, m/z 4,977 was identified as thymosin beta 4 (TYB4). TYB4 showed expression differences also in clinical samples using a tissue microarray of normal mucosa, diploid and aneuploid colorectal carcinomas and serve to predict overall survival.

Conclusion: Our data underscore the potential of MALDI-IMS proteomic algorithms to reveal significant molecular details from distinct tumor subtypes such as different ploidy types. Tβ-4 was validated in clinical samples using a tissue microarray to predict overall survival in colorectal cancer patients.

P-CancG-049

Impact of array CGH analysis in hematological neoplasms

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Chromosome banding analysis (CBA) in combination with FISH is the method of choice used in routine diagnostics in hematological neoplasms to establish diagnosis, determine prognosis and guide treatment decisions. In a subset of patients no karyotype abnormalities are detected based on these techniques. Thus, we asked the question whether array CGH (aCGH) can add useful information in these patients. We performed aCGH in the following groups of patients with a normal karyotype: AML (n=159), ALL (n=186), MDS (n=520), CMML (n=100), and CLL (n=136). In all entities abnormalities were detected by aCGH. The frequency varied between different entities and was highest in ALL (81%), followed by AML (24%), CMML (20%) and CLL (15%) and was lowest in MDS (11%). In most entities the majority of abnormalities detected by aCGH are not detectable by CBA due to the small size of the gained and lost regions. However, in ALL a substantial proportion of cases harbored abnormalities in principle detectable by CBA. In detail, 501 of a total 818 abnormalities (61%) detected by aCGH were larger than 10 Mb. They were missed by CBA due to insufficient in vitro proliferation of ALL blasts. 317/818 (39%) were smaller than 10 Mb and thus not detectable by CBA. In contrast, in AML only in 7/159 (4%) patients abnormalities (n=11) were identified by aCGH which in principle would be detectable by CBA. In MDS also only 9/520 cases (2%) harbored copy number alterations >10 Mb in size. In CLL in 12 patients (9%) 8 deletions and 6 gains were detected by aCGH that had a size of >10 Mb. In CMML only in one patient (1%) a deletion >10 Mb was observed. In all entities recurrent submicroscopic abnormalities were detected. In ALL these were losses of 9p21, 7p12, 14q32, 7q34, 12q21, 13q14, 21q22, 5q33, 9p13, 12p13 and 17q11, while no recurrent gain was observed. In AML 6q23 and 8q24 were recurrently gained while recurrent losses were found in 21q22, 5q31, 2q34, 7q22, and Yq11. In MDS recurrently deleted regions were 4q24, 2p23, 12p13, 17q11, 21q22, and Xq25 (deleted in 2 female patients) while no recurrent gains were observed. In CMML also no recurrent gains were detected while the following recurrent losses were found: 4q24 and 13q14. In CLL the following recurrent abnormalities were identified: deletions of 13q14, 1q42.12, 4p16.3, and 7p14 and gains of Xp22.31 and 3q26-28. In conclusion, arCGH provides additional genetic information in all hematological neoplasms analyzed. Submicroscopic recurrently deleted regions were identified in all entities. In ALL aCGH might has to be considered as a routine diagnostic method in cases with normal karyotype by CBA. In all other entities the spectrum of recurrent abnormalities is quite distinct. Thus, it can be discussed, whether a panel of FISH probes might serve as an alternative diagnostic approach. Further studies have to clarify the clinical impact of these abnormalities to provide a basis for changes in the current diagnostic approach.

P-CancG-050

Establishment, marker expression, isolation, and SNP array analyses of glioblastoma stem-like cells (GSCs)

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There is ample evidence of a very considerable level of heterogeneity among glioblastomas, more than listed in the current WHO classification system. The cancer stem cell (CSC) model has been proposed as an alternative to explain tumor heterogeneity. Glioblastoma stem-like cells (GSCs) are described to be responsible for tumor initiating, maintaining, chemo- and/or radiotherapy resisting and recurrence due to the stem cell features they carry. The genetic and genomic data of this small group of cells are still limited. Therefore, genetic analyses and genomic profiling should be carried out in further investigations of GSCs. The aim of this ongoing study is to establish, characterize and isolate the GSCs, combined with genetic analyses.

Fresh primary glioblastoma tumor tissue and peripheral blood were obtained from patient. Tumor tissue derived explant cell culture (supplemented with serum) and serum-free culture (supplemented with serum-free supplements and growth factors) were established. From the serum-free culture, cell subpopulations

were isolated by two stem cell markers CD15 and CD133 through multi-parameter magnetic-activated cell sorting (MACS) technique. The tumor tissue, blood, serum-free culture, marker-positive cells and marker negative cells were sent for SNP array analyses.

We have successfully established serum-free culture of five tumor specimens. GSCs in the serum-free culture were preliminarily characterized by the expression of stem cell markers: Nestin, SOX2, and CD133. We established the particular protocol for the separation of the stem cell marker positive and negative cells from the serum-free culture by CD15 and CD133. The result of isolation was confirmed by Flow Cytometry. The following SNP array analyses showed unique genetic profile (tumor tissue and cell subpopulations, e.g. gain of chromosome 7, loss of 10q23.31, loss of 10q11.1->q26.3, and complete loss of chromosome 10), such as distinct genetic differences between the cell subpopulations and tumor tissue (e.g. loss of chromosome 4, and segmental uniparental disomy of 9p24.3->p21.3, only in cancer stem- like cell subpopulations).

By increasing the markers for isolation of GSCs, we were able to compare GSCs and related tissues more specifically. The finding of genetic aberrations on GSCs in comparison with tumor tissue and blood can help to further understand the tumor heterogeneity of glioblastoma and may be of help to identify novel potential targets for therapy development.

P-CancG-051

BRCA1/BRCA2 carrier status in patients with triple negative breast cancer from the Regensburg Center for Hereditary Breast and Ovarian Cancer

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Triple negative breast cancers (TNBC) (i.e. estrogen-receptor-negative, progesterone-receptor-negative, and human epidermal growth factor receptor 2-negative) comprise approximately 15–20 % of all breast cancers. Up to 20 % of these are reported to be associated with mutations in BRCA1 and to a minor extend with mutations in BRCA2. Thus, it needs to be assessed whether genetic counseling and germline BRCA mutation testing should be offered to women with TNBC.

Between 2011 and 2015 a total of 303 unrelated patients, who met the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer, underwent genetic counseling at the Center in Regensburg. Molecular testing of BRCA1 and BRCA2 was completed in 229 patients. Of these, 186 patients had developed breast cancer (BC). Histological data of the tumor was available in 156 patients. TNBC was diagnosed in 25 patients (16 %) with a mean age of 40,4 years (range27-58). In 3 of the 25 patients (12 %), TNBC was diagnosed below 30 years, in 11 (44 %) between 30-39 years of age, in 6 (24 %) between 40-49 years and in 5 patients (20 %) beyond 50 years of age (51-58 years).

A pathogenic mutation in the BRCA1 gene was detected in 12 of the TNBC patients (48%). One mutation carrier was affected with TNBC with 27 years, 4 patients developed BC between 30-39, three patients at the age of 40-49 years and 4 women at the age of 51 years and older. A pathogenic BRCA2 mutation was detected in one BC patient at the age of 36 years and an unclassified BRCA2 variant was found in a 49 year old BC patient. In the remaining 11 patients (44%), no mutations in BRCA1 or BRCA2 were detected. Five of the TNBC patients (20%) had a history of a second cancer. Three of them developed contralateral breast cancer, one patient suffered from ovarian cancer and one from cervical cancer. Three patients (12%) with TNBC had no family history of breast and ovarian or other cancer types. In 1 of these patients, a pathogenic mutation in BRCA1 was found. In 2 of them, no mutation in BRCA1 and BRCA2 was detected. The other 22 patients showed a family history of breast and ovarian cancer as well as prostate cancer and malignant gastrointestinal tumors.

Among our TNBC cases, the prevalence of BRCA mutation carriers exceeded 48%. This percentage is higher than the average detection rate of 25% in breast and ovarian cancer families. If this data may be confirmed in a larger cohort, the inclusion criteria for genetic testing of the BRCA1 and BRCA2 genes need to be adjusted.

P-CancG-052

Are hyperhaploid uterine mesenchymal tumors a novel genetic subgroup?

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Hyperhaploid karyotypes have been described to occur in subsets of various solid tumors and leukemias. By definition, in these lesions with 24-34 chromosomes monosomy is noted for most of the

chromosomes while a few chromosomes still remain disomic. At least in some tumor entities these remaining chromosomes seem to be non-randomly selected. Secondary duplication of the chromosome complement as well as structural alterations accompanying the reduced chromosome number are frequent findings.

Here we report three cases of of hyperhaploidy in one uterine leiomyosarcoma, endometrial stromal nodule, and epithelioid uterine leiomyoma, each. All three cases were detected by using CNV arrays to characterize rare uterine tumors displaying at lest areas with smooth muscle differentiation. In addition to the hyperhaploidy, the epithelioid leiomyoma presented with uniparental disomy for most of the chromosomes likely resulting from the duplication of the initially hyperhaploid chromosomal complement.

We feel that these cases are of particular interest because despite histological differences, all three tumors showed considerable genetic similarities. Furthermore, these cases consider the existence of a novel genetic subgroup of hyperhaploid uterine mesenchymal tumors with varying histologic differentiation. It seems tempting to speculate that the histologic differences may depend on the presence of additional genetic abnormalities.

P-CancG-053

DigitalMLPA: A Revolution in the detection of large rearrangements in hereditary breast cancer

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Background

Multiplex ligation-dependent probe amplification (MLPA) is the most commonly used technique for the detection of small and large rearrangements in human genes. Since first introduction MRC-Holland developed many assays for the analysis of human genes causing hereditary diseases. The standard format of conventional MLPA containes about 40 target regions and several controls and resulting PCR products are separated by capillary elektrophoreses. Now MRC-Holland developed a new set up called digitalMLPA with which 27 genes predisposing to cancer can be analysed simultaneously. In a single reaction 589 MLPA probes are amplified and amplicon quantification is performed by Illumina Sequencing Systems (e.g. MiSeq, NextSeq). Genes covered include BRCA1, BRCA2, ATM, RAD51C, RAD51D, ChEK2, TP53, CDH1, NBN and PALB2 which are the core genes causing breast cancer and which are analysed by genetic centers belonging to the German Consortium for Hereditary Breast and Ovarian Cancer.

Methods

To evaluate digitalMLPA we compared results from our standard MLPA with those from digitalMLPA for detection of large rearrangements in BRCA1, BRCA2, CHEK2 genes and for the c.1100delC CHEK2 mutation.

In total, 100 german breast cancer patients from our center underwent screening using a digitalMLPA to detect large rearrangements in BRCA1, BRCA2 and CHEK2, followed by confirmation by standard MLPA. We identified in these patients various BRCA1 copy number variations (e.g. deletion of exon 8, exon 17, exon 22, duplication of exon 13), deletion of exon 9 and 10 in CHEK2 and the c.1100delC CHEK2 mutation. In addition we identified several deletions/duplication in additionally tested genes. At the moment we validate these deletions/duplications by qPCR.

Results

Digital MLPA results were 100% concordant with the previous results of standard MLPA. Our data demonstrate that results of digital MLPA are valid and reproducible.

Conclusion

We conclude that digital MLPA can be used for screening in hereditary breast cancer as a fast and safe alternative method to standard MLPA and offers additional possibilities to screen for small and large rearrangements in human genes.

P-CancG-054

Identification of GPRC5A as a novel risk factor for the triple negative tumor phenotype in BRCA1 and BRCA2 mutation carriers

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The *GPRC5A* gene encodes an orphan G-protein coupled receptor highly expressed in lung tissue. Tao et al. (2007) reported a high susceptibility to develop spontaneous lung adenomas and adenocarcinomas in *Gprc5a* knockout mice suggesting a role in cancer predisposition. Within a consanguineous breast cancer (BC) family of Turkish origin, we identified a homozygous frameshift mutation (c.183delG; p.R61Sfs*59) in the first coding exon of the *GPRC5A* gene in two affected sisters (disease onset 26y and 33y, respectively) via exome sequencing and subsequent candidate verification via linkage analysis, suggesting an autosomal recessive trait. Interestingly, Sokolenko et al. (2014) reported a ten-fold increase of the heterozygous *GPRC5A* p.R61Sfs*59 mutation in *BRCA1* c.5266dupC BC patients (5.1%; 6/117) compared to *BRCA1*-negative BC patients (0.5%; 8/1,578). Thus, we hypothesized that *GPRC5A* could act as recessive BC gene and a disease modifier when heterozygously inactivated. While the search for homozygous or compound heterozygous *GPRC5A* mutation carriers is ongoing (international SEARCH study), we genotyped the p.R61Sfs*59 mutation in a large series of *BRCA1/2* mutation carriers (n=1,841), *BRCA1/2*-negative familial cases (n=5,841) and control individuals (n=8.305). A weak association was found in *BRCA1/2*-positive cases (17/1,519, CF=1.12%), but not in *BRCA1/2*-negative cases (40/5,841, CF=0.69%) versus control individuals (71/8,305, CF=0.86%). The detailed analysis of the *BRCA1/2* mutation carrier cohort, consisting of 1,282 BC, 131 ovarian cancer (OC), 106 BC/OC patients and 322 healthy mutation carriers, revealed that the 17 *GRPC5A* mutation carriers solely coincide with the BC subgroup (CF=1,33%). This results in an estimated relative risk of 1.251 (95%CI=0.969-1.255; p=0.037) for patients carrying a pathogenic mutation in *BRCA1* or *BRCA2* and the *GPRC5A* frameshift mutation. Remarkably, the mutation was particularly frequent in *BRCA1/2*-positive triple negative BC cases (11/540, CF=2.04%) and presented a statistically significant association with this BC subtype (odds ratio: 2.362; 95%CI=1.177-4.627; p=0.007). In summary, we provide evidence for *GPRC5A* p.Arg61fs as a disease modifier in *BRCA1/2* mutation carriers, especially in triple negative BC cases. Based on this data, further studies have been initiated to identify additional *GPRC5A* mutation carriers by analyzing 30.000 *BRCA1/2* mutation carriers and 80.000 mostly sporadic BC cases (OncoArray GWAS). Functional Analyses to elucidate the role of *GPRC5A* p.R61Sfs*59 in BC pathogenesis are in progress.

P-CancG-055

Panel sequencing in familial Breast and Ovarian Cancer identifies multiple novel mutations in genes others than BRCA1/2

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Breast and ovarian cancer (BC/OC) predisposition has been associated with a number of high- and low-penetrance susceptibility genes. Advances in sequencing technology has made multigene testing a practical option when searching for genetic variants that may be associated with a risk for BC/OC. We studied 633 patients fulfilling criteria for BRCA1 and 2 testing including 139 with triple-negative tumor (TNBC) using the next generation sequencing based TruSight Sequencing Cancer Panel kit on a MiSeq platform (Illumina). After remapping with BWA to hg19 (GRCh37) data was analyzed using SeqNext software (JSI) for variants in 16 known high and moderate penetrance susceptibility genes (BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6, PMS2, PTEN and STK11). Variants were classified as deleterious if they were previously published as pathogenic, resulted in a premature termination codon or affected the conserved splice site regions. Variants outside the consensus splice sites were further

characterized by RT-PCR analyses. Using this algorithm, 101 deleterious variants were identified in 100 patients (16%). Additionally, 104 variants of uncertain significance (VUS) were identified in 92 (14%) of all patients, including 9 (1.4%) patients with two or more VUS.

A deleterious BRCA1/2 mutation was found in 9.6% (n=61) of all patients and in 13% (n=18) of TNBC patients, respectively. Of these, 15 truncating mutations were not reported in BIC database or other BRCA1/2 associated databases, so far. A total of 6.2% of all patients (n=39) and 3.6% (n=5) of patients with TNBC were found to have a deleterious mutation in a different gene, the most frequently mutated being CHEK2 (n=11 ; 1.7 %), PALB2 (n=7 ; 1.1 %), RAD51C (n=6 ; 0.9%), NBN (n=6 ; 0.9%) and ATM (n=5 ; 0.8%). Whereas in CHEK2 and NBN the most commonly found mutations were the CHEK2 moderate risk allele c.1100delC (n=5) and the NBN low risk allele c.657_661del5 (n=4), respectively, all deleterious PALB2 mutations were found only once. 4 out of 7 were unpublished, including the mutation c.109-12T>A, which inserts a new splice acceptor side 10bp upstream as shown by RT-PCR. In recent literature PALB2 is still discussed as moderate penetrance gene. However, the average age at diagnosis in these carriers was 45 years (35-50). All but one with an isolated TNBC had a strong positive family history with two or more affected relatives and/or with at least one first degree relative being affected before the age of 50 years. If confirmed, this would indicate that penetrance for PALB2 mutations is higher than previously thought. In conclusion, panel testing yields greater information about cancer risk in families than assessing BRCA1/2 alone. Overall, we identified a deleterious mutation in 16% of patients compared to 9.6% of patients with a BRCA1/2 mutation. However, some genes included in panel tests do not yet have established guidelines for further treatment, requiring further collaborative work.

P-CancG-056

Genome editing of a Fanconi anemia core complex gene by the CRISPR/Cas system

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Fanconi anemia (FA) is a rare autosomal or X-linked disease which displays a heterogeneous phenotype. Due to a DNA repair defect the cellular phenotype is marked by hypersensitivity to DNA-crosslinking agents, leading to increased chromosomal breakage, reduced cell survival and G2-phase arrest during cell cycle. Currently, 19 FA genes whose products are members of the FA/BRCA pathway were reported to be causative for FA. The crucial step in the pathway is the monoubiquitination of FANCD2 and FANCI by the FA core complex. This complex consists of seven FA proteins (FANCA, -B, -C, -E, -F, -G, and -L) as well as two FA-associated proteins (FAAP20, FAAP100). Furthermore, it is subdivided into three subcomplexes. One of these is the catalytic module FANCB-FANCL-FAAP100 which includes the E3 ligase FANCL. Hitherto, five individuals are reported with pathogenic mutations in FANCL and around 30 FA-B patients are known. FAAP100 has not yet been found to be mutated in FA patients and is therefore still a candidate gene for FA.

In order to investigate if mutated FAAP100 cells possess a cellular phenotype similar to FA core complex mutant cells we used the recently discovered RNA-guided CRISPR/Cas9 system to generate a knockout cell line. By employing this technology double-strand breaks (DSBs) at a specific genomic target sequence are generated. Subsequently, the activation of the cellular DSB repair machinery will result in DNA restoration either through error-prone non-homologous end joining (NHEJ) or through error-free homology-directed repair (HDR). NHEJ may produce insertions or deletions (indels) that result in reading frame shifts and potentially in premature stop codons.

To disrupt the FAAP100 locus we used two different sgRNAs which were cloned into a pSpCas9 vector. HEK293T cells were transfected with these two different constructs by Lipofectamine® 3000 (Invitrogen™), respectively. Isolation of clonal cell populations was performed by serial dilutions in 96-well plates. Subsequent verification of FAAP100 mutations in single cell lines was conducted by Sanger sequencing. In summary, we could generate three independent cell lines with indel mutations in FAAP100. All mutations result in frameshifts and in premature stop codons. The resulting cellular phenotypes will be examined in functional experiments including immunoblots, cell cycle analysis and survival assays. These results will be compared to properties of FA core complex mutant cell lines and thereby allow a statement about a potential FAAP100 function in the FA/BRCA pathway.

P-CancG-057

Ploidy status predicts overall survival in patients with breast cancer

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Genomic instability is associated with poor survival in almost all solid cancers. For breast cancer, several studies indicate an association between ploidy status and overall survival of patients. This suggests a potential role of the ploidy status as a prognostic biomarker in breast cancer. Therefore, the aim of our study was to examine the impact of genomic instability on disease progression, recurrence and overall survival of patients with breast cancer.

As a measure of genomic instability, the ploidy status had been analyzed in tumor samples from a cohort of almost 6,000 breast cancer patients with clinical follow up data of at least 10 years, collected in collaboration with the Karolinska Institutet, Stockholm. A subset of 200 patients was included in our statistical analyses: 100 patients with metastasis compared to 100 age- and stage-matched patients without metastasis. The ploidy status was categorized as di-, tetra- or aneuploid.

Statistical analyses showed that the patient survival correlates significantly with the ploidy status of the tumor. While hormone receptor negativity correlates with a significant higher rate of aneuploidy than hormone receptor positivity, no association with menstruation state was observed. Against this background, the overall survival of hormone-receptor-negative and –positive patients was evaluated separately. In this analysis, the overall survival in both subgroups still showed significant association with ploidy status.

These results will be validated by analyzing ploidy data of all 6,000 patients. In addition, associations with further clinical data including treatment regimens of the patients will be performed. Taken together, our results confirm a correlation of ploidy status with survival of breast cancer patients and hence support a role for ploidy status as a prognostic biomarker.

P-CancG-058

Exome sequencing identified potential causative candidate genes for hyperplastic polyposis syndrome

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Purpose: Hyperplastic polyposis syndrome (HPS), also known as serrated polyposis syndrome (SPS), is a still poorly defined colorectal cancer (CRC) predisposition characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. A serrated polyp-CRC sequence (serrated pathway) has been postulated; however, to date, only few molecular signatures of serrated neoplasia (*BRAF*, *KRAS* mutations, CpG Island Methylation, microsatellite instability) have been described in a subset of HPS patients and neither the etiology of the syndrome nor the distinct genetic alterations during tumorigenesis have been identified.

Methods: To uncover predisposing causative genes, the exomes of 31 clinically well characterized HPS patients (27 unrelated index patients with sporadic appearance and 2 presumed familial cases, each with one additional affected relative) were sequenced (Illumina HiSeq platform) using leukocyte DNA. The variants were filtered for rare (homozygous/compound heterozygous: MAF≤ 1%, heterozygous: 0.01% according to dbSNP, EVS, and ExAC), truncating (nonsense, frameshift, highly conserved splice sites), and missense germline mutations (predicted to be pathogenic by at least 2/3 prediction tools) assuming a monogenic disease model. For data analysis and variant filtering the GATK software and the Cartagenia Bench Lab NGS Software were applied. In a first preliminary analysis, we focused on known cancer genes and candidate genes which are assumed to be involved in molecular and cellular functions relevant for tumorigenesis.

Results: After stringent filtering steps, comparison with large datasets from population-based controls, and detailed manual inspection of the variants, potentially biallelic variants were found in 289 genes. However, none of the known cancer genes contained a biallelic mutation that met the filter criteria. All in all, 943 genes harbored heterozygous mutations in at least two patients, nine of which are cancer genes (*ATM*, *CHEK2*, *ERCC4*, *JAK2*, *KIAA1549*, *MET*, *NUP214*, *RNF43*). *ATM* and *RNF43* harbored recurrent mutations in two patients each. Each patient had variants in several cancer or candidate genes (mean: 7, range: 4-11).

Conclusions: Preliminary data indicate that exome sequencing might identify potentially relevant genes for HPS, some of which are recurrently mutated. However, the number of variants per patient is also in line with a more oligogenic etiology of polyp predisposition. The current work-up includes the inclusion of non-cancer genes, validation of variants by Sanger sequencing, testing of relatives to determine the phase of assumed biallelic variants and segregation with the phenotype where applicable.

P-CancG-059

Identification of DNA methylation based biomarkers in rectal cancer for early disease detection

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DNA methylation has been identified as regulator of the transcription machinery with a link between aberrant DNA methylation and a variety of diseases – especially cancer. Tumour-specific DNA methylation signatures have emerged as promising targets for biomarker development for several malignancies including rectal cancer and will be of use for early detection and prognosis of cancerous diseases and for therapy response prediction. Rectal Cancer comprises ~30% of colorectal cancers (CRC) and is characterized by its specific anatomic localization. Aberrant DNA methylation is more prominent in proximal compared to distal colorectal cancers. However, already a number of methylation markers were identified for colon cancer, yet few are available for rectal cancer. Therefore, DNA methylation differences of patients with rectal cancer were assessed by a targeted DNA-microarray for 360 marker candidates. We investigated 22 fresh frozen rectal tumour samples and 8 controls and validated the findings by microfluidic high-throughput and methylation sensitive qPCR in fresh frozen tumours (n=18), fresh frozen adjacent tissue (n=18), PBMCs (n=8) as well as in an independent sample cohort of 129 formalin fixed paraffin embedded (FFPE) tissues, respectively. We identified and confirmed two novel 3-gene signatures that can distinguish tumours from adjacent tissue (TMEFF2, PITX2, TWIST1) as well as from blood (TFPI2, DCC, PTGS2) with high sensitivity and specificity of up to 1 and an AUC of 1. Sensitivity, specificity and AUC in FFPE were up to 0.92, 0.85 and 0.91, respectively.

Therefore these methylation signatures could be of value for minimal invasive and early diagnostics of rectal cancer.

P-CancG-060

Functional investigations of RUNX1 variants to guide clinical decision making in familial leukemia

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Haploinsufficiency and dominant negative variants of *RUNX1* cause autosomal dominant familial platelet disorder with associated myeloid malignancies (FPDMM). This platelet disorder is characterized by thrombocytopenia, prolonged bleeding, and impaired aggregation. Associated leukemias are mainly of myeloid origin, however, sporadically T-cell leukemias are also observed. The mutational spectrum includes point mutations, mainly within the highly conserved RUNT domain, and deletions affecting single exons or the entire *RUNX1* gene. By drawing a clinical utility gene card for FPDMM, we recently started an interdisciplinary initiative to develop guidelines for the care and surveillance. Here, we exemplarily report on a family with FPDMM and childhood MDS to illustrate the future utility of functional assays for the characterization of variants of unknown significance.

Due to progressive cytopenia in a 5-year-old boy diagnosed with MDS (RAEB), haematopoietic stem cell transplantation (HSCT) was scheduled. Screening for potential familial donors led to the detection of a mild thrombocytopenia and platelet dysfunction in the father. There was no bleeding history or leukemia reported in the family. FPDMM was suspected and *RUNX1* investigations were initiated. Using a custom made eArray to detect intragenic copy number changes in genes associated with familial leukemia, relevant copy number changes of the *RUNX1* locus were excluded. By Sanger sequencing an unclassified heterozygous missense variant (c.350T>A; p.Leu117Gln) was identified both in the patient and his father. In the healthy 3-year-old sister not carrying the missense variant Leu117Gln, investigations identified another unclassified variant

within the RUNT domain (c.178G>T; p.Gly60Cys), which was of maternal origin. Due to progressive cytopenia in the patient and no other available donor, the sister's cells were used for HSCT. The patient has full donor chimerism and good hematological recovery. In silico tools (SIFT and MutationTaster), predicted that both variants affect function. For functional evaluation, HEL cells were cotransfected with an *NR4A3*-promoter luciferase reporter construct and pcDNA3.1 vectors expressing *RUNX1* wild type or one of the missense variants. In accordance with the clinical phenotype, the Leu117Gln variant showed significantly reduced induction of the *NR4A3* reporter. In contrast, no reduction was seen for the Gly60Cys variant compared to the wild type protein.

Further investigations are required to address the clinical relevance of both missense variants. We are presently developing iPS-cell-based differentiation assays and FACS-FRET-based techniques to characterize FPDMM-associated *RUNX1* variants. Since *RUNX1* is known as a master regulator of human hematopoiesis that is frequently mutated also in sporadic leukemias, investigations of FPDMM-associated *RUNX1* variants can help to further characterize hematopoiesis and leukemic transformation.

P-CancG-061

Reanalysis of FANCD2 pseudogenes and customized regional enrichment strategies improve FANCD2 mutation identification

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Fanconi Anemia (FA) is a rare genetic disease characterized by progressive bone marrow failure, cancer predisposition and a variety of congenital malformations. Gene products of nineteen genes (FANCA, -B, -C, -D1/BRCA2, -D2, -E, -F, -G, -I, -J/BRIP1, -L, -M, -N/PALB2, -O/RAD51C, -P/SLX4, -Q/ERCC4, -R/RAD51, -S/BRCA1, -T/UBE2T) function together in the FA/BRCA DNA repair pathway that is essential for maintaining genomic stability. A central event is the monoubiquitination of the FANCD2/FANCI heterodimer by a multisubunit complex consisting of eight FA proteins which in turn activates downstream effectors and initiates DNA repair. Only approximately 3% of FA patients belong to subtype D2. Two reported pseudogenes that show extensive sequence similarity to the functional gene are held responsible for difficult genomic mutation detection in this key member of the FA/BRCA pathway (Kalb et al, 2007). Here, we re-evaluated pseudogene regions of the FANCD2 gene and used pertinent information for mutation analysis in 12 new FA-D2 patients from 10 different families.

We used an automated pseudogene identification pipeline (PseudoPipe) to identify pseudogene regions and Clustal Omega for both sequence alignments and similarity analyses. FANCD2 immunoblotting assigned patient cell lines to complementation group FA-D2. Mutations in the FANCD2 gene were either identified by classical Sanger sequencing or by customized enrichment panel and next-generation sequencing (NGS) approaches. Predicted effects of potential splice site mutations were verified by cDNA sequencing.

We were able to discern five individual FANCD2 pseudogenes that are located on the plus strand of the same chromosome (chr. 3) in proximity to the functional gene. They cluster in two discrete regions up- and downstream of FANCD2 spanning a genomic region of altogether about 1.9 Mb. Sequence similarities to the active gene ranges from 93.48% to 90.89%. This information facilitated FANCD2 sequencing of patients' genomic DNA and revealed one small deletion and 12 distinct single base pair substitutions. Six of them affect canonical splice sites and result in aberrant splicing. Nine mutations of our cohort are private whereas four are recurrent that have been observed repeatedly in other FA-D2 patients or represent founder mutations in patients of Turkish and Asian origin.

Extended sequence similarity between FANCD2 pseudogenes and the functional gene may mislead sequence analysis, in particular when using commercial enrichment kits for NGS. Customized enrichment and appropriate filtering defaults allow for discrimination of pseudogene sequence admixture from recurrent mutations. This resulted in the identification of all authentic FANCD2 mutations. Some results from previous studies are confirmed: The clustering of FANCD2 pseudogenes, a high proportion of splicing mutations, the presence of recurrent and founder mutations, and the proneness of this gene to revertant mosaicism.

P-CancG-062

Aberrant DNA methylation in the diagnostics of lung cancer

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DNA methylation is a reversible enzymatically introduced DNA modification controlling gene activity and DNA accessibility. Altered DNA methylation patterns e.g. induced by adverse environmental or lifestyle factors (e.g. exposure to tobacco smoke, asbestos or air pollution) are a hallmark of malignant tumors including lung cancer. Therefore, this study aimed at identifying alterations in the DNA methylation pattern of various lung cancer entities and to subsequently validate their putative diagnostic value in both surgical samples, limited material collected during bronchoscopy as well as liquid biopsies.

As reported previously we applied a 450k BeadChip approach to 40 surgical lung cancer specimen and the corresponding controls to identify ~900 loci (~370 genes) aberrantly methylated in lung carcinomas as compared to controls (FDR < 1 x 10-23, σ/σmax > 0.4). Moreover, entity specific DNA methylation was detected, differentiating between adeno- and squamous carcinoma of the lung, the most predominant histological subtypes of non-small cell lung cancer.

Subsequently, paired biopsies (n=80), which were collected during bronchoscopy, were used for data validation. Additional data validation has been performed using bisulfite pyrosequencing.

Furthermore, we performed Whole Genome Bisulfite Sequencing of cfDNA, isolated from plasma of patients suffering from adenocarcinoma (n=5) and squamous carcinoma (n=4) to detect entity specific DNA methylation patterns. Asthma patients (n=20) were used as a control group. For data validation MS-PCR of selected selected loci has been performed.

Our results suggest that DNA methylation analysis might become a valuable diagnostic tool for diagnostics of lung cancer.

P-CancG-063

Spike-in of LR-PCR products: avoiding pseudogene detection in next generation sequencing.

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The human genome contains at least 14447 pseudogenes (GENCODE, March 2015 freeze, GRCh38) and many more regions that are highly homologous to smaller parts of coding genes, e.g. *BRCA1*. Mainly due to the still – compared to Sanger sequencing – relatively short read length and the preferred hybridisation-based enrichment method for larger panels or whole exome sequencing, the proper analysis with next generation sequencing (NGS) of genes for which homologous regions exist can be very difficult or impossible.

One of those genes is *CHEK2*, for which several pseudogenes with up to 98% homology for the exons 11-15 are known (Sodha et al. 2000 Science). Mutations in this gene, like the founder mutation 1100delC, moderately increase the risk of developing breast cancer (Zhang et al. 2011 Lancet Oncol) with a lifetime risk of approximately 25% (Narod et al. 2010 Clin Genet).

To circumvent the above-mentioned shortcomings, *CHEK2* exons 11-15 were amplified by a specific long-range PCR (LR-PCR) and spiked into genomic DNA prior to library preparation (TruSight Rapid Capture, Illumina). While library preparation gDNA and the added LR-PCR product are fragmented by tagmentation, the excess of fragments derived from LR-PCR inhibit the binding of oligonucleotide probes to the *CHEK2* pseudogenes, resulting in less contamination by pseudogene sequences. Without addition of LR-PCR products prior to library preparation, up to 40% of reads in exon 11, which harbours the 1100delC mutation, originate from pseudogenes. In three positive test sample (heterozygous *CHEK2* 1100delC mutation), the mutation was called only in approximately 19% of the total reads. With the addition of LR-PCR products, >45% of all reads exhibit the 1100delC variant and overall reads derived from pseudogenes in exons 11-15 could be decreased from 30-65% (depending on the region) to <1.5%. Therefore, the described method can be used to analyse genes with known pseudogenes like *CHEK2* by using the "normal" library preparation without the need to do additional time-consuming sequencing of the LR-PCR products by Sanger sequencing.

P-CancG-064

Rare pathogenic mutations causing Hereditary Breast and Ovarian Cancer beyond diagnostic genes.

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Hereditary Breast and Ovarian Cancer (HBOC) is mainly caused by pathogenic mutations in BRCA1 and BRCA2. Though several additional genes were linked to HBOC, only few are part of current diagnostic genetic testing. Here we present data from 575 patients with family history suspicious for HBOC that underwent genetic testing at our institute between May 2013 and May 2015. Next-Generation-Sequencing combined with a panel of 68 genes was applied and analyzed for each patient. Pathogenic mutations were validated using Sanger sequencing. The present study shows the results of all but the diagnostic genes (TP53, ATM, NBN, PALB2, CDH1, CHEK2, RAD51C, RAD51D, and BRCA1and BRCA2). In total 2190 variants were detected. All variants were filtered according to frequency (MAF<1% in common databases), mutation effect (coding) and in-house database. The remaining 987 variants were classified using a 5-class system (Plon et al 2008). In 4.7 % of patients pathogenic and likely pathogenic mutations (class 4/5) were found in genes other than the diagnostic genes including: FANCA (5 mutations), FAM175A (2 mutations), BARD1 (3 mutations), MRE11A (2 mutations), RAD51B (1 mutation), BRIP1 (1 mutation), and MUTYH (12 mutations). Co-segregation of these variants with cancer within families is examined as well as cDNA analysis for splice site variants to provide evidence for pathogenicity of the variants. Our data suggests that pathogenic mutations can be found in genes other than standard diagnostic genes and that these mutations may be of relevant to the families.

P-CancG-065

New insights in the mutation spectrum and the genotype-phenotype relationship of patients with paraganglioma (PGL) / pheochromocytoma (Pheo) syndrome

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Objectives.

Aim of the study was to design and evaluate an NGS panel to offer extensive, time and cost efficient genetic testing to PGL/Pheo patients (up to 40% suffer from an autosomal dominant syndrome) and expand knowledge on genotype-phenotype correlations. Genetic testing of the well-known susceptibility genes SDHA, SDHB, SDHC, SDHD, SDHAF2, MAX, TMEM127, RET, VHL and EGLN1 was enlarged by sequencing of EGLN2, NF1, EPAS1, KIF1B, BAP and FH.

Methods.

A total of 488 samples were subjected to targeted resequencing of 16 PGL/Pheo susceptibility genes. 70 unrelated, clinically evaluated patients, diagnosed with PGL, Pheo or medullary thyroid cancer (MTC) carrying 55 different mutations (57 substitutions, 7 small deletions, 6 CNVs) in one of the susceptibility genes SDHA, SDHB, SDHC, SDHD, SDHAF2, MAX, TMEM127, RET, VHL and EGLN1 which were identified by Sanger sequencing or MLPA (positive controls “mut”); 177 PGL-/phaeo/MTC-patients with negative Sanger sequencing and MLPA results for the above- mentioned genes (“PP”) and 124, partly also affected by PGL or phaeo, relatives of “mut” originating from 34 different families (“FU”) which were previously investigated for the index- mutation only. In addition, we sequenced 5 “negative controls” composed of four MEN1 mutation carriers and one sample being a control for pseudogene amplification of SDHA. One supplementary wild type sample was used for internal control purposes. A hybridization-based targeted resequencing approach capturing exons, splice- sites and untranslated regions (UTR´s) of the 16 candidate genes was carried out on an Illumina MiSeq instrument. Bioinformatic analyses were performed combining the output of NextGENe® with in-house PERL scripts. New results were confirmed by Sanger sequencing.

Results.

NGS resulted in an average of 97% matched reads with a mean coverage of 572 reads in ROI/sample. Approx. 2 interesting variants/patient were defined by the bioinformatic procedure and further evaluated. All expected mutations in the “mut” collective were detected. In the “PP” collective 6 probably disease-causing and 4 known mutations were identified. Genotyping of the family members reveals 8 additional mutations. Though assumed to be already completely characterized, 8 further known mutations/most likely pathogenic variants were found in the “mut” group, suggesting the existence of disease- causing mutations on the one and penetrance- affecting variants on the other hand. Sequencing of tumor DNA in case of germline-negative patients and LOH analysis in tumor samples is still pending.

Conclusion.

Further research is necessary to establish the significance of 3 mutations in PGL/Pheo patients. NGS is not only reliable, time and cost efficient, and facilitates extensive genotyping, it may also help to identify patients with a particularly high disease risk.

P-CancG-066

Unclassified pleomorphic sarcoma in the shoulder of a patient with a PTEN-germline mutation

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PTEN hamartoma tumor syndrome (PHTS) or Cowden Syndrome is an autosomal dominant hereditary cancer syndrome causing increased risk for breast, thyroid, renal, uterine, and other cancers as well as benign neoplasias and is also associated with neurodevelopmental delay.

We report the first case of a PHTS patient with an unclassified pleomorphic sarcoma in the shoulder. The 28-year old patient sought genetic counseling after having multiple symptoms in different body regions. At the age of 24 years the patient developed an unclassified pleomorphic sarcoma in the right shoulder. Since adolescence he has been diagnosed with Scheuermann's disease. Starting at age 18 the patient developed multiple axillary and inguinal fibromas which have increased in number and size over the years. Several nodules can be found the mucosa of the mouth. The patient presents with facial although histologically not proven trichillemmomas on the nose. Additionally the patient has a hyperpigmentation of the penis. His head circumference is 63 cm and therefore above the 97th percentile.

Several hot thyroid nodules were detected using scintigraphy. A large tumor was identified behind the thyroid gland but it remains steady in size and has not been removed yet. The levels of thyroid and parathyroid hormones remain normal. Finally, a colon adenoma has been removed.

His father has been diagnosed with renal cell cancer at the age of 41 years and died. The paternal uncle has an ACTH-producing pituitary adenoma.

Analysing the PTEN gene, a class 5 frameshift mutation (c.1012delT, p.Ser338Leufs*6 in exon 8) was identified. Segregation analysis is pending.

To our knowledge an unclassified pleomorphic sarcoma has not been previously described in patients with PHTS. However, there are several reports of pleomorphic sarcomas with somatic mutations in the PTEN gene. The acknowledgement of unclassified pleomorphic sarcomas as part of the spectrum of the PTEN hamartoma tumor syndrome may lead to the diagnosis in previously unrecognized patients. The diagnosis of PHTS has implications for preventive medicine and family testing.

P-CancG-067

TP53 mutations and deletions in different hematological malignancies

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TP53 is a tumor-suppressor gene and the most frequently mutated gene in cancerTypically one allele is mutated and the other deleted. Mutations and deletions in TP53 have been described in hematological malignancies, however with lower frequencies than in solid tumors. The aim of this study was to determine TP53 mutation/deletion in a large cohort of patients with different hematological malignancies (n=3383), including AML (n=858), MDS (n=943), ALL (n=358), Burkitt lymphoma (n=25), CLL (n=1148) and T-PLL (n=51).

Overall, alterations in TP53 were detected in 361/3383 cases (11%). Regarding the respective entities, the highest frequency of TP53 alterations was observed in patients with Burkitt lymphoma (total: 56%, mut+del: 12%, mut only: 44%, no case del only). Alterations in TP53 also occured with a high incidence in patients with T-PLL (total: 30%; mut+del: 10%; mut only: 4%; del only: 16%) followed by cases with ALL (total: 19%; mut+del: 6%; mut only: 8%; del only: 5%) and AML (total: 13%; mut+del: 5%; mut only: 7%; del only: 1%). However, TP53 alterations occurred less frequently in patients with CLL (total: 8%; mut+del: 4%; mut only: 3%; del only: 1%) and MDS (total: 7%; mut+del: 1%; mut only: 5%; del only: 1%). Missense mutations were found to be the most abundant mutation type in all entities analyzed with a frequency ranging from 71% - 88%. In all entities mainly one mutation per case was detected; however, MDS cases were found to harbour a statistically increased proportion of cases with two mutations compared to the other entities ($p = 0.003$). TP53 alterations were correlated with a complex karyotype in AML, ALL, MDS and T-PLL. Regarding age dependency of TP53 alterations, TP53mut was significantly more frequent in patients ≥ 60 vs < 60 years in AML (9% vs. 2%, $p < 0.001$) and ALL (12% vs. 6%, $p < 0.001$). By contrast, no such differences were observed for patients with CLL, MDS, T-PLL and Burkitt lymphoma and for cases with TP53del in all entities.

Moreover, TP53 alterations and especially TP53mut+del had a significant negative impact on OS in AML (36 vs. 6 months / 2 months, p<0.001), ALL (not reached vs. 24 months / 11 months, p<0.001) CLL (not reached vs. 64 months / 32 months, p<0.001) and MDS (65 months vs. 15 months / 4 months, p<0.001). In T-PLL and Burkitt lymphoma no negative impact was observed which is most probably due to the overall short OS or the lower number of cases.

We conclude that the frequency of TP53 mutations/deletions in hematological malignancies is lower than in solid tumors. However, the frequency substantially varied between different entities (highest in Burkitt lymphoma (56%), lowest in MDS (6%)). Moreover, alterations in TP53 are correlated to a complex karyotype and to a short OS in most entities. The increasing frequency of TP53 mutations with age suggests that these are most likely acquired abnormalities. However, the role of germ line TP53 mutations in hematological malignancies warrants further studies.

P-CancG-068

Constitutional mismatch repair deficiency syndrome caused by one de novo and one familial MSH6 mutation

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Biallelic germline mutations in one of the four mismatch repair (MMR) genes cause an autosomal recessive childhood cancer susceptibility syndrome termed constitutional MMR deficiency (CMMRD). Typically, CMMRD patients inherit each of the two mutations from one parent. The risk for Lynch syndrome-(LS-)associated cancers in parents who are usually considered to be obligatory carriers of a heterozygous MMR mutation depends in part on which of the four MMR genes is affected. Overall, *MLH1* and *MSH2* mutations confer a higher risk than *MSH6* and *PMS2* mutations. This difference is considered to be one of the reasons why CMMRD patients, the majority of whom carry biallelic *MSH6* and *PMS2* mutations, often lack a clear family history of LS-associated cancers.

The broad tumor spectrum and the lack of unequivocal disease specific features complicate the clinical diagnosis of CMMRD syndrome. Therefore, a 3-point scoring system for tumors and non-neoplastic features was developed according to which any childhood or young adult cancer patient reaching a minimum of three points should be suspected of having CMMRD (Wimmer et al. 2014; PMID:24737826). An 8-year old boy clearly fulfilled these clinical criteria by reaching six scoring points with a pre-B cell lymphoblastic leukemia (1 point), a pre-T cell lymphoblastic lymphoma (2 points), multiple café au lait spots and other signs reminiscent of neurofibromatosis type 1 (2 points) and reduced IgA levels (1 point). There is no evidence of LS-associated tumors in either family of the non-consanguineous parents. Massive parallel sequencing revealed two different frameshift *MSH6* mutations, c.1135_1139del and c.2277_2293del, in the patient. To formally prove that they are located *in trans*, both parents were tested for the mutations. The novel mutation c.2277_2293del was detected in the mother, but the recurrent mutation c.1135_1139del was absent in the father and the mother. Since genotyping for STR-markers confirmed the reported familial relationship, there is strong evidence that the recurrent mutation c.1135_1139del occurred *de novo* in our patient. Experiments to prove that it is located *in trans* with the maternal mutation are ongoing.

To our knowledge, this is the first CMMRD case with one proven *de novo* mutation. This explains the lack of LS-associated cancers at least in the paternal family and underscores the importance of testing the carrier status of parents of CMMRD patients. Currently, the *de novo* mutation rate of *MSH6* and other MMR genes are largely unknown. Recent reports show that half of the LS-like patients in whom no germline mutation is found carry biallelic mutations in the neoplastic tissue. This suggests that a substantial proportion of MMR mutations are postzygotic mosaic mutations. Ongoing experiments test for a possible mosaicism of the *de novo* mutation in our patient.

P-CLINICAL GENETICS, GENETIC COUNSELLING AND PRENATAL DIAGNOSIS

P-ClinG-069

A de novo 1q23.3-q24.2 deletion combined with a GORAB missense mutation causes a distinctive phenotype with cutis laxa

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Gerodermia osteodysplastica is a recessive segmental progeroid disorder mainly characterized by wrinkled skin, generalized connective tissue weakness, infantile onset osteoporosis and absence of intellectual disability. Coding mutations in *GORAB*, localized on chromosome 1q24.2, were shown to be the cause of this disease. 1q24 microdeletions underlie a spectrum of disorders with intellectual disability and ear abnormalities. Here we report on an individual from Azerbaijan showing short stature, cutis laxa, frequent fractures, facial dysmorphism, cup-shaped ears and intellectual disability. We analyzed the *GORAB* gene and found the known missense mutation p.Ser175Phe, seemingly in a homozygous state. This mutation was inherited from the clinically unaffected mother, but was not detectable in the healthy father. We performed copy number investigations by high-resolution Array-CGH and PCR approaches and found an approximately 6 MB de novo deletion spanning 1q23.3-q24.2 (chr1:164037509-170654598bp (hg19) in the affected boy. This novel combination of genetic defects very well explains the phenotype that goes beyond the usual presentation of gerodermia osteodysplastica. Our data provide new insight into the phenotypic spectrum of 1q24 deletions and shows that the combination with another pathogenic allele can lead to a more severe clinical presentation.

P-ClinG-070

Overestimation of the positive predictive value of NIPT for Turner syndrome underlines the need for genetic counselling prior to NIPT

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Turner Syndrome has a prevalence of 1/3000 - 1/5000. Prenatally diagnosed it is not considered an indication for termination of pregnancy in Austria if there are no associated malformations like hydrops fetalis or severe heart failure. Without these conspicuous features diagnosed via prenatal ultrasound examination, pregnancy is often uneventful. As mental development is usually within the normal range, females with Turner syndrome remain often unrecognized unless short stature or fertility issues lead to karyotyping.

A 31 year old pregnant woman presented for genetic counseling with suspected Turner syndrome of the fetus by non-invasive prenatal testing (NIPT). Fetal ultrasound examination had been normal. A Combined-test showed a low risk for trisomy 21, 18 and 13. Amniotic fluid had already been sent to our laboratory for validation of the positive NIPT result. The woman strongly expected confirmation of the Turner syndrome by the amniocenteses and wished a termination of the pregnancy even before the results of amniocentesis were completed.

FISH examination, with a centromere probe for the sex chromosomes, revealed a normal female sex chromosome status of the fetus in 100 interphase nuclei which was confirmed by routine karyotyping.

With an estimated prevalence of 1/3000-1/5000 in a pregnancy with normal ultrasound examination results, a sensitivity of 90,3% and a specificity of 99,77% we calculated a positive predictive value (PPV) of only 7-12% for Turner syndrome in NIPT.

There are rare medical indications for assessment of the fetal sex e.g. in mothers who are carriers of X-chromosomal inherited diseases or even suspected Turner syndrome by ultrasound examination. In this case, assessment of the fetal sex by NIPT caused overestimation of the clinical consequences of the test result and lead to the women's request for amniocentesis and consecutive termination of the pregnancy.

NIPT was designed for trisomy 21, 18 and 13 screening. According to actual recommendations NIPT is offered to pregnant women with intermediate risk after early risk assessment. In case of a positive result, amniocentesis is considered to be necessary for confirmation prior to elective termination of pregnancy. At the moment genetic counselling is not mandatory prior to NIPT.

As shown in our case, capability of NIPT can be overestimated and misunderstood by women if they don't receive adequate counselling. Additional testing of sex chromosomes can create the impression of screening for fetal pathology that would justify elective abortion based on the common lack of information

about sex chromosome aneuploidies. For these reasons we want to highlight the importance of adequate genetic counselling prior to NIPT, especially when NIPT is used for screening beyond the recommended diagnostic algorithm. To summarize there is no benefit of prenatal sex chromosome testing without indication.

P-ClinG-071

No evidence that maternal upd(6,16,20)mat are common causes for Silver-Russell syndrome like phenotypes

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Silver-Russell syndrome (SRS) is a congenital growth retardation disorder with a broad molecular and clinical spectrum. The phenotype presents with primary pre- and postnatal growth restriction, a relative macrocephaly at birth, body asymmetry, frontal bossing and feeding difficulties. Patients that fulfill four of six of these criteria are clinically diagnosed as SRS according to the recently published Netchine-Harbison score. In about 60% of these SRS patients (epi)genetic imprinting disturbances on chromosome 11p15.5 or an maternal uniparental disomy of chromosome 7 (upd(7)mat) can be identified.

Furthermore maternal uniparental disomies of chromosome 16 and 20 have also been identified in these patients with a typical SRS phenotype and therefore raise the question on their contribution to the etiology of Silver-Russell syndrome. Additionally, aberrant methylation at the imprinted 6q24 locus has been identified in single cases.

We designed a methylation specific assay for the imprinted ZNF597 locus on chromosome 16 and validated our assay using proven cases of upd(16)mat.

We analyzed 54 patient that were referred for Silver-Russell testing for upd(16)mat. Additionally, we screened for upd(6)mat, upd(14)mat and upd(20)mat using a multilocus MS-SNuPE assay or MS-MLPA. In our cohort we did not identify carriers of maternal upd of chromosomes 6, 16, or 20 or other aberrant methylation pattern at the analyzed loci. One carrier of an epimutation of the MEG3/IG-DMR on chromosome 14 was identified.

Considering reports from the literature and based on our own data it becomes obvious that (epi)mutations on chromosome 14 represents a significant alteration in SRS-like patients. Maternal upd(20)mat can rather be regarded as a new imprinting syndrome with a clinical overlap with SRS. The assignment of upd(6)mat and upd(16)mat to a specific phenotype or common clinical feature remain unclear. Nevertheless, for upd(16)mat an association with IUGR has been observed and it can be assumed that imprinted genes on chromosome 16 contribute to the phenotype of growth restriction. Thus, if they occur in patients with growth restriction, isolated or in combination and a Silver-Russell like phenotype, both UPDs can be regarded as causative for the clinical features. Nevertheless, based on the rarity of the observation upd-testing for upd(6,16,20)mat should not be considered in routine workup while the analysis of chromosome 14 (epi)mutations is reasonable.

P-ClinG-072

Novel DNMT3A mutation in a patient with overgrowth and intellectual disability

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Recently, mutations in the DNA methyltransferase gene *DNMT3A* have been identified as a cause of a new human overgrowth disorder termed 'DNMT3A overgrowth syndrome'. So far, 13 patients have been described by Tatton-Brown et al. (2014), all featuring a tall stature, a distinctive facial appearance with a round face, heavy horizontal eyebrows, short palpebral fissures and most of them moderate intellectual disability (ID). Congenital heart defects, scoliosis, seizures and umbilical hernia can be associated.

Here, we present a four-year-old boy with congenital overgrowth and macrocephaly, developmental delay, behavioral problems and facial features compatible with those described previously. Conventional cytogenetic analysis, CGH array and sequencing of X-linked ID-genes showed unremarkable results. A novel heterozygous de novo frameshift mutation in *DNMT3A* (c.1156delG / p.(V386fs)) was identified by whole exome sequencing leading to a premature stop codon and most probably resulting in a truncated and

functionally impaired protein. The mutation was confirmed by Sanger sequencing and analysis by different prediction tools supported its putative pathogenic relevance. Furthermore, it is not annotated in the Exome Aggregation Consortium database and the Residual Variance Intolerance Score was negative. In contrast to the mutations described by Tatton-Brown et al. it is not located within one of the three functional domains of *DNMT3A*. Instead it affects the region of interaction between *DNMT3A* and *DNMT1* as well as *DNMT3B* which is essential for maintaining DNA methylation marks after DNA replication and establishing new methylation marks during embryonic development, respectively.

Just like *DNMT3B*, *DNMT3A* plays an important role in establishing sex-dependent methylation marks for imprinted genes and new methylation marks after the erasure of the parental methylation pattern. Interestingly, somatic mutations in *DNMT3A* can be detected in about one third of cytogenetically normal patients with acute myeloid leukemia (AML). The *DNMT3A* mutational spectrum found in overgrowth patients so far differs from that detected in hematological neoplasms. Protein structure modeling suggests that the mutations found in overgrowth syndrome patients might disrupt de novo methylation by interfering with histone binding but the exact mechanism of pathogenesis is still unknown. *DNMT3A* therefore resembles *NSD1* and *EZH1*. Constitutional mutations in these genes cause the overgrowth syndromes Sotos and Weaver, respectively, while somatic mutations are also associated with hematological malignancies. At this point in time it remains unclear if constitutional mutations in *DNMT3A* confer a predisposition to malignancy. Characterization of more affected patients is needed to better define the clinical spectrum of this new overgrowth syndrome.

P-ClinG-073

DLG3 mutation in a family with X-linked intellectual disability

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Mutations in *DLG3* are a rare cause of X-linked intellectual disability; only eight mutations have been reported to date. Male patients had moderate to severe but otherwise non-syndromic intellectual disability (ID). The majority of female *DLG3* mutation carriers were reported to be healthy, and they had random X-inactivation.

We report on a 13-year-old boy with moderate ID, behavioral problems, neonatal failure to thrive and subtle dysmorphic signs (triangular face, micrognathia, frontal upsweep, Marfanoid habitus, sandal gap, broad great toes). His mother had learning difficulties, and two sisters of the mother had ID (severe ID in one aunt and mild ID in the other).

Next-generation sequencing analysis using the TruSight One Panel (Illumina®) revealed a novel nonsense mutation in the penultimate exon of *DLG3*: [c.2266C>T; p.(Arg756*)] in the index patient. This mutation was also present in his mother and in his two maternal aunts. X-inactivation in the mother was skewed (96:4); the aunt with more severe ID had a complete skewing, and the aunt with mild ID had a ratio of 73:27.

In contrast to previous observations, this is the first family in which skewed X-inactivation could be demonstrated in all female carriers of a *DLG3* mutation, and in which all female mutation carriers had cognitive defects.

P-ClinG-074

A microdeletion encompassing X-linked *SSR4* gene in a boy with complex clinical presentation further delineates the molecular and clinical phenotype of *SSR4*-related CDG

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Congenital disorders of glycosylation (CDG) represent a growing group of disorders characterized by altered protein or lipid glycosylation. Most of the known CDG types are inherited in autosomal recessive manner. Non autosomal recessive CDG include autosomal dominant mutations in *EXT1* and *EXT2* genes, while the CDG caused by mutations in *TUSC3*, *ALG13*, *SLC35A2* and *SSR4* constitute a group of X-linked disorders. Until today, over one hundred CDG have been identified and more than 50 involve N-linked glycosylation. Patients with N-linked defects can present with a wide variety of manifestations, ranging from

severe developmental delay and hypotonia with involvement of multiple organ systems, to hypoglycemia and protein-losing enteropathy with normal development. Using different biochemical methods for testing of serum transferrin glycosylation the majority of patients with N-linked CDG are identified but not all. Clarification of certain CDG type requires molecular genetic testing. Here, we report on a 16-year-old boy presenting with intellectual disability, developmental and speech delay, proportionate short stature, dysmorphic facial features, hand stereotypies, conductive hearing loss, strabismus, hyperopia, frontal lobe epilepsy and abnormal brain imaging, ataxic gait, patent foramen ovale, delayed bone age and joint hypermobility Trio whole-exome sequencing identified a hemizygous de novo 25.3kb deletion in Xq28 encompassing four genes: *PLXNB3*, *SRPK3*, *IDH3G* and *SSR4*. *SSR4* (*signal sequence receptor protein 4*) encodes a subunit of the heterotetrameric translocon-associated protein (TRAP) complex composed of four subunits (SSR1-4). Along with two other complexes, SEC61 and OST, TRAP complex specifically localizes to endoplasmatic reticulum (ER) membrane sites and is involved in the process of translocation of nascent secretory proteins into ER lumen, where they can undergo co- and/or posttranslational N-glycosylation. Recently, *SSR4* loss-of-function mutations were described in in total nine patients with CDG. Eight patients bear de novo or inherited point mutations or small deletions and one previously described patient bears a 73.4kb deletion in Xq28. Clinical data of our patient and previously reported males show several overlapping but unspecific phenotypic features, such as developmental delay, intellectual disability, facial dysmorphism, epilepsy, cardiac anomalies, visual and skeletal involvement, as well as abnormal brain imaging. In our patient and in nearly all reported patients, testing of serum transferrin glycosylation showed values only slightly above the accepted normal cutoff range. Therefore, one could hypothesize that some cases with CDG might be overlooked during biochemical investigations. However, whole exome sequencing can compensate the shortcomings of the biochemical work-up. This is particular true in individuals showing unspecific clinical features and suffering from an atypical CDG.

P-ClinG-075

A de novo mutation in ROR2 and a large de novo deletion within KMT2D cause atypical Kabuki syndrome that mimics CLPED1

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We present the puzzling case of a girl from a non-consanguineous Turkish family who presented with the facial features of Kabuki syndrome (KS), a large VSD, left renal agenesis, and left hip dislocation. In addition, she showed brachydactyly, especially of the terminal phalanx of the 5th finger, bilateral cleft lip/palate (CLP), and hyperkeratosis of the feet. The latter two features evoked a differential diagnosis of cleft lip/palate-ectodermal dysplasia syndrome (CLPED1), but because of the striking facial features, KS was considered the primary clinical diagnosis. Sanger sequencing of the two KS-associated genes KMT2D and KDM6A yielded a negative result. Trio whole-exome sequencing identified a de novo heterozygous deletion of exons 28-54 of KMT2D as the cause of KS and a heterozygous de novo missense mutation in ROR2 as the cause of brachydactyly type B1 (BDB1). No mutations or copy number changes were identified in genes associated with CLPED1 or ectodermal dysplasia. Thus, the phenotype of the child appears to be the result of a combination of these two de novo events. However, although cleft lip/palate is part of the Kabuki syndrome spectrum, bilateral CLP as in our patient is rare and hyperkeratosis is not a typical symptom of the disorder. ROR2 is a tyrosine-kinase-receptor involved in WNT-signaling, and there is compelling evidence of a bidirectional regulatory loop between proteins of the histone lysine methyltransferase (KMT) group and WNT-signaling. We conclude that the presence of two genetic alterations in related signaling systems may cause a modulation of known phenotypes that leads to unexpected clinical presentations.

P-ClinG-076

Mutational spectrum and phenotypic characterization in 94 patients with X-linked hypohidrotic ectodermal dysplasia (Christ-Siemens-Touraine syndrome)

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Here we report the phenotype and mutational spectrum in 62 male and 32 female patients aged 20 months – 68 years with X-linked hypohidrotic ectodermal dysplasia (XLHED), which is caused by *EDA* gene mutations and represents the most common type of ectodermal dysplasia. XLHED is characterized by

severe hypohidrosis, hypoplasia of sweat, sebaceous, submucous, meibomian and mammary glands, sparse hair and eyebrows, and oligodontia.

24 previously undescribed *EDA* mutations could be detected. Detailed clinical data encompassing 40 single features regarding teeth, hair, sweating, current facial symptoms, eyes, skin, nails, breast development, airway infections, hyperthermia, seizures, and developmental outcome were available from 56 male and 22 female patients and will be demonstrated. Although there is no general genotype-phenotype correlation, we could show that the severity of symptoms may grossly depend on kind and localization of the mutation. Furthermore, we were able to demonstrate or exclude associations between single symptoms. Thus we found for example, that the risk for motor developmental delay or disturbed mental development, which we observed in 12.5% and 5.4% of our male patients respectively, is strongly associated with the occurrence of febrile seizures only.

P-ClinG-077

Predictive Genetic Testing of BRCA1/2- mutations: Expectations and needs of positively tested mutation carriers. A qualitative study.

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Background: Little is known about the views, needs and expectations of BRCA1/2 mutation carriers who are confronted with a lot of information throughout the process of genetic testing. Especially mutation carriers who do not yet have symptoms of cancer need effective genetic counselling. Our aim was to explore the experiences of men and women who underwent predictive genetic testing for BRCA1/2.

Methods: Using a semi-structured questionnaire we interviewed 4 women and 2 men who were healthy mutation carriers of a mutation in BRCA1/2. They differed in age and time of their genetic testing. All interviews were audio recorded, transcribed and independently analyzed using interpretative phenomenological analysis (IPA). This was followed by a cross-case analysis to ascertain emergent themes across cases.

Results: The participant's age ranged between 22 and 52 years; all underwent genetic counselling and predictive genetic testing in the time interval of 2012 to 2015 at the Institute of Human Genetics in Luebeck. Participants reported to what extent their family history of cancer influenced their own coping behaviour concerning the test results. The reaction to the fact of being a mutation carrier and therefore at risk of getting cancer was diverse. Most of the participants were satisfied with the information being offered during genetic counselling. Networking and an improved information transfer between the different specialists would be of great help.

Conclusions: Although the number of interviewed is small, the results show that there might be a need of positive mutation carriers of being supported and informed individually after predictive genetic testing, taking into consideration their social and psychological background.

P-ClinG-078

Splitting versus Lumping: Temple-Baraitser and Zimmermann-Laband Syndromes

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KCNH1 mutations have recently been described in six individuals with Temple-Baraitser syndrome (TMBTS) and six individuals with Zimmermann-Laband syndrome (ZLS). TMBTS is characterized by intellectual disability (ID), epilepsy, dysmorphic facial features, broad thumbs and great toes with absent/hypoplastic nails. ZLS-affected individuals show facial dysmorphism including coarsening of the face, large nose and large ears, gingival hyperplasia, ID, hypoplasia of terminal phalanges and nails and hypertrichosis. In this study, we present four additional unrelated individuals with de novo KCNH1 mutations from ID cohorts. We report on a novel recurrent pathogenic KCNH1 variant in three individuals and add a fourth individual with a previously TMBTS-associated KCNH1 variant. Neither TMBTS nor ZLS were suspected clinically. KCNH1 encodes a voltage-gated potassium channel, which is highly expressed in the central nervous system and seems to play an important role during development. Clinical evaluation of our mutation-positive individuals revealed that one of the main characteristics of TMBTS/ZLS, namely the

pronounced nail hypoplasia of the great toes and thumbs, can be mild. Clinical comparison of all published KCNH1 mutation-positive individuals revealed a similar facial but variable limb phenotype. The KCNH1-related phenotype comprises severe ID, neonatal hypotonia, hypertelorism, broad nasal tip, wide mouth, nail a/hypoplasia, a proximal implanted and long thumb and long great toes. We show that the phenotypic variability of individuals with KCNH1 mutations is more pronounced than previously expected and we discuss whether KCNH1 mutations allow for “lumping” or for “splitting” of TMBTS and ZLS.

P-ClinG-079

Ring syndrome in a boy with complete ring chromosome 4 and short stature, microcephaly, pelvic kidney and normal psychomotor development

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Constitutional ring chromosomes can be found for all human chromosomes and are very rare chromosomal abnormalities. Two mechanisms lead to ring formation with loss and possibly gain of genetic material: Breakage in one or both arms of the chromosome with subsequent fusion of the ends or an inversion-duplication-deletion rearrangement. A complete ring chromosome without loss of genetic material results from fusion of subtelomeric regions or telomere-telomere fusion. In cases of complete ring chromosome - independently of the chromosome involved - an increased incidence of severe growth failure with no or only minor anomalies has been observed and attributed to ring syndrome. Ring syndrome is thought to be caused by "dynamic mosaicism" due to ring instability: Sister chromatid exchanges during cell division result in secondary chromosomal abnormalities with a high cellular death rate and consequent growth retardation. Surviving aneuploid cells and epigenetic factors may contribute to the phenotype.

We report a 5-year-old boy with de novo ring chromosome 4 and typical characteristics of the ring syndrome, e.g. proportionate severe growth failure, microcephaly and some minor anomalies (right-sided pelvic kidney, discrete facial dysmorphism). Cytogenetic and molecular studies showed complete ring chromosome 4 without loss of genetic material. Instability of the ring chromosome was demonstrated by an increased frequency of secondary aberrations and monosomic cells due to ring loss. The parents of the boy are contemplating growth hormone therapy. In the literature, growth hormone therapy and its possible effect on dynamic mosaicism in patients with constitutional ring chromosomes has not been studied. Therefore, it cannot be excluded that growth hormone therapy may lead to an increase in secondary aberrations with a possible negative impact on the phenotype. Our case supports the theory of dynamic mosaicism due to ring instability. We suggest that cytogenetic monitoring of the rate of secondary aberrations in patients with ring chromosome undergoing growth hormone therapy might be feasible.

P-ClinG-080

Van der Woude and Popliteal Pterygium Syndromes: Broad Intrafamilial Variability in a Three Generation Family with Mutation in IRF6

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Van der Woude syndrome (VWS) is usually displayed with cleft lip, cleft lip and palate or with cleft palate only. In contrast to non-syndromic cleft lip and/or palate, VWS typically is characterized by bilateral, paramedian lower-lip pits. Popliteal pterygium syndrome (PPS) shares features with VWS, but, in addition, is characterized by an eponymous popliteal pterygium, genital anomalies, cutaneous syndactyly, and a characteristic pyramidal fold of skin overlying the nail of the hallux. In some patients with PPS filiform synechiae connecting the upper and lower jaws or the upper and lower eyelids are present. VWS and PPS are autosomal dominant inherited allelic disorders caused by heterozygous mutations in *IRF6*. We present a three generation family with tremendous intrafamilial phenotypic variability. The newborn index patient had a clear-cut diagnosis of PPS. The mother presented with a classic VWS, while the maternal grandfather had VWS as well as minor signs of PPS. In all three affected the pathogenic mutation c.265A>G;p.Lys89Glu in *IRF6* was identified. While interfamilial as well as intrafamilial variability has been described in *IRF6*-related disorders, however, the occurrence of a typical VWS without any other anomalies as well as a clear-cut diagnosis of PPS in the same family is a very rare event. We will present the clinical and molecular data and discuss the impact of this case concerning genetic counseling in families with VWS or PPS.

P-ClinG-081

Targeted next-generation sequencing in search for monogenic causes of intellectual disability in children

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Childhood intellectual disability (ID) as a cause of developmental delay and regression comprise a genetically heterogeneous group of disorders. Although numerous causative genes have been identified for the various disease groups in many cases, a specific genetic diagnosis remains elusive even after extensive molecular testing. Next-generation sequencing has opened up new possibilities especially in the search for disease-causing mutations in disorders with common clinical features but a heterogeneous genetic background. Identification of underlying genetic defects provides a clear diagnosis for patients more and more influencing their management and occasionally even their therapy, while it is the prerequisite for prenatal or preimplantation decisions in the affected family.

127 children with syndromic and non-syndromic ID, seen at the genetic counselling unit of our institute, were analyzed with targeted exon enrichment and NGS. The so-called MPIMG-1-Test provides panel diagnostics for over 1200 brain related genes. For enrichment a TruSeq Custom Enrichment Kit was used. Sequencing was carried out on an Illumina MiSeq system (2x300bp PE). A modified version of the Medical Resequencing Analysis Pipeline (MERAP, Hu et al, 2014) was used to check all detected variants against dbSNP138, the 1000 Genomes Project, the Exome Variant Server, the OMIM catalog and the Human Gene Mutation Database. For exclusion of technical artifacts and segregation testing of all likely disease-causing variants, PCR and Sanger sequencing were performed according to standard protocols.

Confirmed disease-causing mutations have been identified in 29 out of the 127 patients. We found 5 cases with mutations in genes associated with syndromic ID (i.a. HRAS, ITPR1, GAMT, SGSH, PTPN11) and 3 cases associated with non-syndromic ID (i.a. NDST1, DYRK1A, ARID1B). All variants were previously reported and recognized as causing of a disorder or a protein truncating mutation in a gene matching the patient's phenotype (category 5). 9 other genes where likely associated with the syndromic ID phenotype (STIL, ZEB2, MLL2, KDM5C, ATRX, CEP152, GLI3, KIF4A, PHF6), but with a previously unreported or not yet clear pathogenic sequence variation (category 4). Another 4 variants are classified as category 3 because segregation analysis is ongoing at the moment. Pathogenicity of the variants was supported by prediction tools (SIFT, PolyPhen2, MutationTaster) and conservation scores. Classification of pathogenicity was carried out according to the standards of Richard et al. 2015. All variants were confirmed by Sanger sequencing.

Our findings so far support the clinical utility of targeted next generation sequencing in identifying monogenic causes of a common clinical symptom associated with childhood developmental delay often taking the burden of guilt from the family and offering possibilities for risk prediction and prenatal and preimplantation diagnostics in the family.

P-ClinG-082

Phenotypic overlap in patients with DDX3X variants and Toriello-Carey Syndrome

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De novo variants in DDX3X (MIM 300160) have recently been reported as a common cause of unexplained intellectual disability (ID) in females (1-3%). They have been shown to be associated with ID, hypotonia, movement disorders, behavior problems, epilepsy, corpus callosum hypoplasia (CCH) and other features.

Whole exome sequencing (WES) data of a small group of female patients with unexplained syndromic ID were reanalyzed by filtering for X-linked de novo heterozygous variants. Reanalysis showed a previously unconsidered DDX3X variant in the patient presented here, previously clinically diagnosed with Toriello-

Carey syndrome (TCS; MIM 217980). The clinical features of both diagnoses are compared with the focus on differences between the TCS core phenotype and DDX3X related phenotypes.

Patient 1 was born with multiple congenital anomalies: anal atresia, congenital heart defect, vertebral malformations, laryngomalacia and brain anomalies including corpus callosum hypoplasia (CCH). She showed postnatal microcephaly (-3 SD), short stature (-3 SD), dysmorphic facial features, experienced neonatal respiratory distress and developed infantile spasms at 6 months of age, hypotonia and severe ID. Cytogenetic analysis, Affymetrix 500K SNP array and targeted sequence analysis of UBE3B showed normal results. Exome sequencing showed a de novo variant c.1703C>T; p.(P568L) in DDX3X (NM_001356.4), confirmed by Sanger sequencing. The clinical features of this patient are very similar to those of published females with DDX3X mutations and in particular one individual carrying the identical mutation. Both have in common severe ID, microcephaly, hypotonia, seizures, CCH, ventricular enlargement, visual problems, and scoliosis, and do not show cleft lip (CL) or cleft palate (CP). However, there is also concordance of most of the clinical features of patient 1 and the TCS phenotype reviewed in 2003 in 45 patients. TCS is a condition with many overlapping phenotypes, in some patients caused by cytogenetic alterations4, variants in UBE3B5 and, considering patient 1, by DDX3X variants. The underlying gene for the core phenotype of TCS, however, is still unknown. Comparing patient 1 to the children originally described with TCS, they differ with regard to two main features: Patient 1 neither has CP nor CCA (but CCH). If there are core genes associated with TCS, they might primarily be associated to TCS phenotype patients with CP and partial or complete CCA.

We conclude that X-linked de novo heterozygous DDX3X variants should be considered not only in undiagnosed females with ID, but also in patients with a clinical diagnosis of TCS. This report provides evidence that the TCS phenotype not only significantly overlaps with phenotypes caused by cytogenetic alterations and UBE3B variants but also with clinical features associated with DDX3X variants. More X-linked genes might cause ID with de novo heterozygous mutations in females.

P-ClinG-083

MIDAS (Multiple Integration of Data Annotation Study) – Integration of genotype data from NGS and Sanger sequencing with HPO-coded phenotype data in a diagnostic setting

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In order to integrate Next-generation-sequencing data, Sanger sequencing data and phenotype data in a diagnostic laboratory, we developed a software tool called MIDAS. MIDAS integrates patient data from the Laboratory Information Management System (LIMS), data from the routine Sanger sequencing workflow, as well as phenotype data based on the Human Phenotype Ontology (HPO) with NGS results. In particular, Genotype-Phenotype correlations identified in one patient are made available for all other cases, to aid the interpretation and build a comprehensive knowledge base.

For the NGS panel analyses, exonic regions of more than 1200 custom selected genes are enriched in parallel by oligonucleotide hybridization and capture (Agilent QXT), followed by massively-parallel sequencing on the Illumina NextSeq platform. By providing various different templates for smaller subpanels, only genes from the requested indication are selected for data analysis, to limit interpretation to relevant genes, while simultaneously minimizing the possibility of incidental findings. Data analysis is performed using the CLC Genomics Workbench and custom developed Perl scripts. Target regions which fail to reach the designated coverage threshold of 20X are re-analyzed by Sanger sequencing. Identified candidate mutations are independently confirmed. All detected variants are imported into the MIDAS database, which may be queried via a web interface for dynamic data analysis and filtering. Information from all analyzed genes (~1200) is used in an anonymized way for internal variant frequency calculation, quality control and the detection of potential sequencing artifacts.

We have applied this approach to more than 400 samples from a variety of different genetic disorders and will continue this implementation for future processing of our NGS and Sanger sequencing results. So far, our approach has been implemented for the diagnostic of arrythmogenic cardiac disorders (LQTS, HCM, DCM), connective tissue disorders (EDS, TAAD), rare kidney disorders (Nephrotic Syndrome, CAKUT), neurological disorders (RASopathies, Microcephalies, Epilepsy), metabolic disorders (MODY diabetes), hereditary hearing loss and coagulopathies and has recently been expanded to retinopathies, immunologic disorders, ataxias, congenital heart defects, skeletal dysplasias and intellectual disability.

P-ClinG-084

New EMQN best practice guidelines for the molecular genetic testing of chromosome 11p15 Imprinting Disorders – Silver-Russell and Beckwith-Wiedemann syndrome

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Molecular genetic testing for the 11p15-associated imprinting disorders Silver-Russell and Beckwith-Wiedemann syndrome (SRS, BWS) is challenging because of the molecular heterogeneity and complexity of the affected imprinted regions. So far, there has not been a standardized and evidence-based molecular diagnostic testing and reporting strategy, and as a result, the final outcome of the first external pilot quality assessment scheme organised by the European Molecular Quality Network EMQN in 2014 was not satisfactory. Based on the results from this scheme and in context with activities of the European Network of Imprinting Disorders (EUCID.net) towards a consensus in diagnostics and management of SRS and BWS, best practice guidelines have now been developed. Members of institutions working in the field of SRS and BWS diagnostics were invited to comment, and in the light of feedback amendments were made. The final document was ratified in the course of an EMQN best practice guideline meeting for 11p15.5 imprinting disorders on October 3rd 2015 and is in accordance with the general SRS and BWS consensus guidelines which are in preparation. These guidelines are based on the knowledge acquired from peer reviewed and published data, as well as observations of the authors in their practice. However, these guidelines can only provide a snapshot of current knowledge at the time of manuscript submission.

P-ClinG-085

Atypical Manifestation of TGDS-related Catel-Manzke syndrome

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An additional phalangeal ossification center leading to clinodactyly, Robin sequence and heart defects are pathognomonic for Catel-Manzke syndrome. We recently identified mutations in TGDS as the cause of this disorder, but the pathomechanism remains poorly understood. Here we present a fetus with Pierre-Robin sequence, talipes, and bilateral clinodactyly of the index and middle fingers. Due to these malformations, pregnancy was terminated in the 22nd week. Radiographs showed V-shaped displacement of the hypoplastic 2nd and 3rd basal phalanges of both hands as well as hypoplastic first metatarsals and abnormal phalangeal bones of the halluces. Based on the phenotypic overlap we suspected Catel-Manzke syndrome in the fetus. Sanger sequencing of TGDS identified two different sequence alterations: The known variant c.298G>T (p.Ala100Ser) and the so far undescribed variant c.895G>A (p.Asp299Asn). The variant c.895G>A is classified as disease causing due to the high conservation of the amino acid position Asp299 which is located in the predicted substrate binding site of TGDS next to a known amino acid change. The mother is carrier of one variant, consistent with autosomal-recessive inheritance. The father's DNA was not available. This is the first report of a fetus with compound-heterozygous mutations in TGDS and anomalies of the middle finger and halluces. Our findings expand the clinical spectrum of TGDS mutations and give insight into the fetal development of the Manzke dysostosis.

P-ClinG-086

De novo truncating GRIN2B mutation causes epileptic encephalopathy

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Epileptic encephalopathy is an early infantile epileptic disorder with a broad range of potential disease causes. Therefore, the molecular diagnostic poses a challenge.

Here, we report a 6 year old, male patient with epileptic encephalopathy. The first seizure (focal seizure) occurred before age 1 and was maybe provoked by an infection. Later, generalized and myoclonic,

treatment-resistant seizures appeared. Additionally, early development was delayed, as he walked and used first words around 16 months and 23 months, respectively.

Analysis of the most relevant genes (SCN1A, GABRG2, SCN2A, FMR1) according to the clinical symptoms of our patient yielded no mutation. An additionally performed array CGH analysis detected a 52kb deletion in the WWOX gene. Since, WWOX-related encephalopathies are inherited in an autosomal recessive manner; another mutation on the other allele is required. However, a sequencing analysis of the WWOX gene did not reveal a second mutation. Therefore, we screened 91 candidate genes encompassing a vast spectrum of genes known to contribute to epileptic encephalopathy by massive parallel sequencing. Towards interpretation of this high throughput data only one potential mutation was detected. We revealed a novel mutation c.2479dupG causing a frameshift and creating a premature stop codon (p.Ala827Glyfs*84) in the GRIN2B gene. Neither the mother nor the father carried this mutation. The GRIN2B gene encodes the NR2B subunit of the N-methyl-D-aspartate (NMDA) receptor, which is composed of two NR1 und two NR2 subunits. This receptor is expressed in the cerebral excitatory synapses and modulates excitatory postsynaptic potentials determining the efficiency of synaptic plasticity, a mechanism for learning and memory. De novo missense mutations in the GRIN2B gene have been identified to be causative for epileptic encephalopathies. Nevertheless, we describe for the first time a frameshift mutation in relation with an epileptic disorder. However, truncating mutations in the GRIN2A gene, coding for another NR2 subunit of the NMDA receptor, are well known reasons for epileptic encephalopathies.

There is a strong evidence for the disease causing effect of the truncating mutation in the GRIN2B gene, due to the deleterious impact of the mutation and the segregation analysis. Although we cannot exclude a secondary mutation modulating the phenotype.

P-ClinG-087

Clinical scoring for Silver-Sussell-syndrome as a prerequisite for genetic testing? Experiences from a cohort of 41 positively tested patients.

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A consensus paper on diagnosis and treatment of Silver-Russel Syndrome (SRS, OMIM #180860) will shortly be published that classifies SRS as a clinical diagnosis. The clinical diagnosis can currently be confirmed in about 60-70 % of patients by detecting the molecular basis. Of these SRS patients, 50-60 % show hypomethylation of the paternal allele of the 11p15 imprinting centre region 1 (ICR1) regulating the imprinted IGF2/H19 locus. Additional 10% of patients carry maternal UPD7 (upd(7)mat), and in rare cases cytogenetic abnormalities or other genetic causes can be identified. However, the underlying molecular causes remain unknown in 30-40%.

Several clinical scoring systems have been published which basically consider the pre- and postnatal growth retardation but differ for the number and the rating of characteristic SRS features. In the course of the recent consensus meeting on SRS, the Netchine-Harbison clinical scoring system (NH-CSS, Azzi et al., J Med Genet. 2015 Jul;52(7):446-53) has been defined as the common clinical score on the basis of a huge literature review. The NH-CSS includes six clearly defined factors: Prenatal (1) and postnatal (2) growth retardation, relative macrocephaly at birth (3), protruding forehead as toddler (4), body asymmetry (5) and feeding difficulties and/or low BMI (6). Patients with 4 out of these 6 factors are classified as `Likely-SRS`, and individuals with less fulfilled criteria as` Unlikely-SRS`. Azzi et al. retrospectively validated the NH-CSS by scoring a cohort of molecularly confirmed and deeply phenotyped SRS patients. As a result, all 35 patients with ICR1 hypomethylation (100 %) and 11 of 12 upd(7)mat patients (91.7%) were classified as `Likely – SRS`.

Applying the NH-CSS score to our patients referred for SRS routine genetic testing (25 patients with 11p15 hypomethylation; 16 patients with upd(7)mat), we were able to confirm its high suitability for detection of 11p15 hypomethylation with a percentage of 94 % `Likely-SRS` patients. However, only 54% of patients with upd(7)mat had a positive SRS-likely score of at least 4/6. The difference in the percentage of `Likely SRS` patients between Azzi et al. and our cohort might be explained by the way of ascertainment, but as a consequence, we raise the question whether a uniform scoring in the clinically and genetically heterogeneous Silver-Russell syndrome is suitable to detect patients with upd(7)mat, especially in case of milder phenotype.

P-ClinG-088

Expanding the phenotype of PIK3CA Related Overgrowth Spectrum (PROS) and NGS-based analysis of postzygotic mosaicism

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Recently, the term PIK3CA Related Overgrowth Spectrum (PROS) was proposed to encompass the known and emerging clinical entities with germline and somatic *PIK3CA* mutations. *PIK3CA* encodes the p110alpha protein, a catalytic subunit of the class I Phosphatidylinositol 3-kinases (PI3K). Activating somatic *PIK3CA* mutations are associated with overgrowth of single tissues or organs or a more generalized form manifesting with cerebral overgrowth and vascular malformations.

In a group of four male patients with clinical signs of PROS, we performed diagnostic PIK3CA testing in different materials: DNA from buccal mucosa in all patients, from blood leukocytes in three, from tumor material in one (patient 2). From all parents lymphocyte DNA was available. In addition, we investigated various tissues for mosaicism by NGS based targeted sequence analysis. The patients were originally diagnosed with Megalencephaly-Capillary Malformation syndrome (MCAP) (patients 1,3,4) or Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome (patient 2). By Sanger sequencing, we identified three previously described mutations in patient 2 (c.241G>A; p.(Glu81Lys)), 3 (c.1133G>A; p.(Cys378Tyr)) and 4 (c.1093G>A; p.(Glu365Lys)), respectively. All mutations were *de novo*. Mosaicism in peripheral blood leucocytes was confirmed by deep sequencing in patient 3 with 17% novel allele reads (105/632).

In patient 2 (CLOVE syndrome), mutation c.241G>A was only detected in tumor material, but not in leukocytes or buccal mucosa, with 37% (326/878) novel allele reads in NGS based sequencing indicating cellular mosaicism. Previously, the same mutation was associated with MCAP syndrome in two patients indicating that genotype phenotype correlation is difficult.

Patient 1 had a novel, most probably activating mutation (c.333G>C; p.(Lys111Asn)). This variant affects an amino acid neighboring a recently described mutation (c.335T>A; p.(Ile112Asn)), both located in a linker region adjacent to the p85 binding domain (encoded by *PIK3R2*). Both patients had overlapping clinical features such as congenital megalencephaly and mild developmental delay. In contrast, the other two MCAP-patients (3, 4) showed typical developmental delay. The mutations locate in the C2 domain, a domain harboring almost exclusively mosaic mutations. Carriers of those mutations are known to have more severe developmental delay indicating that those mutations have a stronger activating effect.

In conclusion, we could further expand the clinical phenotype in patients with PROS towards patients lacking the typical segmental overgrowth. Since most patients have postzygotic mosaic mutations, analysis of different tissues is required. To ensure the detection of low level mosaicism we recommend deep sequencing as diagnostic tool.

P-ClinG-089

Diagnosis of CoPAN by Whole Exome Sequencing: Waking Up a Sleeping Tiger's Eye

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Neurodegeneration with brain iron accumulation (NBIA) is a group of neurodegenerative disorders characterized by iron accumulation in the basal ganglia. Distinct subtypes of NBIA have been identified which are caused by mutations in 10 different genes. Recently, mutations in COASY have been identified as a cause of a novel NBIA subtype (COASY Protein-Associated Neurodegeneration, CoPAN) in two unrelated patients with early-onset spastic-dystonic paraparesis followed by parkinsonian features, cognitive impairment, obsessive-compulsive behavior and axonal neuropathy. We have identified compound heterozygous COASY mutations in an eight year old girl and her seven year old brother by whole exome sequencing (WES). Both patients showed global developmental delay, as well as ataxic gait, and later

developed progressive signs of spasticity, hyperactive and aggressive behavior. The “eye-of-the tiger-sign”, a characteristic hypointense spot within the hyperintense globi pallidi on T2-weighted MRI found in the most common subtype of NBIA (Pantothenate Kinase-Associated Neurodegeneration, PKAN), could not be detected in our patients and was only subtly present in one of the two individuals with CoPAN reported previously. In our patients, bilateral hyperintensity and swelling of caudate nucleus, putamen and thalamus was present. A similar MRI pattern was observed in an early stage of disease in one of the other patients. The present report not only illustrates that WES is a powerful tool to elucidate the etiology of rare genetic diseases but also identifies unique neuroimaging findings that may be the key feature for an early diagnosis of CoPAN.

P-ClinG-090

Congenital myasthenic syndromes: Efficiency and pitfalls of phenotype-based gene panel testing.

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Congenital myasthenic syndromes (CMS) are characterized by a neuromuscular transmission defect and display as clinically and genetically heterogeneous disorders. As genetic disorders CMS are exceptional in that they are highly treatable. However, the appropriate drug treatment depends on the underlying genetic defect. This implicates the special importance of genetic testing. The constantly growing number of known disease-associated genes has enlarged the molecular basis of CMS and is limiting single gene testing. We report on a cohort of 50 CMS patients tested by diagnostic gene panels. Diagnostic efficiency of a focused and a broader phenotype-based selection of CMS genes are compared respecting diagnostic yield and precision.

Patients were consecutively tested for 14 genes, focused on the phenotype of CMS, a congenital myopathy panel (35 genes), and for a comprehensive neuromuscular panel (370 genes). Target enrichment with a custom Agilent SureSelectXT Kit was followed by massive parallel sequencing (Illumina NextSeq500). Data analysis was performed with a bioinformatics pipeline consisting of BWA, SAMtools, snpEff and Alamut-Batch. Diagnostic quality criteria assured a coverage depth of more than 30 sequences per base pair in at least 98% of the analyzed regions with adequate sensitivity and specificity (>99,85%) in variant calling. For the CMS genes more stringent criteria were demanded (i.e. 100% sequencing of the coding region and flanking intronic sequences at >30-fold).

Analysis of the CMS panel revealed a diagnostic yield of about 35%. Being quite low as compared to other CMS cohorts it may reflect the spectrum of patients sent in for diagnostic testing to evaluate a potentially treatable disorder. Recent findings in CMS research point towards an overlapping phenotypic spectrum of CMS with congenital myopathies. However, we failed to significantly increase the diagnostic yield by a second-tier (congenital myopathies) and third-tier (neuromuscular disorders) analysis of larger gene panels. As an unsolicited side effect there is a significant increase in the number of variants with unknown significance if large panels far from the core phenotype are tested. We conclude that even in the era of next generation sequencing detailed clinical data as well as the inclusion of parental genetic data are prerequisite for a precise molecular diagnosis.

P-ClinG-091

Targeted next-generation sequencing in search of monogenic causes of behavioral disturbance in children

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An early diagnosis of developmental delay/cognitive deficiency leads to more efficient and personalized early intervention, and enables genetic counseling of the family. Children with developmental delay are at increased risk for behavioral defects and psychiatric diseases. Behavioral disturbance is often the presenting symptom of genetically determined cognitive deficiency caused by de novo dominant mutations, mild forms of rare recessive diseases, and pathogenic copy number variations (CNV). Next-generation sequencing

(NGS) has opened up new possibilities especially in the search for disease-causing mutations in disorders with common clinical features but a heterogeneous genetic background.

After excluding common genetic causes of developmental delay (chromosome and FMR1 analysis, chromosome microarray) 28 children with various degrees of developmental delay and behavioral disturbance as the presenting symptom, seen at the joint clinical genetic and psychiatric outpatient clinic of the Rheinhessen Clinic of Pediatric Psychiatry, were analyzed with the MPIMG-1 test, which provides panel diagnostics for over 1200 central nervous system related genes. For enrichment a TruSeq Custom Enrichment Kit (Illumina Inc., San Diego, USA) was used. 2x300 basepair paired-end sequencing (Illumina MiSeq Reagent Kit v3) was carried out on an Illumina MiSeq system. Read alignment was performed with SOAP version 2.2. A modified version of the Medical Resequencing Analysis Pipeline (MERAP, Hu et al, 2014) was used to check all detected variants against standard databases. For segregation testing Sanger sequencing were performed.

In three out of the 28 cases, pathogenic or likely pathogenic CNVs were found. A monogenic cause was identified in 7 out of 28 cases. We found 5 cases with mutations in genes associated with autosomal dominant neurodevelopmental syndromes, either previously reported and recognized as causing a disorder matching the patient's phenotype (PTPN11, SETBP1, sequence variation category 5), or likely associated with the phenotype (GRIN2B, ZEB2) but with a previously unreported sequence variation (category 4). In siblings with absent speech, intellectual disability, aggressive behavior and seizures, a homozygous ALG1 mutation was found associated with a mild form of a congenital disorder of glycosylation (CDG1k). In a further 4 patients, likely gene disrupting not yet reported sequence variants (category 3) were identified in known disease-causing genes (ASXL1, BRCA2, MBD5).

Our findings so far support the role of targeted NGS in delineating monogenic causes of a common clinical symptom associated with childhood developmental delay. A definitive diagnosis can aid in helping the family come to terms with the condition, and enables adequate personalized treatment and care. Furthermore a genetic diagnosis often helps caretakers to accept that behavioral problems can be caused by recognizable genetic defects rather than by parental failure.

P-ClinG-092

Distinctive Behavioral and Psychological Features in Tripe-X-syndrome

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Triple-X syndrome (47,XXX) is a relatively common chromosome aneuploidy with a prevalence of about 1 of 1000 females. The literature is ambiguous concerning the corresponding phenotype. Girls with triple-X are described typically quiet and passive; they are at increased risk for a delay in their motor and linguistic development as well as for learning impairment. They tend to have more problems in relationships, leisure and work. For putative mental health problems, limited data are available. In previous studies, increased rates of anxiety, depression and adjustment disorders have been described. Psychiatric disorders seem to be more common in girls and women with trisomy X compared to normo-karyotypic subjects.

The aim of our study was to investigate whether girls and women with Triple-X differ from normo-karyotypic girls and women with respect to their social-behavioral phenotype (behavior, emotions, self-esteem, relationships, competences in school) and traits related to mental health. We investigated three subgroups of girls and women with triple–X and corresponding healthy control groups: very young girls up to seven years, children and adolescents between 8 and 17 years, and adult women.

72 girls and women with triple-X and 69 normo-karyotypic girls and women were included in the study. Five different psychological and behavioral questionnaires were chosen and allocated to the subgroups.

We found differences between triple-X carriers and controls for all subgroups. For young girls, we found smaller effects with significant group differences for social as well as attentional problems and school competences. For the group consisting of children and adolescents between 8 and 17 years, we found major significant group differences concerning school competences and competences in social relationships, as well as for behavioral problems. Furthermore, we could show that the girls with triple-X have significant smaller general self-esteem, especially in relation to school and to their family. For the adult group, we found significant group differences concerning overall personality structure. Moreover, we could show that women with triple-X are affected in their daily life in matters of psychological distress. We could show that in all subgroups triple-X and normo-karyotypic subjects differ regarding their social-behavioral phenotype and mental-health-related traits. Our findings suggest that triple-X syndrome is relevant for mental-health issues and well being across the life span.

P-ClinG-093

Novel ADAMTSL2-Mutations in a patient with Geleophysic Dysplasia Type I

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Geleophysic dysplasia type I (GD1) is a rare autosomal recessive disorder characterized by short stature, small hands and feet, stiff joints and thickened skin. GD1 is caused by biallelic mutations in ADAMTSL2. We report on a male child with severe short stature, brachydactyly, characteristic dysmorphic features and developmental delay in whom we identified two novel biallelic mutations in ADAMTSL2 by massive parallel sequencing. This patient broadens the spectrum of GD1-associated ADAMTSL2 mutations and contributes to a more detailed genotype-phenotype correlation and a better understanding of this rare disorder.

P-ClinG-094

Mutant mice suffering from eye diseases show also alterations in their behaviour

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Introduction:

Many genes that are active during eye development are also expressed in the brain. Therefore, we tested the hypothesis that alterations in the structure and function of the eye are associated with changes in the behaviour of the same mice.

Materials & Methods:

To test this hypothesis, we used phenotypic annotations from 294 mutant mouse lines taken from the public Phenomap database (as of Nov. 30, 2015) of the German Mouse Clinic (http://www.mouseclinic.de/phenomap/phenomap.html) representing the overall assessment of more than 7 million single phenotypic records. We analyzed the frequency of clearly altered phenotypes in the eye and compared this to clear phenotypic differences between wild-type and mutants observed in other routine screens of the German Mouse Clinic.

Results:

36 (12.2%) of the 294 mutant mouse lines of the Phenomap database showed a clearly changed phenotype affecting eye and vision. It is surprising that 3 of the 36 affected genes are not yet listed in OMIM, and just in 11 of the 36 genes mutations are known to be causative for human diseases. For the other 25 genes (=70%) no human disorder has been described so far. Among these 36 mutant lines, 23 lines showed also clear differences to the wild-type phenotype in behaviour and clinical chemistry; an overlap to changes in neurological phenotypes is given in 17 mutant lines indicating that eye diseases might be used as biomarkers for neurological or psychiatric diseases.

Among the three mutant lines whose genes are not listed in OMIM (*Wsb2*, *Spryd3* and *Zfp119a*), *Wsb2* (WD repeat and SOCS box-containing 2) and *Spryd3* (SPRY domain containing 3) showed alterations in their behaviour and in neurological features beside the eye: the *Wsb2* mutants showed thinner retinae and a reduced number of fundic blood vessels, decreased rearing activity in the open field (exploratory behaviour), decreased acoustic reactivity and more tail elevation. The *Spryd3* mutants showed reduced eye size, sex-specific changes in the locomotor activity, increased center time (indicating decreased anxiety) and decreased prepulse inhibition (indicating alteration in sensorimotor gating like in schizophrenia).

Conclusions:

In humans, eye diseases can be observed much easier than behavioural alterations; examples for this aspect have been previously discussed for *PITX3* for cataracts and Parkinson's disease and *CRYBB1* or *CRYBB2* for schizophrenia (Graw, J.: Exp Eye Res. 2015; doi: 10.1016/j.exer.2015.11.006. Therefore, ophthalmologists might be aware of neurological problems in their patients.

P-ClinG-095

Mutation Spectrum in German Patients with Familial Hypercholesterolemia – An Update

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Familial Hypercholesterolaemia (FH) is characterized by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) and a dramatically increased risk to develop cardiovascular disease (CVD). Current data suggest that the prevalence of heterozygous FH (HeFH) is about 1:200-300 and about 1:160.000-300.000 for homozygous FH (HoFH) indicating that FH is clearly underdiagnosed in most countries. Mutations in three genes have been associated with the dominant form of FH: LDL receptor gene (LDLR), apolipoprotein B gene (APOB) and proproteinconvertase subtilisin/kexin 9 gene (PCSK9).

In our study we investigated the mutation spectrum in 205 patients (~56,6% females), of which 191 were apparently unrelated, clinically diagnosed in five specialized lipid clinics in Germany with possible or probable FH, based on the Dutch Lipid Clinic Network Criteria. In a three step mutation screening procedure (direct DNA sequencing) we sequenced the coding region of the LDLR gene, followed by screening for the site of the major disease causing mutation in the APOB gene, c.10580G>A (p.Arg3527Gln), and finally the coding region of the PCSK9 gene.

We found pathogenic mutations in 91 patients (44%) in one of the analysed genes. As expected, most of the mutations were identified within the LDLR gene (about 90%). The missense mutation c.10580G>A (p.Arg3527Gln) in the APOB gene was detected in eight patients (about 10%) and no mutation in the PCSK9 gene was found.

In 74 unrelated patients we detected a total of 80 LDLR mutations of which 49 were missense mutations. In addition we identified seven frameshift and one in-frame mutation, ten splice-site mutations, twelve nonsense mutations and one large duplication of four exons. Six of the 74 patients were probable compound heterozygous. Ten of the identified mutations have not been described before: c.340_344delTTTCG (p.F114LfsX12); c.455delG (p.S152TfsX54); c.540G>A (p.W180X), c.1072T>A (p.C358S); c.1277delT (p.M450X); c.[1561G>A; 1562C>T] (codon: GCC>ATC, p.A521I); c.1649_1668del20 (p.V550DfsX2); c.1880C>A (p.A627D); large duplication of Exon 14-17, c.2390-3C>T (splice-site).

We will give a comprehensive view on the FH mutation spectrum in Germany by including data from four previous studies on German patients in our analysis resulting in data from a total of 464 unrelated FH patients with 199 identified mutations.

P-ClinG-096

Prenatalis® NIPT: Accredited High Resolution Non-invasive Prenatal Testing by Using Massive Parallel Ultra-Deep Sequencing

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Prenatalis® is a non-invasive prenatal test (NIPT) based on Illumina´s Verifi technology that recently obtained as the first NIPT in Europe a DIN EN ISO 15189 accreditation by the German accreditation body DAkkS. The Prenatalis® NIPT provides an adjusted specific risk for trisomy 13, 18 and 21 and is recommended in pregnancies with intermediate risk values following first trimester screening or maternal age > 35 y. Prenatalis® is utilizing up to 3-fold higher sequencing coverage (20 Mio reads) compared to other test procedures, resulting in an improved performance in situations of fetal fraction below 4%. Here we report the results of 1000 analyses performed at a gestational age from 10th to 24th week. The fetal fraction averaged at 9,5% (SD: 4,6%) and included 17 samples of low fetal fraction (2,7% - 4%). A Z score-based NCV (normalized chromosome value) was calculated for each chromosome tested after normalization of the sequence read number to a sample internal reference chromosome set. This algorithm allows a high dynamic range of NCVs and therefore facilitates the classification regarding the presence of a numerical chromosomal aberration. The specificity of the test equals or is higher than 99,94% for all chromosomes tested. The sensitivity for the detection of trisomy 13, 18 and 21 was 99,14%, 98,31% and 98,15% respectively. The positive predictive value (ppv) for trisomy 21 (trisomy 18) tested in a high risk cohort was

99,4% (91,0%). In a low risk cohort, that is characterized by lower trisomy prevalence, the test demonstrated a 10x-fold increased ppv above first trimester screening (45,5% vs. 4,2%). Like any other genetic test, NIPT requires a priori genetic counseling and should be carried out under the supervision of a physician. Due to the complexity of prenatal diagnostics, we strongly recommend embedding NIPT in a medical, prenatal environment including professional medical interpretation and counseling. The Prenatalis® consortium (www.prenatalis.de) is a network initiative offering a platform for information exchange and continuous medical education for prenatal medicine professionals aiming at highest quality standards in patient care and analytics.

P-ClinG-097

NPC1/2 mutations are significantly enriched in a cohort of unexplained early onset ataxia.

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Patients with early onset ataxia (EOA; onset age <40 years) show a wide range of clinical symptoms. EOA is mostly caused by mutations in one of the many rare autosomal recessive (AR) genes within the rapidly expanding spectrum of ataxia genes. Niemann-Pick disease type C (NP-C) is an example for such a multisystemic neurodegenerative EOA disease, genetically caused by mutations in NPC1 and NPC2 genes. The clinical symptoms of NP-C patients are diverse and the initial differential diagnosis is often missed due to incomplete, atypical or missed phenotypic presentations. Therefore, a method was needed to identify NP-C patients before being undetected in adult patients of unexplained neurodegenerative disease.

In order to identify potential disease-causing mutations in NPC1 and NPC2 a series of EOA patients were compiled with a family history corresponding with AR disease. Patients were excluded having a known mutation in a gene initially suspected to be diseases-causing or having a pathogenic triplet repeat expansion in genes for SCA types 1, 2, 3, 6, 7, 17 and FRDA. The final cohort of 96 samples was processed for target genes of a custom-build ataxia specific HaloPlex panel and DNA libraries were sequenced paired-end (2x150bp) on an Illumina MiSeq. Data were analyzed with our in-house bioinformatics pipeline. Raw variant lists were filtered for rare NPC1 / NPC2 variants with following criteria: non-synonymous, MAF <1 % in open-access databases, ≤5 observations in in-house database. Variants were classified according to an updated version of the NP-C disease gene variation database. The final report listed only variants of uncertain significance (VUS3), possible pathogenicity (VUS4) and probable pathogenicity (VUS5). EOA patients with either homozygous or compound heterozygous known or novel disease-causing mutation were defines as “NP-C positive” patients.

On the technical side ≥99 % of NP-C target region (exons ± 20bp) was covered ≥20 x with a medium depth of 247 reads. On the analytical side 2 EOA patients with either 1 homozygous c.3019C>G NPC1 (VUS5) mutation or 2 compound heterozygous c.2861C>T (VUS5) and c.1822A>C NPC1 (VUS3) mutations were found. Also 4 EOA patients with only 1 heterozygous mutation were found [3x NPC1: c.3019C>G (VUS5), c.3477+4A>G (VUS3), c.2731G>A (VUS3) and 1x NPC2: c.292A>C (VUS3)]. The frequency of allelic observations in this EOA cohort (8/196 = 4,17 %) was significantly enriched in contrast to ESP6500 control population data (203/12962 = 1,57 %).

By using a disease specific gene panel we identified 2 NP-C positive patients (2,1 %) in our cohort of 96 EOA patients which is an obviously higher amount as in the general population (1/120000 in live births). Especially mutations in NPC1 are substantially enriched in this high-risk population. Our findings show that panel sequencing is able to identify NP-C cases in EOA patients which were missed by differential diagnosis due to phenotypically unspecific disease signs.

P-ClinG-098

Identification of a COL4A1 mutation in a boy with developmental delay, brain malformations, and bilateral cataract allows for prenatal testing in a subsequent pregnancy

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A 40-year-old pregnant woman was referred for genetic counselling by her gynaecologist to evaluate a potential risk of recurrence of the disease present in her older son: This 6-year-old boy had been born at 42

weeks of gestation, body weight 3330 g [P25-P50], length 52 cm [P50], head circumference 34 cm [P10-P25]; APGAR 5‘/10‘ 10/10. Due to delayed motor development, cMRT had been carried out revealing a complex pattern of malformations including closed-lip-schizencephaly, irregular configuration of the right ventricle, and bilateral cysts of the basal ganglia. Possible reasons discussed for these features were hypoxia/ischemia, haemorrhage or inflammation. Later, ocular involvement was recognized (strabism, bilateral cataract, hyperopia) and seizures developed. A final diagnosis could not be established at this stage. Over time, a profound developmental delay as well as microcephaly became obvious [head circumference 48 cm, 1.8 cm below P3 at 5 9/12 y]). The boy’s clinical features were re-evaluated on the occasion of his mothers‘ request for providing an estimate for a potential recurrence risk. This re-evaluation led to the speculation that a COL4A1-related disorder might be present and therefore molecular genetic analyses were carried out. A heterozygous mutation in the COL4A1 gene (c.2662G>A; p.Gly888Arg) was identified. This particular mutation has been described earlier as a cause of encephalopathy and brain haemorrhage. Both parents of our patient were proven not to be carriers of this mutation. Due to the possibility of germ line mosaicism, prenatal analysis was offered. The COL4A1 mutation was not detected in the fetus.

COL4A1 mutations were first described in 2005 as a cause of perinatal cerebral haemorrhage and porencephaly. Since then, the phenotypic spectrum has broadened and includes entities such as autosomal-dominant porencephaly, brain small-vessel disease with haemorrhage, hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC-syndrome). Ocular findings are congenital cataracts, microcornea, glaucoma, retinal arterial tortuosity, and others. The COL4A1 protein is part of the heterotrimeric collagen helix that is found in almost all basement membranes, for example in small vessels, the eye and other organs. COL4A1 mutations cause structural instability of this collagen molecule leading to phenotypic features mentioned above. So far, approximately 150 patients with COL4A1-related disorders have been reported in the literature. The phenotype of COL4A1-related disorders is variable even within the same family and reduced penetrance has been described, so that genetic counselling for these diseases is challenging.

P-ClinG-099

Focal Dermal Hypoplasia: Case report of patients with mutations in PORCN

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Focal Dermal Hypoplasia (FDH) (MIM #305600) or Goltz-Gorlin Syndrome constitutes a rare genetic multisystem disorder of the skin within the large spectrum of ectodermal dysplasias (Goltz et al., 1962) and is transmitted by an X-linked dominant inheritance. FDH is caused by mutations in the PORCN gene encoding a protein-cysteine N-palmitoyltransferase involved in Wnt pathway, which has an impact on embryonic development. Thus the syndrome affects tissues deriving from the ectoderm and the mesoderm and is characterized by a combination of malformations and dysplasias, mainly affecting the skin, skeleton, teeth and eyes (Goltz et al., 1962; Gorlin et al., 1963) with considerable variation in the clinical features. Hemizygous PORCN mutations are supposedly lethal in men. Therefore male patients (10% of entity) with FDH represent postzygotic mosaic cases or a Klinefelter syndrome (karyotype 47 ,XXY). Until now about 300 cases have been reported in literature.

Here we describe a large series of 10 patients with clinical characteristics of FDH of which 5 patients are definitely carrying mutations in PORCN. We detected 3 missense, 1 nonsense and 1 splice site mutation. The latter (c.720-1G>C) presents a novel mutation and is not yet described.

For two patients we additionally analyzed the parents and show in agreement with literature, that both mutations are generated de novo. 95% of described cases of FDH are known to carry de novo mutations.

The occurrence of genetic mosaicism in this syndrome poses a challenge for diagnostics: whether the proportion of mutated blood cells in genetic analysis is too low, it is of great significance to analyze DNA from the affected skin. In this regard we found mutations in 4 patients by sequencing PORCN based on blood cells. For one further patient a mutation could be only detected by sequencing the DNA isolated from biopsy that was not present in blood. The remaining 5 patients respectively show negative results by sequencing DNA based on blood cells and they don’t carry deletions or duplications in PORCN. If biopsies will be available, we could sequence the DNA out of it and probably find more causative mutations.

For diagnostics it implicates the necessity to sequence at first the DNA from blood cells, then to analyze the respective genomic region for deletions or duplications and in case of negative results to examine additionally the DNA from affected cells of a biopsy.

P-ClinG-100

Impact of pregnancy and postpartum period on disease course in 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. AA affects both sexes and all age groups. AA is divided into three main clinical types on the basis of the degree of hair loss and the sites affected: (i) patchy AA; (ii) AA totalis, affecting the whole scalp; and (iii) AA universalis, affecting the whole body. The course of disease is highly variable and nearly unpredictable. Patients might experience spontaneous and complete remission, but also sudden relapse at any time, a chronic course, or even rapid progression towards AA totalis or AA universalis.

Given the lack of reliable treatment options and the uncertain prognosis of disease course, AA often results in pronounced psychological distress. In particular, female sex and young age are risk factors for significant impairment in quality of life. The course of AA during pregnancy and postpartum is therefore a clinical important question and a matter of urgent concern for many female patients. However, there are no systematic data on the effects of pregnancy on AA.

This study was the first to systematically collect reliable information regarding influence of pregnancy on AA and to examine maternal and fetal outcome. We performed a retrospective study of 453 pregnancies in 252 German women with a dermatologist-assigned diagnosis of AA.

Data obtained by detailed questionnaires were analyzed, comprising more than 70 items including i) clinical picture of AA, ii) detailed information on disease course before pregnancy and on pregnancy-related and postpartum disease course, iii) comorbidity profile, iv) information on pregnancy and delivery, v) information on pregnancy complication and vi) information on fetal outcome.

Our results highlight the risk of pregnancy-related relapse as well as exacerbation of AA and point to an increased risk of relapse during postpartum period. Disease worsening as well as relapse of AA was most pronounced at late pregnancy whereas activity of disease remained quite stable in early pregnancy. After delivery, no significant worsening of existing disease was observed but a significant increased rate of women with relapse with strongest effects from 6 months postpartum onward. Our results point to pregnancy-related and postpartum flares, suggesting that pregnancy as well as time after delivery confer increased susceptibility to enhanced disease activity. Furthermore, the risk for adverse maternal and fetal outcome is found to be slightly increased.

In summary, our findings provide strong evidence for an unfavorable course of AA during pregnancy and postpartum. It will be a challenge for the future to develop effective drugs that are also compatible with pregnancy. Furthermore, our data point to a potentially increased risk of adverse maternal and fetal outcome which implicates the need of careful medical advisement during pregnancy.

P-ClinG-101

A novel patient with mental retardation and seizures due to a mutation in UPF3B identified by next generation sequencing

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Mutations in UPF3B are known to cause X-linked intellectual disability (XLID) with variable associated features such as seizures and autism or behavioural problems.

We report on a 16-year-old boy with intellectual disability, seizures and an atrial septal defect. He, born at term, developed feeding problems at the age of 4 month, making a tube feeding necessary. He had no speech but conversation with nonverbal interaction was possible. Verbal comprehension was slightly better. Seizures started at the age of twelf years. Facial dysmorphic features were mild including a high nasal bridge with prominent columnella and hypoplastic alae nasi. The face appeared long and thin.

The affected boy had a healthy, one year older brother and a healthy maternal half brother of two years age. There was no history of mental retardation in the extended family.

CGH array and cytogenetic analysis as well as Fragile X syndrome gave normal results, sequencing of MECP2 and CRESBBP did not reveal any pathogenic mutation. However, X-inactivation analysis of the

mother detected a skewing with a ratio of 97:3. In suspicion of X-linked intellectual disability (XLID) next generation sequencing was performed in 110 X-linked genes.

A VUS 4 variant was found (c.1188_1189del; p.Glu396fs). Segregation analysis showed that the variant was detected in the mother and not in the healthy maternal uncle. This variant is leading to a premature termination codon. There are only a few cases of UPF3B mutation reported in literature. Most of the recently reported patients with a mutation in this gene do not show a recognizable phenotype. A long and thin face and a high nasal bridge seemed to be associated facial features.

The combination of X-inactivation and next generation sequencing is helpful in making the right diagnosis in patients with unspecific intellectual disability.

P-ClinG-102

BRAT1 mutations are associated with infantile epileptic encephalopathy, mitochondrial dysfunction and survival into childhood

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Via next generation sequencing of the disease-associated genome followed by phenotype-driven bioinformatic analysis we identified compound heterozygous mutations in BRAT1 exons 5 (c.638_639insA) and 8 (c.1134+1G>A) in one of two affected brothers with focal seizures of early onset, severe progressive postnatal microcephaly, muscular hypertonia, feeding problems and bouts of apnea, only minimal psychomotor development as well as death in childhood. Sanger sequencing confirmed these mutations in this patient and his similar affected brother who died in neonatal age as well as a heterozygous status in the parents. Whereas the frameshift mutation (c.638_639insA) has been described in one family, the splice site mutation (c.1134+1G>A) is novel. Recently, four independent exome sequencing projects identified homozygous and compound heterozygous mutations in BRAT1 in individuals from four families with early onset epilepsy. Because of additional severe muscular hypertonia and early death, this condition was called lethal neonatal rigidity and multifocal seizure syndrome. In contrast to all cases published so far, one of our patients showed a considerably milder clinical course with survival into childhood. The reduced cytochrome c oxidase activity found in muscle tissue of patient 2 indicates an important role of BRAT1 in mitochondrial metabolism. Our data expands the clinical and mutational spectrum of the BRAT1 associated phenotype.

P-ClinG-103

3M syndrome - a report of two families

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3M syndrome is a rare autosomal recessive disorder characterized by severe growth retardation, dysmorphic features, large head circumference but normal intelligence and endocrine function. This syndrome also has characteristic radiological features, such as slender long bones and tall vertebral bodies. 3M syndrome can be caused by mutations in the CUL7, OBSL1 and CCDC8 genes.

Here we present two boys from two unrelated families with the characteristic features and radiological findings of the 3M syndrome. The main features in our cases were prenatal growth retardation, low birth weight, severe postnatal growth retardation, characteristic facies (relatively large head, triangular face, hypoplastic midface, fleshy nose tip, long philtrum, prominent mouth and lips, pointed chin) and tall vertebral bodies. Both children have no spina bifida or hypogonadism.

In the first boy compound heterozygosity of a known missense mutation and a previously undescribed splice site mutation, which may result in skipping of exon 24, were detected. In the other boy a known stop mutation was found in homozygous state. Heterozygosity for these mutations were confirmed in their parents, respectively.

3M syndrome is a rare but important differential diagnosis in children with severe short stature and mild skeletal changes. This syndrome may be underdiagnosed because of the phenotypic overlap with other severe growth retardation syndromes. Growth hormone treatment might be beneficial in improving stature in affected children.

P-ClinG-104

Mutation analysis in a cohort of 680 families with clinical diagnosis of ARCI

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of rare disorders of keratinization characterized by generalized abnormal scaling of the skin. The main skin phenotypes of ARCI are harlequin ichthyosis (HI), lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). Minor variants of ARCI are self-improving collodion baby (SICB) and bathing suit ichthyosis (BSI). Eight genes are currently known to be associated with ARCI: TGM1, ALOXE3, ALOX12B, NIPAL4 (ICHTHYIN), ABCA12, CYP4F22, PNPLA1 and CERS3.

In this study we present a large cohort of 680 families with ARCI. This cohort includes patients with ichthyosis symptoms at birth and no signs of systemic disease. Families with an autosomal dominant mode of inheritance were initially excluded. For mutation analysis we performed Sanger sequencing of ARCI genes and/or next generation sequencing with gene panels. Variants were investigated in several databases (e.g. dbSNP, EXAC, HGMD Professional, PubMed) and bioinformatic tools (e.g. SIFT, Polyphen, Mutationtaster). ARCI-associated gene mutations were found in 73.5% of the cases. In 26.5% no mutation was found, this implies that further loci for ARCI must exist.

TGM1 mutations were detected in 26.0% of all cases in which we found mutations. This result confirms the previous findings that TGM1 mutations are the most common cause for ARCI. The second most common cause are mutations in NIPAL4, which affect 17.0% of our ARCI patients, followed by mutations in ALOX12B in 16.0% of the cases. Mutations in other ARCI genes were found less often: We detected mutations in CYP4F22 in 10.5%, in ABCA12 in 8.5%, in ALOXE3 in 6.5%, in PNPLA1 in 3.5% and in CERS3 in 2.0% of our cases. In 10% we detected mutations in other genes: Most of them show mutations in CGI58/ABHD5, which can lead to Chanarin-Dorfman syndrome, a syndromic form of ichthyosis. In occasional cases we found mutations in the following genes with decreasing frequency: STS, ALDH3A2, FATP4, KRT10, DSG1, FLG, KRT2, KRT1, SPINK5, ST14 and GFT2H5. We further discovered novel mutations in several genes.

In our study we show the distribution of ARCI mutations in a large cohort of 680 families. Our results can help to improve the molecular diagnostic procedure, since in many ARCI patients several genes were analyzed consecutively. The distribution of ARCI mutations can help to define an order of genes for analysis and therefore can reduce time and costs of analysis. It is notable that in 10% of our cases an initial diagnosis of ARCI had to be revised since we found mutations in other genes. It is important for mutation analysis to get clear and precise information about the patients' phenotype. In cases where no mutation was found, further genes should be analyzed. Gene panels with ARCI genes and further genodermatoses genes are consequently an ideal method for fast and low-cost analysis that includes a large number of genes in which mutations could be found.

P-ClinG-105

Limb girdle muscular dystrophy due to mutations in DYSF and FKRP in one patient

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We report on a 37 year old female with unspecific muscular complaints and unsuspicious family history. First symptoms were noted as underperformance in school sports. Since the age of 16 years muscular pain and cramps appeared after short-time exercises, especially in the large joints. Besides, muscular pain also emerged at rest affecting the whole body including the masticatory muscles and lasting for days. Cardiological examinations were unremarkable. CK-level was extremely elevated (>60 times) at last investigation, calf and shoulder muscles were enlarged.

A muscle biopsy at age 27 years exhibited unspecific dystrophic changes with an increased spectrum of myofiber diameter and without evidence of inflammation. Immunohistochemistry revealed regular expression of sarcolemma- and membrane proteins. Thus the patient´s symptoms fitted limb-girdle muscular dystrophy (LGMD) but differentiation was difficult due to the large number of mainly autosomal recessive genes associated with this condition. Therefore sequencing using the TruSight One Sequencing Panel was performed. Unexpectedly, compound heterozygous missense variants were detected in two autosomal recessive genes, DYSF and FKRP. After Sanger validation segregation analysis revealed one mutation in each gene in the mother confirming compound heterozygosity. Both FKRP mutations (p.(Leu276Ile) and p.(Val300Ala)) were repeatedly described as causative for LGMD2I, with p.(Leu276Ile) being known as a founder mutation. Dysferlin variants (p.(Y1494H) and p.(R1859C)) have not been reported in the literature, so far, but affect two highly conserved amino acids. Computer modelling predicted deleterious effects for both variants. Dysferlinopathies include a spectrum of muscle diseases with one main phenotype being limb-

girdle muscular dystrophy type 2B (LGMD2B). Of note, clinically and histomorphologically presentations in this individual was compatible with either LGMD2I or LGMD2B.

Dysferlin is a transmembrane protein located at sarcolemmal membranes implicated in muscle fiber repair, while FKRP localizes at medial Golgi apparatus and plays a critical role in posttranslational modification of alpha-DAG. The fact that this individual is compound heterozygous for two genes but is not more severely affected than patients with mutations in each gene alone suggests that both proteins have no additive effects. This is in accordance with two Hutterite brothers with limb girdle muscular dystrophy and mutations in Dysferlin and TRIM32 (LGMD2H). These boys did not differ in age at onset or mode of presentation or serum CK levels compared to age-matched individuals with only LGMD2I or LGMD2H alone.

It is unclear, if this finding of double compound heterozygosity is a rare event or might be more common, since previously only single genes were analysed consecutively based on a clinical suspicion and mutations in a second gene may have been overlooked. These could have important consequences for diagnostics, genetic counselling, therapy and prenatal diagnosis.

P-ClinG-106

23 years of predictive HD testing at the Huntington Centre in Bochum (Germany) since 1993.

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The discovery of the mutation causing Huntington's disease (HD) in 1993 allowed direct mutation analysis and predictive testing to identify currently unaffected carriers with a sensitivity and specificity of virtually 100%. We comprehensively profiled the participants who sought predictive testing for HD in our Huntington Centre between 1993 and 2009. Using a retrospective design, we analyzed the written documentation of the counselling sessions for all referrals for predictive mutation testing in this time span. Six hundred sixty-three individuals at risk for HD requested predictive testing. Roughly half (n = 333) completed the protocol and asked for their test result. In general our findings are in accordance with other reports: most participants share an a priori risk of 50% (91.1%); more females request testing (58.5%); and those who request the result are mostly in their 30s (mean = 35.1 years). Of those at 50% or 25% prior risks, 47.4% and 22.7%, respectively, tested positive in accordance with the respective risk of inheriting HD. Generally, more participants with an affected mother than father sought genetic testing (52.5% versus 47.5%). Interestingly, this difference was especially evident in the group of females who finally withdrew from testing (59.1%, p = 0.040). Men, in particular those who decided in favour of the test, were more often accompanied by their partner in the pre-test counselling session than vice versa (67.9% versus 44.7%, p = 0.003). On the other hand, significantly more men who were being tested did not have a companion in the pre-test session as compared with men who decided against the test (40.0% versus 25.7%, p = 0.012). During the first four years of predictive testing (1993–1996) more participants completed the protocol and received their test result as in later years. Yet, in this early time span significantly fewer females finally decided in favour of the test (48.4%, p = 0.005). We discuss these findings longitudinally and extend the evaluations concerning gender-specific aspects of decision-making until the end of 2015.

P-ClinG-107

Mosaic KRAS mutations in patients with oculoectodermal syndrome and encephalocraniocutaneous lipomatosis

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Oculoectodermal syndrome (OES) and encephalocraniocutaneous lipomatosis (ECCL) are rare disorders that share many features such as epibulbar dermoids, aplasia cutis congenita / focal alopecia, pigmentary changes following Blaschko lines, bony tumor-like lesions and others. About 20 cases with OES and more than 50 with ECCL have been reported in the literature. Both were long proposed to represent mosaic disorders, but only recently whole genome sequencing has led to the identification of somatic KRAS mutations, p.(Leu19Phe) and p.(Gly13Asp), in affected tissue from two individuals with OES. Here we report

the results of molecular genetic studies in two patients with OES and one with ECCL. In all three cases, Sanger sequencing of the KRAS gene in DNA from lesional tissue detected mutations affecting codon 146 (p.(Ala146Val) and (p.Ala146Thr), respectively) at variable levels of mosaicism, while the mutation was absent from leukocyte DNA. Our findings thus corroborate the evidence of OES being a mosaic RASopathy and confirm the common etiology of OES and ECCL. Our three cases suggest KRAS codon 146 as a specific mutational hotspot for these conditions. KRAS codon 146 mutations as well as the previously reported OES-associated alterations are known oncogenic KRAS mutations, predominantly observed in intestinal cancers, and have distinct functional consequences. Considering the phenotype and genotype spectrum of mosaic RASopathies these findings suggest that the wide phenotypic variability is not only depending on the tissue distribution but also on the specific genotype.

P-ClinG-108

A CADASIL-like phenotype in patients with cysteine-sparing mutations in the NOTCH3 gene

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Clinical manifestations of CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) are migraine with aura, recurrent ischemic strokes, mood disturbances and cognitive impairment. The age of onset and the clinical picture are highly variable between and within families. White matter hyperintensities in magnetic resonance imaging (MRI) precede the onset of clinical symptoms. This arteriopathy is characterized by degeneration of vascular smooth muscle cells and deposits of granular osmiophilic material (GOM). CADASIL is caused by mutations in *NOTCH3*. Almost all mutations result in the loss or gain of a cysteine residue in one of the 34 epidermal growth factor-like repeat domains. There are just a few reports on cysteine-sparing mutations and it is still a matter of debate, especially for protein truncating mutations, whether or not these alterations also cause CADASIL or a CADASIL-like phenotype.

Here we report three patients with suspicion of CADASIL and *NOTCH3* mutations not altering the number of cysteine residues.

Patient 1 is a 36 year old woman who presented with paroxysmal headache, cognitive impairment, and leukoencephalopathy as well as further symptoms (hyposmia, resting tremor, muscle pain and cramps, narrowing of the visual field, loss of hair). A skin biopsy showed no evidence of GOM. In molecular genetic analysis of the *NOTCH3* gene (NM_000435.2) the heterozygous sequence alterations c.2293C>T, p.Gln765* and c.3328-8C>A were detected. cDNA analysis revealed a residual expression of 5-20 % for the c.2293C>T allele and no aberrant splicing regarding c.3328-8C>A.

In patient 2, a 67 year old male, the suspicion of CADASIL was raised due to the leukoencephalopathy in MRI. He also exhibited vertigo for the past 5 years, progressing gait disturbances, headache, fatigue and personality change. In the *NOTCH3* gene the heterozygous sequence alteration c.2853_2857del, p.Pro952Leufs*9 was detected.

Patient 3 is a woman aged 56 with ischemic strokes, MRI anomalies suggestive of CADASIL and positive family history. In this patient the recently described heterozygous sequence alteration c.239A>G, p.Asp80Gly in the *NOTCH3* gene was detected (Wollenweber et al. 2015 Stroke Mar;46:786-92).

Especially the relevance of mutations predicted to cause premature protein truncation has to be further elucidated. In line with some previous reports of patients with *NOTCH3* alterations not affecting cysteine residues as well as in Notch3 -/- mice, GOM deposits seem not to be an obligate hallmark.

P-ClinG-109

First case of maternal uniparental isodisomy of chromosome 8 in Meckel Gruber Syndrome

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Meckel Gruber Syndrome (MKS) is a rare autosomal recessive, pre- or perinatal lethal disorder characterized by the combination of central nervous malformation (usually occipital encephalocele), ductal plate malformation of the liver, multicystic dysplastic kidney and postaxial polydactyly. For MKS causative mutations in at least 12 genes have been identified to date and *MKS1*, *MKS3*/*TMEM67*, *MKS4*/*CEP290* and *MKS6*/*CC2D2A* represent the most frequently mutated genes.

We report molecular characteristics of a nonconsanguineous German family in which antenatal fetal ultrasonography during the 14th gestational week showed multicystic dysplastic kidneys, enlarged cisterna magna and was suggestive of occipital encephalocele. Meckel Gruber syndrome was suspected and parents opted for termination of pregnancy. Fetal DNA was retained, but parents declined fetal pathological examination.

A NGS-based panel diagnostic including the four genes *MKS1*, *MKS3*, *MKS4* and *MKS6* was performed in the fetus and revealed an apparently homozygous missense mutation c.1010A>G (p.Tyr337Cys) in exon 11 of the *MKS3* gene. This variant has not been described in the literature and is predicted to be pathogenic based on PolyPhen, SIFT and Mutations Taster. It has not be found in the SNP databases, has been reported with a allele frequency of 1.657e-05 (2/120722) in the databases of the Exome Aggregation Consortium (ExAC) and the affected residue is highly conserved across species. Thus we suggest that this variant is likely to be disease-causing.

Segregation analysis showed that only the mother was heterozygous for the mutation. A genome wide SNP array was carried out and showed complete uniparental isodisomy of chromosome 8, probably the product of postzygotic endoduplication. The maternal origin of both chromosomes 8 was confirmed by haplotype analysis with microsatellite markers.

To our knowledge there are only four reports of chromosome 8 complete maternal uniparental disomy and only one case of complete maternal isodisomy of chromosome 8 that unmasks autosomal recessive mutations. Notably, uniparental disomy (UPD) has never been described associated with MKS. Even if rare, awareness of UPD and comprehensive work-up in case of unexpected homozygosity for a recessive mutation is essential for accurate genetic counseling as recurrence risk is low or negligible in case of UPD.

P-ClinG-110

A novel form of cutis laxa with signs of a congenital disorder of glycosylation

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Cutis laxa is a hallmark of a variety of congenital disorders that often present with a progeroid aspect. So far only two of those syndromes are associated with glycosylation defects: ATP6V0A2-related autosomal recessive cutis laxa type 2A (OMIM # 219200), and RIN2-related macrocephaly, alopecia, cutis laxa, scoliosis syndrome (MACS; OMIM # 613075). We here describe a male child from a consanguineous Turkish couple with normal birth parameters who developed macrocephaly due to hydrocephalus and displayed Wormian bones, delayed fontanel closure, and a hypoplastic corpus callosum. The face looked progeroid with prominent, arched eyebrows, sagging cheeks, malar hypoplasia, and a short nose with anteverted nares. Skin was lax and translucent with paucity of subcutaneous fat tissue. Hyperextensible joints and bilateral inguinal herniae furthermore underlined a generalized connective tissue involvement. Psychomotor development was delayed with head control achieved at five months and no sitting at the last examination at the age of seven months. Transferrin isoelectrofocussing revealed a mild type 2 glycosylation deficiency. Sequencing of ATP6V0A2 and RIN2 did not reveal any mutations. We suggest that this phenotype represents a novel subform of cutis laxa, most likely with autosomal recessive inheritance.

P-ClinG-111

Psychological Impact of Carrying a BRCA1/2 Mutation - An Explorative Study

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Breast cancer is the most common malignancy in women in Europe and the United States.

BRCA1 and BRCA2 are major genes associated with hereditary breast cancer and are transmitted in a dominant fashion. Mutations in these highly penetrant genes implicate further stress-related actions for the patients, like chemo-and radiotherapy as well as surgery and testing of close family members.

In this cross-sectional study we examined the effect of BRCA1/2 test results on the psychological distress of breast cancer patients in a German sample. Women with a pathogenic mutation of the BRCA1/2 gene(n=22) and without(n=52) participated in the study. All underwent prior genetic consulting and molecular testing. The patients completed self-report questionnaires measuring sociodemographic data, metacognitive beliefs (MCQ-30), quality of life (EORTC C-30 & EORTC BR-23), anxiety and depression

(HADS),followed by a brief telephone interview. The preliminary results indicate that there seems to be no significant difference between the psychological well-being of women with breast cancer carrying the BRCA1/2gene mutation compared to the ones without a pathogenic mutation.

P-ClinG-112

Exome sequencing identifies a novel heterozygous TGFB3 mutation in a disorder overlapping with Marfan and Loeys-Dietz syndrome

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Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS) are clinically related autosomal dominant systemic connective tissue disorders. Although mutations in several genes of the TGF-beta signalling and related pathways have been identified in the past (e.g. FBN1, TGFBR1, TGFBR2, SMAD3, TGFB2), there are still many individuals with "marfanoid" phenotypes in whom no causative mutations are identified. We performed whole exome sequencing in two of three affected individuals from a family with phenotypic features overlapping MFS and LDS. The two affected children and their affected father had tall stature, arachnodactyly, hyperextensible joints, hypertelorism, bifid uvula, but no cardiac involvement, aortic dilation or eye involvement. We detected a novel heterozygous mutation in TGFB3, c.898C>G, predicting the missense substitution p.Arg300Gly. Sanger sequencing confirmed the mutation and its segregation with the phenotype. The first two TGFB3 mutations were reported previously in two unrelated individuals with marfanoid features: one individual with growth retardation carried a heterozygous mutation (c.1226G>A; p.Cys409Tyr; Rienhoff et al., 2013), whereas a child with overgrowth carried a mutation in the same codon as the mutation identified in the three affected individuals reported here (c.899G>A; p.Arg300Gln; Matyas et al., 2014). The mutations at codon Arg300 presumably lead to increased TGF-beta signalling, suggesting that the short or tall stature seen in patients with TGFB3 mutations may result from opposing effects of mutations on TGF-beta signalling. Thus, we add a novel human TGFB3 mutation, contribute to the clinical delineation of the emerging connective tissue disorder meanwhile called Rienhoff or Loeys-Dietz syndrome 5 and compare the data with a recent report by Bertoli-Avella et al. (2015) on TGFB3 mutations associated with aortic aneurysms or dissections.

P-ClinG-113

Utility of a next-generation sequencing-based gene panel investigation in German patients with genetically unclassified limb girdle muscular dystrophy

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Limb girdle muscular dystrophies (LGMDs) are genetically heterogeneous and the diagnostic work-up including conventional genetic testing using Sanger sequencing remains complex and often unsatisfactory. We performed targeted sequencing of 23 LGMD-related genes and 15 genes in which alterations result in a similar phenotype in 58 patients with genetically unclassified LGMDs. A genetic diagnosis was possible in 19 of 58 patients (33 %). LGMD2A was the most common form, followed by LGMD2L and LGMD2I. In two patients pathogenic mutations were identified in genes that are not classified as LGMD genes (glycogen branching enzyme and valosin containing protein). Additionally, in 6 patients heterozygous variants of unknown pathogenicity were identified. Thus, a focused next-generation sequencing-based gene panel is a rather satisfactory tool for the diagnosis in unclassified LGMDs.

P-ClinG-114

Mutations in WNT9B are associated with Mayer-Rokitansky-Küster-Hauser syndrome.

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Mayer–Rokitansky–Küster–Hauser syndrome (MRKHS), which is found in approximately 1 out of 4,500 women, is a complex malformation pattern of the Müllerian ducts (MDs) characterized by congenital absence of the uterus and vagina. Mutations in *LHX1*, *TBX6* and *WNT4* have been reported in a minority of patients with MRKHS. However, most cases still remain unexplained at a molecular level. Since female *Wnt9b*-/- mice show a MRKHS-like phenotype, *WNT9B* has emerged as a promising candidate gene for this disease.

We performed retrospective sequence analyses of *WNT9B* in 226 female patients with disorders of the MDs including 109 patients with MRKHS, as well as in 135 female controls. 14 patients with signs of hyperandrogenism were additionally screened for *WNT4* mutations.

One nonsense mutation and five likely pathogenic missense mutations were detected in *WNT9B*. Five of these mutations were found in cases with MRKHS accounting for 4.6% of the patients with this phenotype. No pathogenic mutations were detected in the control group (p=0.017). Interestingly, all of the MRKHS patients with a **WNT9B** mutation were classified as MRKHS type 1, representing 8.5% of the cases from this subgroup. No mutation in *WNT4* was detected. In previous studies, two of the patients with a *WNT9B* mutation were found to carry either an additional deletion of *LHX1* or a missense mutation in *TBX6*.

Mutations in *WNT9B* were frequently associated with MRKHS in our cohort. Some of the cases with MDs anomalies may be explained by a digenic disease-model.

P-ClinG-115

Hermansky-Pudlak syndrome genes are frequently mutated in patients with albinism from the Arabian Peninsula

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Albinism is clinically and genetically heterogeneous and characterized by the complete or partial absence of pigment in the skin, hair and eyes. Ocular affection varies and may include nystagmus, reduced visual acuity and photophobia. Oculocutaneous albinism (OCA) affects the eyes, skin and hair, whereas ocular albinism affects the eyes only. Inheritance is autosomal recessive except for X-linked isolated ocular albinism (OA1). Rarely, albinism is part of systemic disease such as Hermansky-Pudlak syndrome (HPS). HPS is a ceroid lipofuscin storage disease characterized by albinism, bleeding diathesis, platelet abnormalities, and sometimes fibrosis of various organs, and results from mutations in at least nine genes (*HPS1-9*). To date, there has been no systematic analysis of the genetics of albinism in the Arab world, where consanguinity is common. We conducted next-generation sequencing of a panel of 16 albinism genes for a cohort of Saudi Arabian patients from 19 families who had been referred for low vision and diagnosed with albinism affecting the eye. Nineteen different homozygous or hemizygous albinism gene mutations were identified in all 19 families: eight in HPS genes, five in *GPR143*, four in *TYR*, one in *C10orf11*, and one in *TYRP1*. All but three albinism gene mutations have not been previously reported. Of note, OCA due to *C10orf11* has so far been restricted to families from the Faroe Island, and to one patient from Lithuania. The finding of HPS gene mutations in 8/19 families is remarkable and clinically relevant. Although virtually all patients with homozygous mutations in one of the HPS genes appeared non-syndromic by history, they are still at risk for subclinical and/or later extraocular manifestations for which they require targeted clinical follow-up.

P-ClinG-116

Novel missense mutations in GUCY2C and NAA10 in a patient with meconium ileus, ataxia and global developmental delay

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We present here a nine year old boy with global developmental delay, ataxic gait, and history of meconium ileus. He is the first child of healthy unrelated parents of European descent. Already at 20 weeks of gestation a hyperechogeneity of the gut suggestive of ascites, followed by hydrops fetalis and polyhydramnion were evident. He was born at 33 weeks of gestation with normal birth weight, length and occipitofrontal head circumference. In addition to meconium ileus, complications such as ileal atresia, ileal perforation and volvulus were observed at birth, for which he received surgery several times. We first saw the boy at the age of 7 years. Clinical examination revealed a friendly though restless boy with short stature, ataxic gait, slurred speech, global developmental delay, delayed bone maturation and facial dysmorphisms including hypertelorism, orofacial hypotonia with open mouth appearance and Salivation, and a long face. To elucidate the genetic cause of his condition we performed trio whole exome-sequencing. Notably, we identified both a novel homozygous missense mutation in GUCY2C, p. Asp662Tyr, and a novel hemizygous missense mutation in NAA10, p. Cys17Gly. Both of which are located within evolutionary conserved regions and are predicted to be probably damaging by Polyphen2. In addition, both were not observed in dbSNP136, the ExAC database or the 1000 Genomes data, indicating that they are very rare in the population and unlikely to be disease-unrelated alterations. Heterozygous mutations in GUCY2C were previously identified as the cause of an autosomal dominant form of early-onset chronic diarrhea associated with increased susceptibility to inflammatory bowel disease, small-bowel obstruction, and esophagitis. Moreover, biallelic mutations in GUCY2C were previously associated with isolated, non-cystic fibrosis related, meconium ileus, in all together 3 families. On the other hand, mutations in NAA10 have so far been described in four distinct phenotypes, all of which are characterized by global developmental delay. These syndromes could commonly be referred to as N-terminal acetyltransferase deficiency (NATD) syndromes. Above further expanding the clinical variability of NATD syndromes, our findings further highlight the power of trio-WES in dissecting the genetic components of seemingly monogenic human diseases.

P-ClinG-117

Multi-Gene Panel Analysis in the Primary Diagnosis of Limb-Girdle Muscular Dystrophy

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Limb-girdle muscular dystrophies (LGMD) are characterized by great clinical and genetic heterogeneity. Mutations in a number of genes lead to overlapping phenotypes that cannot always be differentiated clinically or through muscle biopsy. Molecular genetic testing is therefore becoming increasingly important to the initial diagnosis of potential dystrophinopathies with significant clinical and familial consequences.

Our cohort comprised more than 80 patients with a suspected differential diagnosis of Becker-Kiener muscular dystrophy and negative test results for 1) DMD gene dosage analysis by MLPA, and 2) DMD gene point mutation analysis by Next-Generation-Sequencing (NGS). As over 700 neurogenetic/neuromuscular genes were captured during the course of the technical analysis by NGS, we were able to perform a second-tier expanded data analysis of a gene panel including more than 140 genes known to be responsible for congenital muscular dystrophies or myopathies. Target enrichment with a custom Agilent SureSelectXT Kit was followed by massive parallel sequencing (Illumina NextSeq500). Data analysis was performed with a bioinformatics pipeline consisting of BWA, SAMtools, snpEff and Alamut-Batch. Diagnostic quality criteria assured a coverage depth of more than 30 sequences per base pair in at least 98% of the analyzed regions with adequate sensitivity and specificity (>99,85%) in variant calling. For the DMD gene more stringent criteria were demanded (100% sequencing of the coding region and flanking intronic sequences at >30-fold).

More than thirty percent of patients tested were found to have a disease-causing mutation in another gene and therefore given a definite genetic diagnosis. Other patients were found to have sequence variants of unknown clinical significance; the potential relevance of these variants to the disease cannot be assessed with certainty without further testing.

Multi-gene panels utilizing next-generation sequencing (NGS) technologies enable the sensitive, cost-efficient, and simultaneous analysis of multiple disease-relevant genes. However, despite the broad analysis, it is not possible to identify pathogenic mutations in all patients. There is also a risk that the analysis of a large number of genes independent of the clinical phenotype may identify variants that can be assessed only in combination with refined clinical or muscle biopsy data and/or segregation analysis.

P-ClinG-118

Whole-Exome Sequencing detects a novel homozygous RYR1 splice-site variant related to a fetal lethal phenotype.in a fetal lethal phenotype.

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Prenatal ultrasonography identifies an increasing number of undescribed fetal malformation phenotypes. Little attention has been paid to use whole-exome sequencing (WES) strategies for gene identification in fetal disorders that are lethal in utero, because they are extremely rare, may appear to be sporadic, and Mendelian inheritance can be easily missed. Some lethal phenotypes, however, indicate an error in early development implying a major malfunction of a gene with a crucial role in cellular and developmental processes.

Hypothesizing that truncating autosomal recessive variants are an important cause of early human lethality, we use WES in order to identify novel variants and genes in families with recurrent fetal phenotypes suggesting autosomal recessive inheritance.

Here we report on presumably non-consanguineous parents of Turkish descent, who had five pregnancies including one early miscarriage and two pregnancies resulting in healthy daughters. The mother reports on decreased fetal movement in both the 4th and 5th pregnancies. In the 4th pregnancy fetal death occurred in the 5th month due to a deteriorating fetal hydrops; no autopsy was performed. The 5th fetus presented with hydrops as well and died in the 24th gestational week. Both fetuses were male and chromosome analysis was normal. The autopsy confirmed a generalized flexion arthrogryposis and revealed an overall important hypoplasia of skeletal and smooth muscle tissues. Family history was otherwise unremarkable.

We used family-based WES in order to determine the cause for this lethal phenotype. We identified homozygous variants in the skeletal ryanodine receptor 1 (RYR1) and the collagen α3 chain (COL6A3) genes and confirmed autosomal recessive segregation in the family. No deleterious compound heterozygous or X-linked variants were identified. The candidate variant in RYR1 is novel and affects an essential splice-site. Mutations in the skeletal ryanodine receptor (RYR1) are described to cause various myopathies. The phenotypes of these myopathies vary greatly between individuals, but decreased movement in utero with arthrogryposis, kyphoscoliosis and polyhdramnios in some fetuses was described. Variants in the COL6A3 gene are known to cause Bethlem myopathy and Ullrich congenital muscular dystrophy. Joint contractures, hip dislocation, micrognathia, clubfeet and generalized weakness are common clinical signs, but the phenotypes are highly variable. We found two homozygous variants in the COL6A3 gene. They were excluded from further investigation, because there are eight healthy, homozygous carriers annotated (ExAC).

The candidate variant in RYR1 is currently under further functional study. Further histological phenotyping of fetal muscle tissue is mandatory for genotype-phenotype correlation. Identifying the causal mutation will improve recurrence risk counseling and allow prenatal diagnosis for future pregnancies in the family.

P-ClinG-119

NSD1 Duplication in a Girl with Silver-Russel-like Phenotype

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Silver-Russell syndrome (SRS) is a congenital growth retardation syndrome that is mainly characterized by intrauterine growth retardation (1), poor postnatal growth (2), craniofacial features such as relative macrocephaly (3) and protruding forehead (4), body asymmetry (5) and feeding difficulties in newborns and toddlers (6). The recently published Netchine-Harbison Score [Azzi et al, 2015] is based on these six characteristics. Patients who fulfill at least four of these six criteria are regarded to have clinically diagnosed SRS. Most commonly SRS is caused by a hypomethylation of the distal imprinting control region (H19/IGF2

IG-DMR, ICR1) on chromosome 11p15.5 (up to 50%) or maternal uniparental disomy of chromosome 7 (upd(7)mat, 10%). Other known causes in SRS or SRS-like patients are rare (IGF2 or CDKN1C mutations, copy number alterations in 11p15.5).

NSD1 loss-of-function mutations or deletions are associated with Sotos syndrome. Sotos Syndrome is characterised primarily by overgrowth, learning disability and a characteristical facial gestalt, like dolichocephalus with a broad and prominent forehead. NSD1 mutations are also found in some cases of Beckwith-Wiedemann syndrome, vice versa in some patients with Sotos Syndrome 11p15 anomalies can be detected.

We present a case of a female infant showing four out of six features of the Netchine-Harbison Score (SGA, postnatal growth failure, protruding forehead, feeding difficulties). Additionally, we found hypotonia and microcephaly, which is unusual for a SRS phenotype.

Genetic analyses (MLPA, molecular karyotyping) revealed a 380 kb duplication including NSD1 on chromosome 5. Other typical chromosomal aberrations of SRS could be excluded. There are a few reports on NSD1 duplications in patients showing growth retardation, microcephaly and developmental delay. These cases show that reciprocal deletions and duplications of NSD1 cause opposite phenotypes. Our case provides additional support for this idea and shows that NSD1 duplication carriers can exhibit a SRS-like phenotype with the exception of microcephaly.

P-ClinG-120

A novel homozygous FOXE3 mutation causes congenital primary aphakia and sclerocornea - report on a German girl

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Mutations in the FOXE3 gene (OMIM 610256) have been identified in patients with autosomal recessive congenital primary aphakia. These patients show complete absence of the lens, sclerocornea, microphthalmia and partial retinal dysplasia, but no other extra-ocular abnormalities. So far, only nine families have been reported with homozygous FOXE3 mutations, mainly from Arab countries. Also, heterozygous FOXE3 mutations have been implicated in autosomal dominant inherited variable anterior segment abnormalities. FOXE3 encodes a transcription factor only expressed in prelens and lenticular tissue, and has been shown to be essentially involved in lense induction in early eye development. Here we report on a girl born to non-consanguineous German parents. At birth, bilateral sclerocornea was noted. Opthalmologic investigation at the age of five days revealed additional anterior segment abnormalities with microcornea, absent lens and iris structures, and microphthalmia, characteristics of primary aphakia. Follow up further revealed bilateral optic coloboma, mild nystagmus, and no fixation but minimal light sensation. Her growth and psychomotor development were normal. Sequence analysis revealed a homozygous novel FOXE3 mutations c.181delC, p.(R61Gfs*163). The mutation was detected in heterozygous state in both healthy parents, as well as in five close relatives lacking abnormal eye findings. This is the first report of a German patient with a homozygous FOXE3 mutation with primary aphakia and sclerocornea. Segregation analysis confirmed autosomal recessive inheritance, according to earlier reports in the literature. Characteristic ophthalmologic findings in the newborn prompted us to analyse FOXE3 the only gene known to cause primary aphakia in humans.

P-ClinG-121

Update on large rearrangements in the renal ciliopathies autosomal recessive and autosomal dominant polycystic kidney disease (ARPKD/ADPKD)

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ARPKD is characterized by bilaterally enlarged polycystic kidneys with fusiform dilations of renal collecting ducts and distal tubuli in combination with an obligate congenital hepatic fibrosis. The disease often manifests prenatally and occurs in 1:20,000 live births. About 30% of these children die within the first year of life. Mutations in the large PKHD1 gene (6p12) are the only known cause so far. The longest ORF contains 66 exons. ADPKD is as well characterized by bilaterally enlarged polycystic kidneys but presents

usually in the second decade of life with a large number of fluid filled cysts of variable size in cortex and medulla. Approximately 50% of patients acquire end-stage renal disease by the age of 60. Cysts in other organs (e.g. liver) are common additional findings. A prenatal manifestation is observed in about 3-5% of cases and therefore ADPKD is an important differential diagnosis to ARPKD. The prevalence at birth is 1:400-1,000. ADPKD is caused by mutations of the PKD1 (16p13.3, 85% of mutations) and PKD2 gene (4q22.1, 15% of mutations). The analysis of PKD1 is complicated by several pseudogenes.

The mutation detection rate is about 83% in ARPKD and about 90% in ADPKD. These data usually do not take into account large deletions/duplications; however the frequency for PKD1/PKD2 has been reported in the literature as 1-3%. For PKHD1 no such data have been established reliably yet.

To determine the frequency of large rearrangements in our ARPKD/ADPKD patient cohort we used a Multiplex Ligation-dependent Probe Amplification (MLPA) approach (Kits P341/P342, P351/P352; supplier: MRC-Holland) to screen almost 70 ARPKD and 35 ADPKD patients for deletions/duplications of PKHD1 and PKD1/PKD2 respectively.

In our ARPKD cohort we detected heterozygous deletions in two cases (3%, n=2/66) in trans with a pathogenic mutation. One patient showed a whole gene deletion. A follow up by SNP array revealed a 985 kb deletion (arr[hg19] 6p12.3p12.2(51,104,541-52,089,559)x1) involving three further genes. The second patient showed a deletion of exons 18-24.

In the ADPKD cohort we detected a previously not described deletion of exon 22 in one case (3%, n=1/33). In one further case – clinically described as early manifestation of ADPKD – we confirmed a TSC2/PKD1 contiguous gene syndrome, which led to the diagnosis tuberous sclerosis/ADPKD. Furthermore in the two additional, preselected cases we detected as well PKD1 deletions of several exons.

To sum up: We determined deletion frequencies in our cohort of ARPKD/ADPKD patients with usual mutation detection rates. Based on our findings we calculate that in ARPKD the frequency of large rearrangement is about 3% or less. Additionally we confirm the literature data on large rearrangement frequencies of about 3% of PKD1/PKD2.

P-ClinG-122

Genetic Counselling in the Federal Republic of Germany and the United States of America. A Comparative Perspective.

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In 1971 at the 4th International Conference on Human Genetics CO Carter stated about the possibilities of genetic counselling as a rapidly developing branch of the medical services:

- To give information to parents and their relatives on the risks to future children, including risk of disease and prognosis for an affected child.
- To alert the medical profession to special risks to an as yet unborn child, thus facilitating early diagnosis and treatment.
- To prevent an increase birth frequency of genetically and part-genetically determined diseases.

These were also the tasks of genetic counselling in the 1950s and 1960s in the Federal Republic of Germany (FRG).

(1) Before 1945, the “Ehegesundheitsgesetz” (Marital Health Law) of 1935 said that people affected with genetically determined diseases are forbidden to marry. Therefore is has to be a counselling with the aim to prevent society of genetic diseases.

(2) After 1945 the first tasks of the new established Institutes of Human Genetics and Anthropology were as well genetic counselling as paternity tests. First, the reason was to avoid affected children, while abortion was not allowed. Second, in the post-war confusion to confirm paternity seemed to be necessary.

(3) For genetic counselling in the FRG the USA were orientation, where in 1951 already ten genetic counselling centres were established. Up to 1974 there were 387 centres listed. Up to 1959 genetic counselling was more an epidemiological and empirical-risk type of exercise. Then Lejeune (1959) discovered Mongolism and on account of this human genetics was brought to the attention of general practitioners.

(4) The classic publication was Counselling in Medical Genetics by Sheldon C. Reed (1910-2003) in 1955. This book was orientation in the FRG. In 1960 Genetic Counselling by W Fuhrmann and F Vogel was published. The main difference between USA and FRG were the legal regulations on genetic counselling.

(5) The method of Amniocentesis was first published in 1966 and necessitated genetic counselling. Further methods of prenatal diagnosis consolidated the consultation, although ethical aspects became more and more important as well in FRG as also in USA.

(6) The increasing technical possibilities in reproduction medicine and knowledge in human genetics added new challenges to genetic counselling in the USA and in the FRG.

Case Example: A grandchild wants to know his genetic load. The result also includes information about the parents and grandparents.

Ethical considerations are:

- People have the right not to know nothing or everything about their genetic load. (autonomy)
- In addition genetic tests can disclose diseases of relatives: prevention of harm and care for the affected person (nonmaleficence and beneficence) versus medical confidentiality.
- Legal and ethical aspects can be controversial, but have to be fair-minded (justice).

Because of these aspects genetic counselling may be confronted nowadays with unsolvable problems.

P-ClinG-123

Recessive truncating mutations in the TTN gene of two patients with muscular dystrophies

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Titin is the largest known human protein consisting of more than 33,000 amino acids. It is expressed in the cardiac and skeletal muscle spanning half of the sarcomere from Z-disc to M-line. Mutations in the giant TTN gene (OMIM 188840) are associated with different muscular disorders, especially cardiomyopathies, limb-girdle or tibial muscular dystrophies, as well as other myopathies.

Here, we report on two unrelated patients – a ten-year-old boy and a 60-year-old woman – both suffering from limb girdle muscular dystrophies since early and later childhood, respectively. The boy repeatedly showed very high levels of serum creatine kinase (CK) of > 10,000 IU/l and central cores in the muscle biopsy. The affected woman also showed elevated CK activity, a mild cardiomyopathy (since 15 years) as well as inflammatory infiltrates and an increase of internally located nuclei in the muscle biopsy.

Both patients were examined for 37 myopathy genes including most known genes involved in limb girdle muscular dystrophies using a gene panel enrichment strategy and next generation sequencing (NGS) on a MiSeq desktop sequencer (illumina). NGS data were analysed by GensearchNGS (PhenoSystems) and detected variants were classified using prediction tools and databases provided by Alamut (Interactive Biosoftware).

In the affected boy, we identified a homozygous nonsense mutation c.105832C>T (p.Gln35278*) in exon 358 of the TTN gene (NM_001267550.2) in combination with three different homozygous TTN missense variants showing very low minor allele frequencies (MAF < 0.02%). The nonsense mutation has been published once in a compound-heterozygous status in a young woman with congenital core myopathy combined with primary heart disease. The premature termination codon leads to a truncated protein lacking about 690 amino acids of the C-terminal M-line domain of titin. The accumulation of homozygous TTN variants in the here presented boy indicates either that his parents are consanguineous or that a deletion of the complete TTN gene occurred on the second allele.

The affected woman showed two heterozygous truncating mutations in the TTN gene: c.52903C>T (p.Arg17635*) in exon 276 and c.107651_107657del (p.Thr35884Lysfs*7) in exon 362. Both mutations have not yet been published to our knowledge and are predicted to lead to premature termination codons in the A-band region and the M-line domain of titin, respectively. An additional missense variant in the RYR1 gene (c.1061G>A, p.Arg3539His) of uncertain significance was detected in this patient which could also contribute to the cause of disease.

In summary, we could identify the molecular cause of the muscular dystrophies in two patients by analysis of a myopathy gene panel. The NGS approach applied for these cases seems to be an efficient method for analysis of large genes, especially TTN, for which Sanger sequencing is not established in routine diagnostics.

P-ClinG-124

A Novel C8orf37 Splice Mutation and Genotype-Phenotype Correlation for Cone-Rod Dystrophy

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Background. To identify the disease-causing mutation in a consanguineous family of Morrocan origin with syndromic autosomal recessive (ar) cone-rod dystrophy (CRD) in two patients and describe genotype-phenotype correlations.

Materials and Methods. Genome-wide homozygosity mapping and direct sequencing of *C8orf37*, located in a homozygous interval, was performed in the family. mRNA analysis revealed the effect of the newly

identified splice-site mutation. For a comparative analysis phenotypic and genetic data of *C8orf37* mutations were extracted from published cases.

Results. The new splice-site mutation c.155+2T>C identified in the family results in a skipping of 82 bp. The CRD phenotypes of our patients were consistent with previous reports. Non-ocular findings in our patients and two previously described patients were postaxial polydactyly present at birth. Both families with additional postaxial polydactyly had splice site mutations affecting intron 1 of *C8orf37*, one at the slice donor and one at the splice acceptor site.

Conclusions. This report extends the genotypic spectrum of *C8orf37*-associated retinal dystrophies and demonstrates for the first time a genotype-phenotype correlation between an arCRD-polydactyly-association and truncating germline mutations affecting the N-terminal region of the protein. Furthermore, our findings underline the ciliary function of C8orf37 protein.

P-ClinG-125

Case report: combined albinism and deafness in an Argentinian family

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Pigmentary disorders are frequently found associated with hearing impairment in many syndromes. Albinism-Deafness syndrome (ADFN; OMIM # 300700) was first described in 1962 by Ziprkowski et al. and Margolis (1962) and was originally considered to be an X-linked syndrome. Major clinical features are congenital deafness and partial or total albinism. So far no gene could be definitely correlated with the ADFN phenotype. Phenotypically several types of Waardenburg syndrome (types 1 to 4 and subtypes), Tietz syndrome and different types of oculocutaneous albinism could be considered for differential diagnosis.

We examined an Argentinian family with a pedigree over 4 generations. The index patient (male infant, *06/2012) is affected by albinism with multiple round pigmented macules on head, trunk and limb extremities and in addition by deafness on one or both ears. The family medical history revealed that the index' parents are siblings. The index' sister has likewise bilateral deafness and has heterochromia iridis with sectorial hypopigmentation, but no albinism. In total the condition was found in 7 out of 19 family members with either hearing impairment or skin anomalies or both features in common. We did not observe other symptoms in the affected family members. The inheritance pattern of the phenotype in this family is not clear as yet since pigmentation anomalies of skin (albinism/ mosaic albinism) and eye (heterochromia iridis with sectorial hypopigmentation) do not consistently segregate with cases of deafness within the affected family members. We further observed a variable expressivity of pigmentation anomalies and variable severity of deafness (one or both ears affected).

To assess the family status in regard of consanguinity we performed a microsatellite analysis for the index and without doubt confirmed that the index' parents are siblings. By whole exome sequencing ─ index, mother, father (unaffected) and grandmother ─ we did not detect any pathogenic variant in the genes *PAX3*, *MITF*, *SNAI2/SLUG*, *SOX10*, *EDNRB*, *EDN3* and *TYR*, being known for the aforementioned syndromes. Nor could we detect a known non-syndromic pathogenic variant for either albinism or sensorineural deafness, which should have constantly (dominantly) occurred in the examined and affected family members (index, mother, grandmother). Further, a reduced penetrance for one or the other trait cannot be excluded. In accordance with the pedigree and the original ADFN descriptions we suggest an X-linked dominant gene defect leading to functional X-chromosomal mosaicism in female carriers which becomes visible in the mosaic pattern of skin and eye. The hemizygously affected male index therefore displays a more severe phenotype. In this case the gene defect must thus be non-lethal for male carriers.

P-ClinG-126

An integrated CASUS® based curriculum for Human Genetics in the Duesseldorf Medicine model course of study

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Competency-based education (CBE) has attracted renewed attention in recent years as a means to ensure that graduating medical students are prepared with the skills required for current medical practice and to meet contemporary healthcare needs. This learner-centered educational paradigm emphasizes outcomes and abilities rather than time in a training program. CBE was established in the Duesseldorf Medicine model course of study of the Medical Faculty of the Heinrich-Heine-University in 2013. The curriculum includes a newly developed longitudinal program of education in Human Genetics. The program

integrates knowledge and practical skills in a case based approach. The back bone of the program is the case based online learning modul CASUS®. Individual cases with genetic problems are presented in a clinical setting encompassing the patients' history, family history and clinical investigations. The system interactively stimulates the students to reflect the decision process to establish a differential diagnosis and to choose specific genetic analyses. The biological and pathophysiological basics of the genetic problems are addressed as well as the respective consequences for the patients and their families. The genetic CASUS®-cases fit into the overall topic of the respective sections of the Medicine model course of study, e.g., muscular dystrophy, Duchenne type, and achondroplasia in the section „musculoskeletal system". The CASUS®-cases are also used in an inverted classroom setting during the fifth year courses, where the students practice genetic counselling in genetic syndromes and hereditary cancer. So far, 15 CASUS®-cases covering the most common and most relevant genetic diseases have been developed and integrated into all eight clinical sections of the Duesseldorf curriculum. The first results of the evaluation of the newly developed program of education in Human Genetics by the students are encouraging.

P-ClinG-127

A microduplication encompassing TBL1XR1 causes a genomic sister-disorder for the 3q26.32 microdeletion syndrome

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Recently a new syndrome of intellectual disability with dysmorphism due to deletions on the chromosomal band 3q26.32 harboring the TBL1XR1 gene was described. Here we report a patient in which Array-CGH analysis of genomic DNA revealed a 309 kb microduplication of genetic material on the same chromosomal locus: 3q26.32: arr [hg19] 3q26.32(176.648.502-176.957.675)x3 encompassing only the TBL1XR1 gene. De novo occurrence of the duplication was verified by real-time PCR of parental DNA.

Our patient showed an identical facial gestalt and a similar degree of intellectual impairment as the previously reported mother and daughter with a 708 kb deletion containing the TBL1XR1 gene. Recognizable facial features included: low-set ears, synophris, thick eyebrows, hypotelorism, short nose with anteverted nostrils, deep philtrum, prognathism with a pointed chin, and dental crowding. In addition our patient also demonstrated a Dandy Walker malformation, congenital heart defects (ASD II and subvalvular aortic stenosis), short stature, and bilateral sensoneurinal hearing loss.

In a third patient a de novo 1.6 Mb deletion encompassing the TBL1XR1 gene as well as the last exon of the NAALADL2 gene had been identified. This patient also presented with intellectual disability and dysmorphic features but no evidence of ASD.

Taken together our data suggest that deletions as well as duplications including the TBL1XR1 gene cause a similar facial phenotype and degree of intellectual impairment and could be from that perspective regarded as a genomic sister-disorder for the 3q26.32 locus. Whether the unique additional syndromal features found in our patient are dose-related to the additional copy of the TBL1XR1 remains to be investigated.

P-ClinG-128

ADNP mutations are a frequent cause of autosomal dominant intellectual disability

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Heterozygous loss-of-function mutations in the activity dependent neuroprotector homeobox gene ADNP, which encodes a transcription factor involved in the SWI/SNF remodelling complex, were recently shown to be associated with moderate to severe intellectual disability (ID). Additional clinical abnormalities which included short stature, muscular hypotonia and facial dysmorphisms had enabled the delineation of a new entity termed Helsmoortel-van der Aa syndrome (OMIM 615873). Since its first report in 2014, studies in large ID cohorts identified mutations in 14 unrelated patients, suggesting that ADNP mutations are a comparatively frequent cause of autosomal dominant ID.

We report on three unrelated German patients (two boys and one girl) in whom ADNP mutations were detected by next-generation sequencing. Clinical problems that are in accordance with those of previously published patients included severe ID, muscular hypotonia, short stature and hypoplastic corpus callosum.

Borderline microcephaly, which had so far only been reported in a minority of patients, was present in two of our patients. One patient had unilateral iris coloboma, which has hitherto not been associated with ADNP mutations.

Our findings corroborate the assumption that ADNP mutations are one of the more frequent causes of ID in sporadic patients, and they expand the clinical spectrum of the probably underdiagnosed Helsmoortel-van der Aa syndrome.

P-ClinG-129

Targeted next generation sequencing for molecular diagnostics of malignant hyperthermia susceptibility (MHS)

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Malignant hyperthermia (MH) is a dominantly inherited rare condition that is usually triggered by exposure to volatile anesthetic agents such as halothane, sevoflurane, desflurane and isoflurane as well as the depolarizing muscle relaxant succinylcholine. The prevalence of MH susceptibility (MHS) is estimated to be up to 1:3,000 and thus markedly higher than the clinical incidence of MH episodes (1:20,000) indicating a reduced penetrance of the disorder. MHS has been causally linked to two large genes, with most of the pathogenic variants detected in the ryanodine receptor type-1 (RYR1) gene encoded by 106 exons on chromosome 19q13.2 and less than 1% of the pathogenetic variants in the calcium channel, voltage-dependent, L type, alpha 1S subunit (CACNA1S) geneencoded by 44 exons on chromosome 1q32. We present the results of targeted next generation sequencing diagnostics performed in our institute to detect pathogenic variants in these two genes in 70 individuals with clinical diagnosis (by in vitro contracture test) of MH or clinical suspicion of MHS.

Target enrichment of all coding exons of RYR1 and from patients´ genomic DNA samples was carried out using an Illumina MiSeq system or a Roche Junior system. Sequences were analysed using the NextGENe v.2.3.3 software and the human reference genome (NBI build GRCh37.3). Pathogenicity of variants was assessed using several in silico predictive algorithms and comparison against population, disease-specific, and sequence databases including the European MH Group (EMHG) RYR1 mutation database. All detected variants were confirmed by conventional PCR and Sanger sequencing.

In 27 patients with clinical diagnosis of MHS or with suspicion of MHS we identified a RYR1 variant. These 27 variants included 7 variants that had been already described as causative in the EMHG database, 5 that had been listed as potentially causative in the EMHG database and 15 potentially causative novel variants. In two patients, we detected a second RYR1 variant, predisposing not only for a MH but also for the RYR1-related congenital myopathies central core disease (CCD) and multiminicore disease (MmD). In addition, we found two novel potentially pathogenic variants in the CACNA1S gene in three patients.

Overall, our results indicate that targeted next generation sequencing offers an efficient and reliable method for the molecular diagnosis of MHS and RYR1-related congenital myopathies.

P-ClinG-130

Teaching history of human genetics to medical students - is it worthwhile?

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In many lectures of human genetics, the history of human genetics serves as an entry point, routinely starting with Mendel and giving up on the historical dimension of today's achievements in human genetics after a few slides. Often, this is not sufficient to explain students the depth and context of contemporary thinking in human genetics. Lectures on ethics, theory and history of medicine are offered to medical students, but usually cover a wide range of topics and are not relevant to oral or written tests. The new „Gegenstandskataloge" GK1 or GK2 of the Institute for Medical and Pharmaceutical Exam Questinairies (IMPP) do not mention the term "history". Given the time constraints for studying human medicine, students tend to concentrate on exam-relevant lectures. We are currently offering a course to third year students, specifically devoted to the history of human genetics. The topics focus on the development of genetic thinking in its social and political context and the link to present day knowledge in this disciplin. Among others, emphasis is given to the formation of eugenic thinking. Such aspects were integrated into exams and evaluation questionaires. As a basis for further deliberations, we will discuss our first experiences with this teaching approach and will present feedback responses from our students.

P-ClinG-131

Returning secondary findings in whole exome or genome sequencing: are we ready for "positive lists"?

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The implementation of high throughput genomic techniques in clinical care and research led to the publication of various recommendations on how to deal with genetic information that is unrelated to the clinical question but might be worthwhile to report back to the patient. The American College of Medical Genetics (ACMG) published a "positive list" of 56 genes that should be actively examined for mutations regardless of the indication and age of the patient. In contrast, working groups of human genetics societies in Europe, including the ESHG and the GfH, propose to balance the benefits and drawbacks for the patient and his relatives in the individual case.

In the research context, many ethic committees at least in Germany demand inclusion of the option of returning secondary findings for severe preventable or treatable disorders. For this, clear guidance or a "positive list" would clearly be required in order to avoid legal uncertainties apart from being of great value for the clinician or researcher who is faced with a huge amount of genetic data. However, as yet there are no universally agreed criteria on what is a relevant finding that should be reported. Various factors such as disease severity, age of onset, penetrance, risk increase, effectiveness of prevention measures, etc. need to be considered for each condition. It is also questionable if handing raw data to the proband, if requested, is a suitable alternative. Finally, cost factors also need to be taken into consideration.

We wish to argue that reporting of secondary findings is comparable to a screening test and should be assessed as such. We do not accept the position voiced in the ACMG recommendations that, because the patient is already under clinical care and that any associated risk has already been sustained for the primary indication, the analysis of secondary findings fundamentally differs from other screening tests. In our opinion the criteria for screening tests originally developed by Wilson and Jungner (1968) should also be applied to the active search for secondary genetic findings. In particular, the analysis should be restricted to genes responsible for an important health problem with proven benefit of early detection and available facilities for diagnosis and treatment. The natural history of the condition, and specifically the prognostic value of identified genetic variants, must be adequately understood, and there should be an agreed policy on whom to treat as patients. This also requires addressing the criteria outlined above, communicating them with the population, and gaining informed consent from the individual tested. Finally, the cost of case-finding (including diagnosis and treatment) should be economically balanced, and respective funds must be made available on a long-term basis. It is a challenge to the genetic societies to address all these criteria in order to give adequate guidance for researchers and clinicians alike.

P-ClinG-132

Bringing NIPT to the next level: detection of fetal trisomy 21 based on quantitative real-time PCR

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Current non-invasive prenatal testing (NIPT) methods for the detection of trisomy 21 (T21) are primarily based on next generation sequencing (NGS) strategies which are quite costly in clinical application and hence are limited to certain patient groups. Here, we describe the results of a blinded comparative study with the aim to validate the diagnostic accuracy of a newly developed NIPT assay based on quantitative real-time PCR for the determination of fetal trisomy 21 (T21 qPCR assay) in comparison to results of non-invasive PrenaTest® based on NGS. In the study, close to 750 maternal blood samples were selected from singleton pregnancies. Cell free DNA was prepared and analysed using new T21 qPCR assay. Results of qPCR based technique were compared with those from NGS procedure. The blinded study demonstrates clearly that the accuracy of the new qPCR-based PrenaTest® is superior to the accuracy of the combined test and comparable to the performance of current non-invasive prenatal tests based on next generation sequencing or microarrays. Our results suggest that the newly developed T21 qPCR assay is a very reliable and robust method suitable for NIPT in clinical routine. While qPCR also presents a more cost-efficient solution over NGS testing, the new assay will also be able to provide results in 72 hours or less. As a consequence, this novel T21 qPCR assay could have the potential to become a NIPT solution on a global basis. Further studies with aim to improve specificity for detection of T21 and to include determination of Trisomy 13 and 18 are currently underway to assess their relevant clinical performance parameters.

P-ClinG-133

Alport syndrome is an important cause of focal segmental glomerulosclerosis (FSGS) in children and adults.

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Primary focal segmental glomerulosclerosis (FSGS) is the most common histopathology finding in steroid-resistant nephrotic syndrome (SRNS). The genetic basis of SRNS is highly heterogenous with almost 30 known genes resulting in isolated or syndromal forms of nephrotic syndrome. Patients with nephrotic syndrome show proteinuria, hypoalbuminemia, hypercholesterolemia, and peripheral edema. Most patients with SRNS suffer from a progressive decline in renal function and are at high risk for end-stage renal disease (ESRD).

Alport syndrome is characterized by hematuria and proteinuria, progressive renal insufficiency and end-stage renal disease (ESRD). Additional clinical features like progressive sensorineural hearing loss and ocular findings like anterior lenticonus might also be present.

Here we report on three patients (two familial female and one sporadic male case) that were initially referred to us for SRNS/FSGS molecular genetic testing. Testing of several genes associated with SRNS/FSGS was unremarkable in these patients. Therefore we asked the pathologist to review the patients' kidney biopsies again. Reanalyses revealed the presence of morphological features compatible with Alport syndrome in all three cases. Immunohistochemical staining of type IV collagen was performed and demonstrated staining pattern characteristic for dominant Alport syndrome in two patients (sporadic male, familial adult female) and recessive Alport syndrome in one patient (female child from a consanguineous family).

Subsequent sequencing analysis of the Alport syndrome genes identified a pathogenic *COL4A5* mutation in the sporadic male case and a homozygous pathogenic *COL4A3* mutation in the female child from a consanguineous family where the father of the maternal line died from unclear renal failure.

In the third patient (adult female with a family history positive for a FSGS-like phenotype) a variant of uncertain clinical significance (VUS3) in the *COL4A5* gene was detected. Segregation analysis of the sequence variant is ongoing.

To sum up, patients presenting with a clinical course suggestive of SRNS/FSGS might instead have Alport syndrome, especially if the hallmark features like hematuria and hearing loss are not present yet or have not been considered.

Therefore, a detailed evaluation of kidney biopsy samples with regard to features of Alport syndrome is indicated in all patients with a clinical suspicion of SRNS/FSGS.

P-ClinG-134

Is KCND1 a new gene for X-linked intellectual disability and/or autism spectrum disorder?

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We describe two half-brothers with moderate intellectual disability, delay of motoric skills, and speech delay without development of active language abilities. Both have severe behavioural anomalies including hyperactivity, temper tantrums, autistic behaviour, and sleep disturbance. The two boys were otherwise in good health. No internal malformations were known, and no specific dysmorphic features were present. All growth parameters were in the normal range.

High resolution GTG-banding showed normal karyotypes, molecular testing regarding Fragile-X-syndrome and molecular karyotyping (array-CGH) were unremarkable. Because of the evidence for a X-linked form of intellectual disability, but the non-specific findings in the 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 of the KCND1 gene in both affected half-brothers. Several in silico tools assessed the deleterious nature of the variant, MutationsTaster and PolyPhen2 classified the mutation as damaging. The variant was not present in public SNP databases (dbSNP, 1000 Genomes Browser, ExAC). Further segregation analysis in the family showed that the mother of the two boys carried the KCND1 mutation, as did the maternal grandmother.

KCND1 is one of three members of the KCND/Kv4 family of voltage-gated potassium channels. Members of the Kv4 channel family are responsible for native, rapidly inactivating (A-type) currents in neurons and heart and show subtype specific expression patterns with significant overlaps. KCND1 is

expressed ubiquitously with highest levels in brain where it is expressed in almost all areas, including cerebral cortex, cerebellum, corpus callosum, hippocampus, amygdala, thalamus, basal ganglia, medulla, and spinal cord. Mutations in the Kv4 family members KCND2 and KCND3 have been correlated with a broad spectrum of neurological disorders. Truncating mutations in KCND2 lead to temporal lobe epilepsy and some rare variants (submicroscopic de novo deletions, translocations, sequence variants) have been identified in individuals with autism and autism spectrum disorder. Loss-of function mutations in KCND3 have been identified causing spinocerebellar ataxias (SCA19/22), whereas gain-of function mutations were implicated in Brugada syndrome and atrial fibrillation. Furthermore an in-frame duplication of 9 nucleotides within the voltage-sensor domain of KCND3 was described in a patient with mild intellectual disability, seizures and cerebellar ataxia.

Considering the identification of a sequence variant in KCND1 in two half brothers with moderate ID and autism spectrum disorder and the correlation of KCND2 and KCND3 with ID and autism we speculate that KCND1 might be a new candidate gene for intellectual disability. To prove the significance of the KCND1 variant functional analysis is pending and further patients with KCND1 variants are desirable.

P-ClinG-135

Pre-implantation Genetic Diagnosis (PGD) Center, MGZ Munich

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Pre-implantation Genetic Diagnosis (PGD) is an analytical method used to identify genetically inherited diseases in an embryo or ovum prior to implantation in the uterus, i.e. before pregnancy. In vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) is a prerequisite for pre-implantation genetic diagnosis. In Germany, PGD is regulated by the Pre-implantation Diagnosis Act (PräimpG), an amendment to the Embryo Protection Act (ESchG). PGD is therefore permitted in Germany under certain preconditions, and can be performed by a government-authorized PGD center following positive assessment by an ethics committee for PGD. On July 1, 2015, MGZ – Medical Genetics Center in Munich was authorized as a PGD center along with three reproductive medical institutes: KITZ.-KinderwunschTherapie im Zentrum in Regensburg, the Kinderwunsch Centrum München, and the kïz) kinderwunsch im zentrum in Munich. After approval by the Bavarian Ethics Committee for PGD, pre-implantation genetic diagnosis can be performed at the MGZ.

Between July and the end of October 2015, patients in our PGD center made 34 requests for pre-implantation genetic diagnosis. Of these requests, 9 applications were for monogenetic diseases and 25 for the exclusion of chromosomal anomalies such as reciprocal and Robertson translocations, inversions, and deletions. This contribution will give an overview of PGD methods and test results at MGZ – Medical Genetics Center Munich.

At MGZ, genetic analysis for monogenetic diseases is performed in two ways, STR marker analysis (with and without direct mutation identification) and karyomapping. Chromosome abnormalities are tested with microarray and next-generation sequencing (NGS). We perform PGD on trophoblasts extracted on day 5 of fertilization.

The diagnostic possibilities for analyzing an embryo have seen vast improvement in recent years due to the new methods available in reproductive medical and human genetics laboratories. International findings reveal that these new methods achieve better and more accurate diagnostic results. Furthermore, microarray and NGS technologies make it possible to combine the pre-implantation genetic diagnosis of monogenetic diseases and the analysis of structural chromosome abnormalities.

P-ClinG-136

Mutation detection rates in a German cohort of patients affected with MYH9-related diseases

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Purpose: Myosin heavy chain 9 (MYH9)-related platelet disorders belong to the group of autosomal-dominantly inherited macrothrombocytopenias caused by heterozygous mutations in MYH9. The MYH9 gene consists of 40 coding exons and encodes non-muscular myosin heavy chain IIA (NMMHC-IIA), a cytoskeletal

contractile protein. The different clinical manifestations of MYH9 gene mutations lead to four overlapping syndromes, known as May-Hegglin anomaly (MHA), Epstein syndrome (EPS), Fechtner syndrome (FS), and Sebastian platelet syndrome (SPS). Since macrothrombocytopenia with inclusion bodies in neutrophils is characteristic for all of them, preselection by immunofluorescence analysis of blood smears is supposed to enhance the mutation detection rate compared to non-preanalysed patients.

Methods: Air dried blood smears from peripheral blood were fixed, permeabilized, stained using a monoclonal antibody against NMMHC-IIA and visualized by immunofluorescence. Mutation analysis of the MYH9 gene was performed by standard methods using PCR and Sanger sequencing.

Results: Molecular analysis was performed for 81 index cases with the suspected diagnosis of inherited macrothrombocytopenia. A pathogenic mutation could be verified in 90% of immuno-positive probands. About 96% of these mutations were found to be localized in the nine hot spot exons described in the literature. The remaining mutations were identified by Sanger sequencing of all coding exons. Large deletions or duplications within the MYH9-gene were not detected by MLPA-analysis.

Conclusions: Immuno-positive but mutation-negative cases should be included in further investigations by gene panel analyses to screen for pathogenic mutations in non-coding regions of the MYH9-gene as well as in other genes involved in thrombocytopenia.

P-ClinG-137

A novel frameshift mutation in *MTM1* in a 50-year-old patient with an adult onset form of X-linked myotubular myopathy

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Background: X-linked myotubular myopathy is a neuromuscular disorder usually characterized by a congenital onset of severe muscle weakness, hypotonia and respiratory problems in affected males. The classic form of myotubular myopathy normally leads to death in infancy or childhood. However, mild forms of X-linked myotubular myopathy exist in a minority of patients. Hemizygous mutations in the gene *myotubularin* (*MTM1*) are the genetic cause of X-linked myotubular myopathy. Truncating variants in *MTM1* are usually associated with a severe phenotype.

Results: Here, we report a male patient who initially presented at 42 years of age with moderate to severe, proximally pronounced paresis of arms and legs: muscle research scale of 3- to 4 in shoulder abduction, elbow flexion, hand grip, hip flexion and ankle dorsiflexion. Scapular winging was present. The creatine kinase was ~2.5-fold elevated above normal. EMG displayed sever myopathic changes and occasional myotonic discharges. Muscle MRI showed muscular atrophy and increased fat and connective tissue in the thigh. The vital capacity was 60% of age-adjusted normal value. The clinical course was slowly progressive over 8 years, when the patient was unable to rise from a chair or walk more than 30 metres without aids and had developed dysarthria and dysphagia, but no cardiac symptoms. Standard genetic testing excluded a range of muscular dystrophies including myotonic dystrophy and facioscapulohumeral muscular dystrophy. The patient's brother was equally affected. A next generation sequencing based gene panel for neuromuscular diseases detected the novel frameshift mutation c.98dupA (p.Ala34Gly*fs**13) in the *MTM1* gene in our patient.

Conclusions: This is a rare example of a frameshift mutation in the *MTM1* gene leading to an X-linked myotubular myopathy with adult onset and a moderate to severe phenotype resembling a limb-girdle muscle dystrophy.

P-ClinG-138

Segregation of a duplication in 8p21.2 in three affected family members identifies STC1 as candidate gene for short stature in humans

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Stanniocalcin-1 (STC1) is the mammalian homolog of a glycoprotein hormone, which has been first identified as a main regulator of calcium and phosphate homeostasis in bony fish. In mammals, STC1 is also involved in the regulation of bone metabolism and growth, including a calciotropic effect on osteogenesis. Transgenic mice overexpressing human STC1 show a significant reduction in birth weight, a reduced adult body size and cranial bone hypoplasia. Studies in human adipose derived stem cells, differentiated to pre-

osteoblast cells, propose an impact of STC1 on calcitonin and calcitonin gene-related peptide during osteoblastogenesis.

Here we report a 27-year-old woman and two of her four children, who present with short stature and microcephaly. Her 5-year-old daughter (height -2,04 SD, head circumference -1,69 SD at the age of 3 11/12 years) and her 1 10/12-year-old son (height -0,34 SD, head circumference -2,82 SD at the age of 1 01/12 years) additionally show global developmental delay (daughter: walking at 20 months; son: no crawling at the age of 12 months). On examination the mother was noted with a height of 159 cm (-1,06 SD) and a head circumference of 51 cm (-2,69 SD). She appears slightly mentally retarded as well (secondary school certificate and finished apprenticeship in horticulture). All three family members were diagnosed by microarray analysis with a duplication of 0,12 Mb in 8p21.2 (arr(hg19) 8p21.2(23,706,968-23,828,047)x3). In contrast, microarray analysis of the other almost 1-year-old son of the proposita, showing normal growth and head circumference, gave a normal result. The 8-year-old, also clinically inconspicuous daughter has yet not been tested.

The duplicated region in 8p21.2 segregating with the phenotype in the family contains the exons 1 to 3 of STC1 gene and has not been noted as benign CNV. Though much larger duplications in 8p21.2 have been reported encompassing the STC1 gene or parts of it, no comparable alteration only affecting the STC1 gene has been cited so far. Nevertheless, one female patient with a duplication of about 0,5 Mb in 8p21.2 (arr(hg19) 8p21.2(23,827,988-24,384,37)x3) immediately centromeric to STC1 has also been described with global developmental delay and short stature and it is intriguing to speculate that this affects STC1 via a regulatory mechanism.

To investigate potential functional consequences of the STC1 alteration in the family described here, we investigated the calcium-phosphate-metabolism in the affected mother. Nevertheless, serum levels of calcium, phosphate, alkaline phosphatase and parathormone were within the reference range, though this does not preclude any effects in earlier life.

We speculate that the partial duplication of the STC1 gene, containing most of its coding sequence, might lead to a gain-of-function which might explain the familial phenotype by analogy to the data from fish and transgenic mice. Further studies are ongoing to delineate the molecular basis.

P-ClinG-139

Diagnosis of monogenetic metabolic hepatopathies in children by next generation sequencing

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Introduction

As neonatal cholestasis is a common infant problem with more than 50 possible differential diagnoses, fast and comprehensive, cost effective and ideally non-invasive diagnosis is desirable to initiate timely therapy to improve patient outcome. Also for other hepatopathies, especially those manifesting in acute liver failure, quick diagnosis is important, as they could constitute contraindication to liver transplantation or can be treated with specific curative therapies. We developed a next generation sequencing (NGS) panel of 21 genes associated with acute and chronic hepatopathies. The panel includes familial progressive intrahepatic cholestasis syndromes, Niemann–Pick disease type C, Alagille syndrome, congenital bile acid synthesis defects, Crigler-Najjar Syndrome, Wilson disease, mitochondrial DNA depletion syndromes, Deoxyguanosine kinase deficiency, Hereditary fructose intolerance and Transaldolase deficiency.

Methods

DNA was extracted from 1-2 mL EDTA peripheral blood samples of 112 patients with hepatopathy (age: 8-18 years; 56 female, 56 male; 23 of them with already genetically proven hepatopathies for validation purposes) after obtaining informed consent of the patients and/or their parents. A TruSeq Custom Amplicon (TSCA) panel of 21 genes related to paediatric or juvenile hepatopathies was used for targeted resequencing on the MiSeq sequencing device (Illumina). Data analysis was performed using Sequence Pilot 4.1 2 software (JSI medical systems GmbH). Interpretation of variants was done with the help of Alamut Visual (Interactive Biosoftware, Rouen, France).

Results

For validating the method, samples of 23 patients with already genetically proven hepatopathies were measured by NGS and blindly analyzed. All NGS results were identical with the previous findings. In 23 out of 89 patients without previous positive genetic findings we could detect disease related or likely disease related variants with the help of the NGS panel. The related diseases include 3x Wilson disease, 8x Alagille syndrome, 3x familial progressive intrahepatic cholestasis, 5x Crigler-Najjar syndrome, 3x congenital bile acid synthesis defect, 1x mitochondrial DNA depletion syndrome.

Conclusion and Outlook

Our NGS based analysis of 21 genes represents a fast and comprehensive tool to diagnose genetically determined paediatric hepatopathies. As a result, next to shortening the hospital stay by reduction of examinations and reduced patient's burden because of smaller required blood volumes, fast and specific initiation of therapy enables a better prognosis.

Up to now more than 100 genes related to paediatric hepatopathies are known. Therefore we aim to perform our future NGS analyses by whole exome sequencing, which also allows us to include genes in our analysis whose relevance for the disease pattern was newly discovered.

P-ClinG-140

A novel intragenic deletion in NSD1 in a boy with Sotos Syndrome born SGA

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Sotos Syndrome is caused by mutations in the NSD1 gene (OMIM *606681). It is characterized by overgrowth, intellectual impairment of variable severity and a distinctive facial appearance with prominent forehead, sparse frontotemporal hair, downslanting palpebral fissures, malar flushing, long narrow face and long chin.

We describe an 18 month old boy with mild motor and speech delay, strabismus, hyperopia and scoliosis. He was born at 39 weeks of gestation after an uncomplicated pregnancy with a low birth weight (2225 g, -3 SD), length (46 cm, -2,6 SD) and head circumference (33 cm, -1,8 SD). Due to feeding difficulties and low weight gain tube feeding was necessary for the first seven days. The patient showed a percentile crossing growth. His current body measurements are within normal range. At the age of 12 months he was able to walk with aid. Up to date he speaks three words. The patient shows distinct facial features like broad prominent forehead, sparse frontotemporal hair and downslanting palpebral fissures. Radiograph of the left hand at the age of 18 months revealed an accelerated and dissociated bone age of the carpus (28 months) and the phalanges (42 months). Because of a characteristic facial gestalt, crossing of growth percentiles and the accelerated and dissociated bone age we initiated analysis for Sotos Syndrome. NSD1 analysis revealed a de novo intragenic deletion of exon 20 and 21.

In summary, we describe a patient, who was born SGA and now shows normal growth measurements, with a distinct facial gestalt of Sotos Syndrome, an X-ray of the left hand with accelerated and dissociated bone age, with a novel de novo intragenic deletion in NSD1. In the literature, almost all patients with Sotos Syndrome show characteristic overgrowth from birth. Hence this case expands the phenotypic spectrum of Sotos Syndrome.

P-ClinG-141

Variations in TBX6 are associated with disorders of the Müllerian ducts

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Malformations of the Müllerian ducts encompass the Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (congenital absence of the uterus and vagina) and fusion anomalies of the Müllerian ducts such as unicornuate uterus, bicornuate uterus, septate uterus, didelphic uterus, and vaginal septum.

MRKH syndrome type I is referred as isolated utero-vaginal aplasia whereas MRKH syndrome type II is associated with other malformations, such as skeletal and renal malformations, hearing defects and rarely cardiac and digital anomalies. MURCS (Müllerian duct aplasia, unilateral renal agenesis, and cervicothoracic somite anomalies) is a severe manifestation of type II MRKH syndrome. The knowledge about the etiology of anomalies of the Müllerian ducts is very limited.

Recently, in approximately 1% of patients with disorders of the Müllerian ducts recurrent aberrations in 16p11.2 have been identified by array-CGH.

The TBX6 gene (OMIM 602427) is located in the deletion interval 16p11.2 and seems to be a good candidate gene, since it encodes a conserved transcription factor playing an essential role in developmental processes like mesoderm formation and specification.

In human, sequence variants in TBX6 are associated with congenital scoliosis in the Chinese Han population and spondylocostal dysostosis. A mouse model for TBX6 is the homozygous Tbx6rv (rib-vertebrae), in which the expression of Tbx6 is down-regulated. The model shows a hypomorphic phenotype, with an occasionally unilateral absence of kidneys and a reduced female fertility. The phenotype of the Tbx6rv /Tbx6rv and the associations between TBX6 mutations in human and scoliosis showed similarities with MURCS.

Sequential analysis of TBX6 in a cohort of 293 patients with disorders of the Müllerian ducts has been performed. In total we identified five different heterozygous variants in a total of fourteen of 293 patients (~5 %).

Two of these variants are listed in the ESP database, but with a very low MAF value of 0.03 and 0.6 %. Both cause an amino acid exchange in the T-box of the protein.

In addition, we found two missense mutations and one nonsense mutation in heterozygous state, respectively. The sequence variants are not listed in the ESP database. The two missense variants cause an amino acid exchange, in one case also in the T-box of the protein. The nonsense mutation generates a premature stop codon. In addition, all five variants are according to PolyPhen predicted to be probably damaging with a score of > 0.9.

Since we detected three potential pathogenic missense mutations in three cases and in addition two variants with increased frequencies in patients with disorders of the Müllerian ducts in comparison with the general population, we conclude that variants of the TBX6 gene seem to be associated with disorders of the Müllerian ducts.

P-ClinG-142

Complex allele in a cystic fibrosis patient: exon deletion and missense mutation with a frameshift deletion in trans

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders, affecting approximately 1 in 2500 newborns in Germany. The disease is caused by mutations within the cystic fibrosis conductance regulator gene (CFTR) coding for a chloride channel in the apical cell membrane of exocrine epithelial cells. To date, about 2000 molecular defects are known, many of them occurring as rare mutations affecting only one family or individual.

Here we report on a 7 years old boy of turkish origin with a classical clinical phenotype of cystic fibrosis (positive sweat test and pancreatic insufficiency). By screening the most common mutations (multiplex-CF-EU2v1 kit, Elucigene), no disease causing mutation could be identified. Subsequently, by sequencing the whole coding region of the CFTR gene, we identified a small heterozygous deletion 306delTAGA (c.174_177del) in exon 3 resulting in a frameshift and a premature stop codon (p.Asp58Glufs*32). Moreover, a second mutation D1154G (c.3461A>G) in exon 18 was identified. However, this is a mild (class IV-) CFTR mutation associated with CBAVD that does not account for the observed clinical phenotype. By MLPA analysis we detected a large exon 2 deletion/insertion (CFTRdele2), which was further verified by breakpoint-specific PCR. Following genetic counselling, carrier status in the parents was confirmed by sequencing and MLPA. This case underscores the importance of mutations detection (including large deletions) using different methods and has important implication for genetic counselling.

P-ClinG-143

Clinical and genetic evidence implying PDZD7 as a non-syndromic hearing loss gene

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Non-syndromic hearing loss is a clinically and genetically heterogeneous disorder with approximately 50% of cases with an underlying genetic etiology. This extensive genetic heterogeneity can make the identification of a second validating case extremely challenging.

Disruption of PDZ domain-containing 7 (PDZD7) was initially identified in 2009 in a then 10 year old proband with non-syndromic sensorineural hearing loss. However, subsequent clinical reports have

associated PDZD7 with digenic Usher syndrome, the most common cause of deaf-blindness, or as a modifier of retinal disease. We present a validating case (proband 1) and review the first described case from 2009 (proband 2) with biallelic disruption in PDZD7.

Proband 1, a female patient last seen at age 12 years, was sequenced using a custom-designed next generation sequencing panel consisting of 151 deafness genes. Bioinformatics analysis and filtering disclosed two PDZD7 sequence variants (c.1648C>T, p.Q550* and c.2107del, p.S703Vfs*20). Segregation analysis confirmed biallelic inheritance (compound heterozygosity). Mutation analysis determined the c.1648C>T mutation as novel and reported the c.2107del deletion as rs397516633 with a calculated minor allele frequency of 0.000018.

Both probands presented with prelingual, mild-to-severe, non-syndromic, sensorineural hearing loss. Longitudinal assessment of pure-tone audiograms was performed for both probands and revealed a single progressive episode in the high frequencies around nine years of age. Proband 2, a male patient last seen at age 15 years, has thus far indicated stable hearing loss. Both probands underwent detailed ophthalmologic testing and disclosed healthy retinas, excluding Usher syndrome-like changes in the eye.

Our data from two independent patients distinctly affirm PDZD7 as a bona fide autosomal recessive non-syndromic hearing loss gene. In both probands, there was no evidence of impaired vision or ophthalmic pathology. As the current understanding of PDZD7 mutations bridge Mendelian and complex phenotypes, we recommend careful variant interpretation, since PDZD7 is one of many genes associated with both Usher syndrome and autosomal recessive non-syndromic hearing loss. Our findings rule out all forms of Usher syndrome with an onset before 12 and 15 years of age in probands 1 and 2, respectively.

P-ClinG-144

A new NOTCH1 splice site mutation in Adams-Oliver Syndrome

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Adams-Oliver syndrome (AOS) is a rare disorder that presents most commonly with scalp defects, which sometimes include underlying bone defects, combined with terminal transversal limb defects. Around 20% of individuals with AOS additionally show cardiovascular malformations. Other clinical findings (e.g. cutis marmorata telangiectatica congenita, ophthalmological or central nervous malformations) can also be present. Sporadic and familial cases were reported, some with incomplete penetrance and variability in expressivity. There are six known genes that are associated with autosomal recessive (*DOCK6*, *EOGT*) and autosomal dominant (*NOTCH1*, *ARHGAP31*, *RBPJ*, *DLL4*) disease transmission, respectively.

Our female patient was born at 38+2 weeks by vaginal delivery and was aged three days when we saw her the first time. She was the first child of nonconsanguineous parents. At birth an area of aplasia cutis was noted at the occiput, without underlying bone defect. She had a brachydactyly of both feet and a hypoplasia of the toe nails II-V. Additionally, cutis marmorata and several teleangiectasias were present. An echocardiography showed a ventricular septal defect that spontaneously closed one month later without therapy. Her father showed cutis marmorata at birth, but no further signs of AOS.

Sanger sequencing of all 34 exons of the *NOTCH1* gene in our patient showed the heterozygous variant c.1669+5G>A in intron 10. The same variant was detected in her father. RNA analysis by sequencing of exons 8 to 12 with customised primer pair for cDNA confirmed the presumption that the variant leads to skipping of the whole exon 10 on mRNA level. The consequence of this loss is an in frame-deletion of 38 amino acids (p.Phe220_Gly557del) which affects the epidermal growth factor-like domains 13 and 14 of the NOTCH1 protein. This alteration is predicted to be deleterious, but its precise consequences on protein function cannot be predicted at this time.

The *NOTCH1* gene encodes a single-pass transmembrane receptor and the signalling pathway is involved in the development of numerous tissues. Besides AOS, *NOTCH1* mutations also cause aortic valve anomalies. Therefore an echocardiography of the father is planed.

In conclusion, we have found a new splice site mutation in the *NOTCH1* gene that causes AOS and confirms that the NOTCH1 pathway plays an important role in the pathogenesis of AOS. Our patients are a good example for variability in expressivity of the clinical abnormalities arising from *NOTCH1* mutations. Additionally it highlights the importance of a thorough examination and an echocardiography of persons with a *NOTCH1* mutation.

P-ClinG-145

Relevance of segregation studies in familial dilated cardiomyopathy: report of a family with different variants in SCN5A and TTN detected by genetic panel analysis.

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Familial dilated cardiomyopathy (DCM) is a genetically heterogeneous disease with an estimated prevalence of 1 in 250 individuals. Up to now, causative mutations in more than 30 genes have been reported to account for only 40-50% of patients with familial DCM. Multi-gene panel testing by massive parallel sequencing is the method of choice to identify causal mutations. Panels that are offered for DCM vary in the numbers of included genes ranging from 15-20 to more than 60. Often a combined panel for various cardiomyopathies is in use. In 2014 a core-panel for cardiomyopathy has been defined in the Netherlands containing 45 “core”genes (e.g. www. Amsterdamgenomedx.com). Relevant issues of panel analysis are the detection of variants of unknown significance (VUS); individuals who may have two or more disease-causing mutations; and individuals with particular genetic variants who do not express clinical disease.

Here, we describe a family with autosomal dominant DCM affecting at least 7 members in 3 generations. Multi-gene panel analysis including 46 genes (45 “core”genes + *PRDM16*) related to cardiomyopathy was performed in the 50 year old index patient who was diagnosed with DCM at the age of 48. His mother became symptomatic from DCM at the age of 45 and underwent heart transplantation three years thereafter. A heterozygous splice variant in the *SCN5A* gene (c.2437-1G>T) as well as a heterozygous VUS in the *PRDM16* gene (c.2452G>A) were detected. Based on its type, the *SCN5A* variant was considered to be likely causative. Surprisingly, neither the *SCN5A* variant nor the *PRDM16* variant was detected in the affected mother. Causality of the *SCN5A* mutation was questioned and the family was re-analyzed in our laboratory with the Illumina TruSightTM Cardio Sequencing Panel. This panel includes the *TTN* gene which was not part of the first investigation. In addition to the confirmation of the *SCN5A* and *PRDM16* variants we detected a heterozygous truncating mutation in the *TTN* gene (c.85090C>T, p.Arg28364Ter) in the patient. This mutation was subsequently also detected in the mother, indicating that this mutation is responsible for the DCM in our family. Further segregation studies in the family are underway for confirmation.

In conclusion, our study underlines the importance of the *TTN* gene for the pathogenesis of DCM which has been attributed to about 10-20% of patients but is difficult to interpret because of its size and the prevalence of variants in the reference population. It also stresses the relevance of segregation analysis before offering predictive testing of at risk family members for DCM. Further studies are required to define genotype-phenotype correlations and the impact of double mutations in hereditary cardiomyopathies.

P-ClinG-146

A relatively mild case of Bohring-Opitz syndrome

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Bohring-Opitz syndrome is an autosomal-dominant disorder, first described in 1999 by Bohring et al. It is caused by mutations in the ASXL1 gene and characterized by intrauterine growth retardation, poor feeding, typical facial gestalt with trigonocephaly, exophthalmos, a prominent metopic suture and nevus flammeus, skeletal abnormalities like flexion of the wrists and ulnar deviation and a severe to profound intellectual disability.

We present a 7 year old boy with mild to moderate global psychomotor retardation and multiple congenital anomalies. He was born as the first child of healthy, non-consanguineous parents at 40 weeks of gestation after an uneventful pregnancy. Postnatally, trigonocephaly due to premature synostosis of the sutura metopica was noted and operated at age 11 months. As further skeletal anomaly mild ulnar deviations of the wrists were detected. Cerebral ultrasound and cMRT showed a small corpus callosum. Cardiac ultrasound revealed an ASD II and bilateral mild stenosis of the main pulmonary artery branches.

During his first months of life, frequent respiratory infections, severe feeding problems and a muscular hypotonia were reported. Delayed development was noted within the first year of life. The boy could crawl and stand at 19 months.

At presentation at age 2 years his head circumference was on the 75th centile, his length below the 3rd centile, and his weight on the 10th centile. Minor facial dysmorphism included a prominent metopic suture,

hypertelorism, epicanthic folds and upslanting palpebral fissures. He did not speak but could follow simple requests and used signs. He didn´t walk unassisted.

Relevant chromosomal aberrations were excluded by CMA. NGS-based sequencing with a TruSight One Sequencing Panel (Illumina) and subsequent Sanger validation revealeda de novo mutation (p.Gln1201Lysfs *16) in ASXL1.

The patient's phenotype is generally in accordance with Bohring-Opitz syndrome. This case shows that Bohring-Opitz syndrome should also be considered in patients with trigonocephaly also in the absence of severe mental retardation.

P-ClinG-147

Report of a rare case: Mediastinal seminoma in an 11-year-old boy with Klinefelter syndrome

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An 11-year-old boy presented with pain of alternating intensity in the right chest after a 5-month history of dry cough and progressive dyspnea. On clinical examination the patient showed signs of orthopnea and tachydyspnea. In addition, we found adiposogigantism with a height of 172 cm (> P97 + 3.17 z) and a body weight of 95 kg (> P97 + 3.3 z). The X-ray study of the chest revealed widening of the mediastinum consistent with a localized tumor mass, deviation of the trachea and atelectasis of parts of the right lung due to obstruction of right main bronchus. MRI scanning was performed and demonstrated a soft-tissue mass with moderate contrast enhancement and an extent of 18 cm (cc) x 18 cm (lr) x 15 cm (ap) encasing the vena cava superior, compressing the trachea and displacing the ascending aorta and the arch of aorta leading to a pulmonary thrombosis. Lymphoma was expressed as suspected diagnosis. For further examination abdominal ultrasonography was performed revealing hepatomegaly and structurally normal testes with a volume of 1.3 ml on each side. Abdominal and cranial MRI scanning showed no pathological contrast enhancement. Cervical lymph nodes were studied by ultrasonography and showed nodes of high and low echogenicity with a size from 18 mm x 9 mm to 33 mm x 21 mm. Excision and histological examination of one lymph node demonstrated features of a seminomatous germ cell tumor without evidence of immunohistochemical markers (AFP, beta-HCG, CD30, Glypikan 3) of a non-seminomatous fraction. Chemotherapy according to protocol MAKEI 96 was initiated. Four PEI courses containing cisplatin, etoposide and ifosfamide were administered preoperatively. Complete tumor resection with lymphadenectomy was performed. The patient received one further PEI course after surgery according to protocol MAKEI 96. MRI scanning of the chest was performed for follow-up. No suspect findings were reported. The patient showed good general condition. Due to body measurements of the patient and small testes cytogenetic analysis was recommended. A karyotype of 47,XXY was detected. Although mediastinal germ cell tumors are common in patients with Klinefelter syndrome, the review of the current literature revealed, that this is the first case of mediastinal seminoma without non-seminomatous fraction reported in a patient with Klinefelter syndrome.

P-ClinG-148

Methods of qualitative research in the analysis of Development and Care in Children with rare diseases

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Background

Structured analysis of the natural history is missing for many rare diseases, e.g. for X-Linked Myotubular Myopathy (MTM) (OMIM # 310400) with an incidence of 2:100.000 male neonates. Most MTM patients die in early childhood, but some survive to school age.

Due to the small number of patients quantitative analyses (classical statistics) are not feasible for rare diseases. Therefore, we applied qualitative methods to better understand disease-related needs. We developed a protocol for collection and analysis of data on development and care of patients with MTM covering general medical data, neurology, genetics, social factors, supportive treatment and education.

Our study aims at analysing the clinical development of patients with MTM and the therapeutical approaches that are tried by families and professionals to promote the physical, motor and cognitive development of these boys. The objective is to understand which support the children need and which possibilities exist for them to attend school.

Methods

We use the descriptive and explorative methods of qualitative research. Structured, problem-centred interviews with families, teachers and physical therapists are conducted according to Witzel (2000). The interviews focus on the development of the children, the experiences with the disease and the possibilities of schooling. Data on medical history were collected using a modified version of the questionnaire from a study on infantile spinal muscular atrophy (SMA) from the Institutes of Human Genetics in Aachen and Cologne. The results of general health check-ups for children were included as well as the medical records. Content analysis according to Mayring is used for evaluation.

Preliminary results:

We contacted 17 families of which 15 agreed to take part in the study. So far, we have performed 15 interviews with parents and 5 interviews with teachers. We have reviewed the medical records of 15 patients. General health check-ups were recorded in 8 patients and a standardized physical examination was performed in 9. Preliminary results indicate complex health needs due to various co-morbidities that show the need of a multi-disciplinary approach when treating patients with MTM. We will present those experiences that are particular challenges in everyday life and are shared by several families. Since medical records rarely contained information on physio- or ergotherapy, we will extend our study by structured interviews with physical therapists.

Conclusion:

Our data show that the methods of qualitative research are a feasible approach for analyzing rare diseases. We suggest that the protocol can be adapted and applied to other rare diseases.

P-ClinG-149

Novel dominant case of de-novo mutation in Ullrich congenital muscular dystrophy

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Collagen is the most abundant protein in humans and a major component of the extracellular matrix and the connective tissue. Among several existing collagen subtypes, collagen VI consists of three subunits α1, α2 and α3, encoded by three different genes *COL6A1* (OMIM # 120220), *COL6A2* (OMIM # 120240) and *COL6A3* (OMIM # 120250). Mutations in all three genes are associated with collagen VI disorders: Bethlem myopathy (OMIM #158810) and Ullrich congenital muscular dystrophy (OMIM # 254090). Bethlem myopathy (BM) is inherited autosomal dominant and described by moderate muscle weakness and atrophy with a variable onset from early infancy to adulthood and slow progression. Ullrich congenital muscular dystrophy (UCMD) follows an autosomal recessive inheritance and is defined by severe progressive muscle weakness, hypotonia and hyperextensibility of distal joints. Recently, a few cases of dominant UCMD were also described.

We report on a 1.5-year old girl suffering from hypotonia and hyperextensible joints. After birth she presented with long, flexible fingers, a long skull and congenital dysplasia of the hip. She was suspected to suffer from Ehlers-Danlos syndrome (OMIM #130000) or congenital Marfan syndrome (OMIM #154700). The girl (index patient) was sequenced as well as her healthy parents.

We analyzed 195 genes associated to myopathy, Marfan syndrome and Ehlers-Danlos syndrome from the TruSight One panel (Illumina), a clinical exome sequencing panel which enriches 4813 disease-associated genes, following sequencing on a MiSeq desktop sequencer (Illumina). Data analysis was performed by GensearchNGS (PhenoSystems) and pathogenicity predictions were made by Alamut (Interactive Biosoftware). Filtering for variants the index patient shared either with only her mother or father revealed no conspicuous variant. Therefore, we searched for a *de-novo* mutation in the index patient and found a heterozygous splice site mutation (c.954+3A>C) in intron 9 of the *COL6A2* gene (OMIM # 120240). All five *in-silico* splice site prediction tools used (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder) predicted the mutation to reduce the efficiency of the donor-splice site of exon 9. We isolated RNA from whole blood to verify the exon-skipping ability of the mutation. Sequencing of the cDNA revealed a missing exon 9 in about one half of the sequences, confirming the heterozygous splice site mutation.

In summary, by using next-generation sequencing we could identify an unknown *de-novo* mutation in *COL6A2*, which affects a donor splice site. RNA sequencing could confirm the pathogenicity of the mutation. The heterozygosity of the mutation would suggest a case of BM. However due to the severe phenotype UCMD is suspected as clinical diagnosis, resulting in a new case of a dominant UCMD mutation.

P-COMPLEX DISEASES, POPULATION & EVOLUTIONARY GENETICS AND GENETIC EPIDEMIOLOGY

P-Compl-150

Contribution of DNMT3A variant rs11887120 to rate of cognitive decline in subjects with mild cognitive impairment

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Alterations in DNA methylation have been related to cognitive decline and Alzheimer's disease. Building on these findings, a recent study in mild cognitive impairment (MCI) reported a significant association between the annual decline in cognitive functioning and the rs11887120 single nucleotide polymorphism located in the DNMT3A gene, which is implicated in DNA methylation. Here, we aimed to replicate this finding in two independent, larger cohorts of MCI patients. In neither of the samples (nor in the pooled dataset) a significant association was found. For further investigation, stratified analyses were performed in subjects converting to dementia during follow-up, and non-converters. While converters showed a steeper decline in cognitive functioning than non-converters, no differential effects with regards to rs11887120 were observed.

P-Compl-151

The RBFOX1 gene: ASD-associated CNVs affect alternative promoters that drive stage specific expression in the mouse brain

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Autism spectrum disorders (ASD) are neurodevelopmental, heterogeneous disorders that occur in 1 of 150 children. Although it is known that ASD have a strong genetic basis only few causative genes have been identified.

The RBFOX1 gene which is located on chromosome 16p13.2 encodes an RNA-binding protein that regulates pre-mRNA splicing events in specific cell types including neurons. RBFOX1 has been found to be constantly under expressed in the brains of autistic patients. Moreover, rare copy number variants (CNVs) in the RBFOX1 gene have been found to be associated with ASD. The RBFOX1 gene contains several alternative promoter regions that drive expression of RBFOX1 transcripts containing the same coding regions but different 5'UTR exons. CNVs detected in ASD patients are located in the 5'region of the gene and are predicted to interrupt the promoter regions of some but not all of the alternative RBFOX1 transcripts. Understanding the tissue specific regulation of RBFOX1 expression by its alternative promoter regions is necessary to predict the pathogenic potential of these CNVs.

Using the mouse as a model we aim at identifying and characterizing the alternative promoter regions that drive Rbfox1 expression in the embryonic and adult brain. We have found that brain specific expression of Rbfox1 is driven from three alternative promoters that are conserved across species. Two of the three promoters are located at the 5'end of the Rbfox1 gene and drive Rbfox1 expression in the embryonic and perinatal brain. The third promoter that is located further 3' is mainly active in the adult brain. At the moment,

we are searching for transcription factors that bind to the alternative Rbfox1 promoter regions and drive Rbfox1 expression in the embryonic and adult brain.

The results of this project will not only enhance our understanding of the transcriptional regulation of Rbfox1 but will also help us to predict the pathogenic potential of CNVs found in the 5'region of the RBFOX1 gene in autistic patients. Our preliminary data suggest that these CNVs may predominantly affect RBFOX1 expression in the embryonic and perinatal brain.

P-Compl-152

Exome Sequencing in patients enriched for poor prognosis identifies new candidate genes for schizophrenia

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Schizophrenia (SCZ) is a multifactorial disorder with a lifetime risk of ~1% and an estimated heritability of approximately 60-80%. The genetic architecture is complex and established risk factors include both common low-penetrant and rare high-penetrant variants. Exome Sequencing allows for the identification of the latter. Due to the large number variants per exome, it is difficult to distinguish between clinically relevant and "phenotype neutral" variants. Focusing on a subgroup of patients with a clinically narrow defined phenotype that implicates a high underlying genetic load (i.e. early age at onset and chronic course of disease with deterioration) increases the likelihood of detecting variants that are relevant for the disorder.

In total, 150 patients with a DSM-IV diagnosis of SCZ were analyzed. The cohort was enriched for the above mentioned phenotype. For DNA enrichment the Agilent SureSelect Human All Exon v5 Enrichment Set was used. Exome-Sequencing was performed on Illumina HiSeq 2500. Data analyses and filtering was performed with the Varbank pipeline v.2.1 and interface. The dbNSFP database (https://sites.google.com/site/jpopgen/dbNSFP) was used to in silico predict the functional relevance of the identified variants. In order to be considered in our downstream analysis, the variants had to: (i) be predicted to be functionally relevant from > five in silico prediction tools and (ii) identified in a gene that was already reported to carry a de novo mutation in SCZ patients (list of ~ 700 genes, data from ~ 950 patients). Additionally, we are cross-checking our results with published de novo studies in patients with autism spectrum disorder.

On average, we identified two variants per individual and confirmed these by Sanger sequencing. We are currently genotyping all identified variants in 1,000 German population-based controls and will exclude variants with a minor allele frequency ≥0.1%. In addition, we performed gene-based tests (as implemented in VEGAS) in the largest genome-wide association study of SCZ to date (containing 35,000 patients and 100,000 controls).

To validate the candidate genes identified in the exome sequencing and assess their generalizability, we will re-sequence these genes in large samples of phenotypically well-characterized unrelated patients with SCZ and controls. For the re-sequencing, we will prioritize those genes with an additional association with common variants (set-based tests) and those that are brain-expressed. As a promising candidate gene for follow-up analyses, we identified a non-synonymous, potentially damaging mutation in the SEC31A gene. Interestingly, de novo mutations in this gene have been reported in two independent SCZ-affected individuals and were additionally identified in our in-house trio dataset. The analyses are ongoing and will be presented at the upcoming conference.

P-Compl-153

Analysis of further genes of the IL-36 pathway in patients with generalized pustular psoriasis from Germany

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Generalized pustular psoriasis (GPP) is a rare, severe inflammatory psoriatic skin manifestation that can be accompanied by a potentially life-threatening multi-systemic inflammation. Bi-allelic loss-of-function mutations in a gene (*IL36RN*) coding for the antagonist of the IL-36 receptor have been identified as a frequent cause for GPP (22% in 50 patients from Germany), indicating this entity as either a Mendelian trait or as major genetic risk factor in contrast to the numerous associated SNPs in classical plaque type psoriasis. The pathogenic model suggest that mutations in *IL36RN* cause an imbalance between the pro-inflammatory cytokines IL-36α, IL-36ß and IL-36γ and its antagonist leading to an excessive inflammatory reaction.

We hypothesized that mutations in genes coding for other members of the IL36-pathway (IL-36 receptor, IL-36α, IL-36ß and IL-36γ) might be disease-causing, as well. Therefore, we analyzed sequencing data of the four genes generated by whole exome sequencing of 25 GPP patients and sequenced the four genes in further 25 patients by Sanger.

Those 50 GPP patients were recruited at 7 German university hospitals, and three further dermatological departments; 47 patients suffered from typical symptoms of GPP, 3 further ones were diagnosed with GPP as a differential diagnosis. The majority of GPP patients were female (68%), age of recruitment was 46.8±17.2 years and age of disease onset was 27.6±21.1 years.

Using the NGS Variant analyzer tool, we identified 219 variants in the four genes. After filtering for rare variants (MAF<1% in publicly available datasets), position in coding exons or neighboring intronic sequences (+/-20bp), two variants remained in two patients, respectively. The first one was a potential splice site variant: c.391+9C>G (rs13429363) in exon 6 of *IL36B*; further analysis revealed low conservation and no evidence for an effect on splicing by using a splice site prediction tool. The second variant was a missense variant: c.1411G>A; p.Ala471Thr (rs75091099) in exon 11 of *IL1RL2* (coding for the IL-36 receptor) with only moderate positional conservation. Prediction of protein function by four different algorithms was ambiguous; furthermore this variant was identified on 3.3% alleles of 603 internal control individuals, indicating that this missense variant is unlikely disease-causing. Sanger sequencing of the four genes in the further 25 patients revealed 13 variants in coding exons and neighboring intronic sequences (+/- 20bp). After filtering for rare variants (MAF<1%), no variants remained. For the next step, we plan quantitative analyses of exons of the four genes in all GPP patients to exclude a possible CNV.

In conclusion, coding variants in the four genes of the IL-36 pathway can be excluded as disease-causing variants in our GPP patients. Other yet unidentified pathways are more likely to play a major role in GPP.

P-Compl-154

The founder mutation p.Y151N in the cystinuria gene SLC3A1 reflects the (demographic) history of Malta

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The present Maltese population (425,000 people) originates from a much smaller population in the past four hundred years. This demographic expansion has been accompanied by the introduction of several founder effects due to admixture with different populations in the Mediterranean. We report on the identification of the founder mutation p.Y151N in SLC3A1 as the major pathogenic variant in Maltese cystinuria patients. Cystinuria is characterized by the disturbed reabsorption of cystine and the dibasic amino

acids in the proximal renal tubule, and it is exclusively caused by mutations in the SLC3A1 and SLC7A9 genes. In SLC3A1, more than 150 autosomal recessive mutations have been reported, some of which show a population-specific distribution. The variant p.Y151N (NM_000341.3:c.451T>A). This variant has been reported only once in an Italian patient (1 out of 336 alleles). In a European control population (n=56,600; rs376910755), one carrier has been reported. By analyzing seven Maltese cystinuria patients we detected the mutation in 13 alleles. Further screening of 100 healthy Maltese control individuals resulted in the detection of one heterozygote carrier. The observation of an accumulation of a rare mutation in the Maltese population reflects the history of Malta and its population. It can be assumed that the mutation p.Y151N was initially a founder mutation the Sicilian/Italian population, and was then transferred to Malta in the medieval ages. With the expansion of the small medieval Maltese population in the last centuries, the mutation expanded as well, becoming the major cystinuria mutation in Malta. Our results furthermore help us to calculate a prevalence of cystinuria of 1 out of 40,000 for SLC3A1 mutations in Malta, which is in the range reported for other populations (1:2,500 in Libyan Jews and 1:100,000 in Sweden). A frequency of heterozygous carriers for SLC3A1 mutations in the Maltese population of 1:100 can be estimated. Our data demonstrate that the genetic composition of a population is affected by its history and demography, and it confirms that small isolated communities help to trace the genetic drift of a mutation and the associated disease.

P-CompI-155

A pilot study revealed strong association of the subphenotype of vulnerable atherosclerotic plaques with common risk variants for coronary artery disease

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Introduction

The risk of atherosclerotic plaques to induce vessel occlusion mainly depends on plaque phenotype. Compared to stable plaques, vulnerable lesions are prone to rupture due to their composition. While several risk alleles have been identified for coronary artery disease (CAD) and the incidence of atherosclerotic plaques, none of these studies analyzed a potential association with plaque phenotype.

Materials and Methods

In a small pilot study, we used trichrome-stained cross-sections of human carotid endarterectomy specimens for classification into groups of stable (n=45) and vulnerable plaques (n=51) based on established parameters, such as the size of the lipid core, fibrous cap thickness, the collagen content, platelet aggregation and intraplaque hemorrhage. DNA was isolated from tissue sections of plaques and genotyped for 32 common SNPs previously associated with CAD. Association analysis was performed using Chi-Square-Test and Fisher`s exact test.

To analyze both, the inflammatory state of the plaques and the degree of neovascularization, cross-sections of 20 stable and 20 vulnerable plaques were immunohistochemically stained for infiltrated macrophages (CD68), T lymphocytes (CD3) and newly formed vessels (CD31). Cell number and vessel density were determined microscopically in different plaque regions (inflammatory plaque shoulder, fibrous cap and lipid core).

Results

Two SNPs showed significant association. rs579459, located on chromosome 9q34 near the ABO blood group showed higher risk allele frequency in patients with vulnerable plaques compared to those with stable plaques (0.43 vs. 0.21, P=0.013) but with a broad confidence interval, OR (95%CI)=1.32 (0.38;1.74). The second SNP, rs11556924, is a synonymous SNP in the coding region of ZC3HC1, encoding an F-box-containing protein that is a component of an SCF-type E3 ubiquitin ligase complex regulating onset of cell division. It exerts its effect on CAD through a yet unknown mechanism. In our study the risk allele frequency in patients with vulnerable plaques was increased compared to those with stable lesions (0.57 vs. 0.29, P=0.014; OR C/T=2.59 (1.39;4.80); OR CC/nonCC=3.45 (1.44;8.27). Compared to stable plaques, macrophages and T cells were significantly increased in the fibrous cap (macrophages: 50±7 vs. 20±6, p<0.001, T cells: 17±4 vs. 6±3, p=0.009) and the inflammatory plaque shoulder (macrophages: 127±9 vs. 47±6, p<0.001, T cells: 24±5 vs. 7±3, p<0.001) of vulnerable lesions, which were also characterized by a higher vessel density (13±2/mm2 vs. 5±1/mm2, p<0.001). Nonetheless, neither SNPs showed any association with the investigated immune cell content.

Conclusions

The risk allele of SNP rs11556924 in ZC3HC1 showed much stronger association to the plaque vulnerability subphenotype than to CAD itself indicating that it might exert some of its effects on CAD also via

the characteristics of atherosclerotic plaques but not their content of immune cells. Our results warrant a larger confirmatory study which is ongoing.

P-Compl-156

Association of genetic risk scores of complex diseases and traits with age-related macular degeneration (AMD)

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Purpose: Age-related macular degeneration (AMD) is the leading cause of vision loss in western societies with strong environmental and genetic components influencing disease development. With regard to the latter, the International AMD Genomics consortium (IAMDGC) recently reported the association of 52 independent genetic signals at 34 loci with AMD risk by analysing over 30,000 individuals. To evaluate a possible overlap with the genetic risk of other complex diseases / traits, we calculated their genetic risk scores and assessed their association with AMD.

Methods: First, we catalogued previously published, genome-wide significant variations for 48 complex diseases/traits and extracted a single variation per independent locus. Second, we calculated a genetic risk score for each trait/disease by calculating the weighted sum of risk alleles for all late stage AMD cases and controls. We then compared the genetic risk scores between late stage AMD cases and controls to identify significant overlaps between AMD risk and the risk for other diseases.

Results: So far, we completed the analysis of 23 out of 48 risk scores and evaluated their association with AMD risk. Highly significant associations (P < 10^-5) were found for risk scores of two complex diseases related to autoimmunity, four complex traits related to cardiovascular diseases, four traits related to lipid metabolism and two traits related to complex eye phenotypes. By restricting the analysis to the 758 variants initially used to compute the risk scores, we identified over 50 novel AMD risk loci so far not recognized by genome-wide association analyses (FDR < 0.01). We show that there is a strong correlation between genetic risk scores within disease/trait clusters as well as between traits and trait clusters. Together, the significantly , Herms S.1,2,4, Hoffman P.5, Fischer S.4, Thiele H.6, Nürnberg P.6, Cichon S.5, Rietschel M.3, Nöthe

Conclusion: We demonstrate a substantial overlap between complex diseases/traits and AMD risk and provide significant evidence for more than 50 additional loci associated with AMD. This highlights the fact that unrelated pathologies may share correlated genetic risk profiles and thus common disease pathways. It further challenges the notion that gene/genome manipulation may be generally applied to eradicate risk for a defined complex disease.

P-Compl-157

Stomach Specific eQTLs represent Risk Factors for the Development of Gastric Carcinoma

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Gastric carcinoma (GC) represents the fifth most common cancer type worldwide and depending on the subtype and stage at diagnosis the prognosis for affected patients remains to be poor. Effort on characterizing the somatic genomic landscape of GC revealed deep insides into the tumor biology, opening ways to new therapeutic approaches. However, essential questions, for example on the tumor subtype development, remain to be solved. One important aspect in this context is the identification of genetic risk factors priming to GC development. To elucidate risk variants and genes, GWAS in Asian populations were performed with success, but so far studies in patients of European origin are scarce and focused on small sets of candidate loci only.

To identify germline variants playing a role in the development of GC in patients of European descent, we genotyped a set of 27 SNPs in a sample comprising 502 GC cases and 507 controls of Lithuanian or Latvian origin. As disease associated variants often reside in gene regulatory elements, we selected the SNPs based on the latest data release of the Genotype-Tissue Expression (GTEx) consortium, focusing on stomach specific expression quantitative trait loci (eQTLs).

Three of the tested SNPs showed at least nominal significance ($P < 0.05$). rs2976397 showed the strongest association signal ($P = 2x10^{-8}$) at the same time influencing the expression of PSCA. This result confirms the most significant finding of the GWAS in Asian patients, which identified PSCA as risk gene for GC development. In addition rs198408 was associated to GC ($P = 1.67x10^{-2}$) and regulates the expression of NPPA, encoding a natriuretic peptide. Furthermore, rs10771539 showed GC association ($P = 2.31x10^{-2}$) and controls transcript levels of KLRB1, a gene involved in regulating the activity of natural killer cells, which may be of interest on the background of an Helicobacter pylori infection.

In summary, in this study we provide a link of stomach specific eQTL findings with the development of GC. Further replication studies in other European samples will be performed to confirm the GC association of the analyzed eQTL loci.

P-CompI-158

Analysis of psoriasis vulgaris associated candidate genes with next-generation sequencing in Families with "dominant-like" disease inheritance

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Psoriasis is a complex chronic immune-mediated inflammatory disease of the skin with a prevalence of about 2-4 % in the Caucasian population. The most common form, psoriasis vulgaris (80-90 % of the patients) is characterized by red and dry lesional plaques predominantly on knees, elbows, scalp and trunk. The exact cause for the development of the disease is unknown but several environmental, epigenetic and genetic factors play an important role. Genetic analysis exposed several genes which are involved in disease development and genome-wide association studies increased the number of susceptibility loci over 40. Furthermore, these studies revealed the importance of different inflammatory innate and adaptive immune system pathways.

Our unique collection of psoriasis samples has been used for familial based analysis. The collection consists of 2096 individuals, thereof 831 affected from 203 different families with mainly "dominant-like" inheritance of psoriasis (80 % penetrance and 5 % phenocopy). To investigate potential genetic factors playing a role in psoriasis disease development in these families, we designed a Haloplex Gene Panel (Agilent Technologies, Santa Clara, CA, USA) assay for next-generation sequencing (NGS) with the MiSeq (Illumina, San Diego, CA, USA). Based on recent literature studies, 54 candidate genes were selected for the assay. All candidate genes are mentioned in at least one publication and most of them are directly associated to psoriasis through a PSORS-locus or GWAS results.

So far, we sequenced 2 affected individuals of 20 different families with MiSeq. The bioinformatics evaluation of the NGS sequencing results was performed through a stringent standard NGS analysis pipeline which was developed by our bioinformatic department. Afterwards, interesting candidate variants underwent a co-segregation analysis with Sanger sequencing. The preliminary results of this family based approach will be presented.

P-CompI-159

Association of miRNA polymorphisms with ischemic stroke in Northeast of Iran, a database structure

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Background: Single nucleotide polymorphisms (SNPs) in miRNA genes can affect their biogenesis and function, therefor may contribute to a pathologic situation. Although association of these polymorphism with a wide range of diseases has been observed, there are few studies investigating such association with ischemic stroke. It has been shown that stroke in Northeast of Iran occurs approximately one decade earlier than in Western countries. So we made a Genetic stroke databases with more than 1000 Stroke patients for this region. 25 gene polymorphisms were analyzed and registries in this database. Furthermore this database is part of INTERNATIONAL STROKE GENETICS CONSORTIUM .The present study aimed to determine if there is any association between 7 SNP within miRNA gene regions (mir-30c rs928508 G/A, mir-125a rs12976445 C/T, mir-146a rs2910164 G/C, mir-221 rs113054794 C/A, mir-34a rs369892834 T/C, mir-499 rs3746444 A/G and mir-608 rs4919510 C/G) and ischemic stroke in population of Northeastern of Iran.

Methods: In this case-control study patients with ischemic stroke and subjects without any history of vascular diseases were recruited. All cases were diagnosed via computed tomography (CT) scans and magnetic resonance imaging (MRI). Mir-499 rs3746444 A/G was genotyped in cases and controls using

PCR-RFLP ARMS-PCR method. Statistical analyses were performed using SPSS v.21 software and a p-value < 0.05 was considered significant.

Results: Following statistical analysis significant association was observed between the G allele for mir-30c rs928508 (p-value=0.023; OR=1.42, 95% CI 1.05-1.93), the C allele for mir-125a rs12976445 (p-value=0.048; OR=1.37, 95% CI 1-1.86), the A allele for mir-221 rs113054794 (p-value=0.001; OR=1.7, 95% CI 1.237-2.475) and G allele for mir-499 rs3746444 (p-value=0.005; OR=1.62, 95% CI 1.44-1.87).

Conclusion: According to results of the present study, mir-30c rs928508, mir-125a rs12976445, mir-221 rs113054794 and mir-499 rs3746444 can be considered as susceptibility genetic markers for ischemic stroke in Northeast of Iran.

P-Compl-160

Deleterious mutations in GTPase related genes in bipolar disorder.

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Bipolar disorder is a common, complex, and heritable psychiatric disorder characterized by episodes of extremely elevated, expansive or irritable mood, grandiosity, flight of ideas, distractibility or agitation. severe mood swings. Heritability estimates range from 58% to 93% with a monozygotic twin concordance rate of about .43. Nevertheless, the etiology of the disease remains unknown. Linkage studies and genome-wide association studies have suggested chromosomal and genomic regions potentially related to bipolar disorder, but the identification of disease causing variants remains largely elusive. Exome-wide sequencing offers now the opportunity to lead these investigations to a new level.

The identification of rare, damaging genomic mutations in families with bipolar disorder could inform about disease mechanisms and lead to new therapeutic interventions. To determine whether rare, damaging mutations shared identity-by-descent indicate potential pathophysiological pathways, exome sequencing was performed in multigenerational families of the National Institute of Health Bipolar Disorder Family Study followed by in silico functional prediction. Disease association and disease specificity was determined using 5,090 exomes from the Sweden-Schizophrenia Population-Based Case-Control Exome Sequencing study. We identified 14 rare and likely deleterious mutations in 14 genes that were shared identity-by-descent among affected family members. The variants were associated with disease compared to healthy controls (Fisher exact $p<0.05$ after Bonferroni correction) and disease specificity was supported by the absence of the mutations in patients with schizophrenia. The mutated genes are related to GTPase activity in neuronal pathways involved in G-protein coupled receptor signaling. This disease mechanism has been implicated in the pathophysiology of bipolar disorder in cell culture and animal studies, indicating that the disease association is plausible and coherent with well-established observations and pathophysiological theories. In addition, we found rare, functional mutations in known disease-causing genes for neuropsychiatric disorders including holoprosencephaly and epilepsy which could be modifiers of the disease phenotype.

The results of our study indicate that rare, deleterious mutations in gene-coding regions could be related to a bipolar phenotype in families, in which the disease is transmitted over several generations. Exome sequencing in multigenerational families with bipolar disorder is effective in identifying rare genomic variants with potential clinical relevance. Our results further support the rare-variant disease model of bipolar disorder. The disease association of the identified mutations need to be replicated and the functional consequences of the mutations validated before the information could be used in clinical settings.

P-Compl-161

Screening for rare disease-causing variants in extended CAD families

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Genome-wide association studies (GWAS) have identified several common variants for coronary artery disease (CAD). However, only around 10% of the genetic heritability can be explained by these common variants so far. It is to be expected that a proportion of this so-called missing heritability can be explained by rare variants. A positive family history is a known risk factor for cardiovascular disease and underlines the strong genetic background of CAD. Hence, families severely affected with CAD, are a suitable starting point to unravel the genetic causes. One successful example is a study were we identified two causal mutations in one of our extended families from the German MI family study.

Here, we aim to identify further rare variants by studying ten extended families. For this, we exome sequenced three affected family members per family. We searched for variants which are rare in the general population and shared by all three family members. This strategy led to the identification of several potential causal variants per family. To further filter the variants, we performed a ranking based on several prediction scores such as SIFT, Polyphen, CADD and MutationTaster. In addition, we included variants that are found in or in proximity to CAD associated GWAS genes. The most likely candidates were checked for co-segregation with disease in each respective family.

Unraveling the genetic cause of CAD proved difficult in some of the investigated families. In our ongoing work we will account for potential phenocopies by also studying variants not shared by all affected family members. Further, we check for combinations of variants that might better explain the disease in the family. We also develop strategies to identify variants with reduced penetrance, a well known phenomena in CAD.

P-CompI-162

Fine-mapping of the RUNX3 locus in psoriatic arthritis localizes the association to variants in the 5' region

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Psoriatic Arthritis (PsA) is a chronic inflammatory joint disease occurring in up to 30% of patients with the most common psoriatic skin manifestation, psoriasis vulgaris (PsV). PsA is considered a T-cell mediated disease with a complex genetic basis. To date, GWAS identified 11 susceptibility loci for PsA including the *RUNX3* locus on chromosome 1. Most of these loci overlap with those identified for PsV, and the respective candidate genes are involved in innate and adaptive immunity.

In a previous GWAS we identified the *RUNX3* locus as a susceptibility locus, and an analysis of several additional SNPs revealed most significant association (p=1.40E-08) to a linkage disequilibrium (LD) block of 2.6kb in the first intron of *RUNX3*. Unfortunately, all SNP genotyping arrays available in the project (Affymetrix 6.0, Illumina 660 BeadChip, and Illumina Immunochip) had insufficient coverage at this locus. Therefore, we decided to fine-map the locus with a set of 32 tagging SNPs covering the whole gene and its neighboring 5' and 3' LD block (overall size 92kb). When investigating those SNPs in 2,489 PsA patients and 6,202 control individuals of the Psoriatic Arthritis Genetics in Europe consortium, we observed most significant association to 5 SNPs (3.17E-08 ≥ p ≥ 9.96E-13) in one LD block of 16kb, located 5' to the previously identified LD block. This newly identified LD block comprises part of intron 1, a short exon 1 as well as 14kb of the upstream region of *RUNX3*.

Analyses of genomic annotations of all SNPs (n=54) in high LD (r2≥0.8) with the 5 associated SNPs indicated that 8 SNPs are located in histone modified transcriptionally active regions in different immune cell subsets relevant in PsA including primary T cells, memory T cells, T helper cells, CD8+ T cells, monocytes and chondrocytes. We are currently pursuing functional studies to test whether those SNPs have an effect on gene expression and whether they alter transcription factor binding sites.

Furthermore, one of our 5 primarily associated SNPs (p=9.96E-13) was an interesting missense variant, as two variant prediction tools indicated a (probably) damaging effect. In contrast, protein modeling did not reveal an impact of the missense allele on protein structure. As the missense variant locates near the exon intron border, we hypothesized alternative splicing of *RUNX3* mRNA. In cDNA from whole blood, though, RT-

PCR experiments revealed an inconsistent expression independent of the SNP's genotype and no evidence for alternative splicing. Studies exploring splicing effects in further tissues and qPCR experiments are in progress.

Thus, our study refined the association signal to a 16 kb LD block encompassing exon 1 with an interesting potential splice variant as a candidate disease-causing SNP. By performing further functional studies, we will identify the disease-causing variant and characterize the molecular mechanism that contributes to the pathogenesis of PsA.

P-Compl-163

Role of Alzheimer's disease risk genes in the progression to dementia and in relevant endophenotypes among subjects with mild cognitive impairment.

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Few data are available concerning the role of risk markers for Alzheimer's disease (AD) in progression to AD dementia and AD endophenotypes in patients with mild cognitive impairment (MCI). We therefore investigated the role of well-known AD-associated single nucleotide polymorphism (SNP) in the progression from MCI to AD dementia and in AD endophenotypes such as cognitive decline over time and cerebrospinal fluid (CSF) biomarkers (aβ, tau, ptau). Four independent MCI datasets were included in the analysis: (a) the German study on Aging, Cognition, and Dementia in primary care patients (n=853); (b) the German Dementia Competence Network (n=812); (c) the Fundació ACE from Barcelona, Spain (n=1245); and (d) the MCI datasets of the Amsterdam Dementia Cohort (n=306). The effects of single markers and combined polygenic scores were measured using Cox proportional hazards models, linear mixed models, and meta-analyses. Three different polygenic scores were constructed. Polygenic score 1 (PGS1) comprised the nine established AD-associated SNPs reported prior to publication of the International Genomics of Alzheimer's Project (IGAP) consortium results in 2013. Polygenic score 2 (PGS2) comprised nine of the 11 novel AD-associated SNPs identified by IGAP. Polygenic score 3 (PGS3) comprised all SNPs from Polygenic Scores 1 and 2. The Clusterin (*CLU*) locus was an independent genetic risk factor for MCI to AD progression (*CLU* rs9331888: Hazard ratio (HR)=1.187[1.054-1.32]; p=0.0035). PGS1 predicted a small effect on the risk of MCI to AD progression in *APOE* ε4 carriers (HR=1.746[1.029-2.965]; p=0.038), whereas PGS2 and PGS3 had no effect on disease progression. In the AD endophenotypes, PGS3 was modestly associated with cognitive decline over time, as measured by changes in mini-mental state examination (β±SE:-0.24±0.10; p=0.012), particularly in *APOE*-ε4 non-carriers, and with tau and ptau (tau: 1.38±0.36; ptau: 1.40±0.36; both p<0.001). After stratification, these associations with the endophenotypes remained significant in APOE-ε4 non-carriers, and in progressors to AD dementia. In conclusion, although SNP-based polygenic risk scores comprising currently available AD genetic markers did not predict MCI to AD progression, we observed a joint effect of AD susceptibility genes on non-amyloid endophenotypes. These findings suggested a link of these genes with neuronal degeneration in general, rather than with Alzheimer-related amyloid deposition. Finally, we conclude that SNPs in *CLU* are potential markers for MCI to AD progression.

P-Compl-164

The alternative serotonin transporter gene SLC6A4 promoter P2 drives expression differentially in the gut and is associated with Irritable Bowel Syndrome

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The serotonin reuptake transporter (SERT) gene *SLC6A4* represents one of the most promising candidates involved in the aetiology of the irritable bowel syndrome (IBS). SERT is responsible for the reuptake of serotonin from the synaptic cleft into the presynaptic neuron and the interstitial space into enterocytes. To date, two distinct promoters of *SLC6A4* have been described, P1 and P2. The short allele of the promoter P1 polymorphism 5-HTTLPR has been found to be associated with IBS in various studies. In addition, its association with depression and anxiety is well established supporting the biopsychosocial model of IBS.

The alternative promoter P2 predominantly drives expression in the gastrointestinal tract.

For unravelling the role of P2 in IBS development, we have performed sequencing analysis of the promoter region P2 in a discovery sample from the UK consisting of 98 patients with diarrhoea (IBS-D), 100 patients with constipation (IBS-C) and 92 control individuals. This revealed several single nucleotide polymorphisms (SNPs) within the respective promoter region to be associated with IBS-C. Interestingly, haplotype analysis uncovered all SNPs to be in strong LD (>0.9) among each other and to be incorporated in two main haplotypes. The tagging SNP rs2020938 (tagSNP) was determined for validation of the association finding in additional case control samples.

The P2 tagSNP rs2020938 has so far been found to be associated in three case control samples: in the initial screening collective from the UK, an US American (197 IBS; 95 controls) and a Greek sample (161 IBS, 143 controls). Currently, additional case control collectives from partners in the COST Action BM1106 GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe, www.GENIEUR.eu) are being tested for association (1590 IBS; 2580 controls). Functional follow-up of the risk/protective haplotypes in luciferase reporter assays in the two neuronal cell lines SH-SY5Y and IMR-32, as well as the gastrointestinal cell lines Caco2 and Colo 320 and the standard embryonic kidney cells HEK293 showed that the risk haplotype leads to enhanced luciferase activity corresponding to increased expression compared to the protective haplotype.

Our hypothesis is that the protective allele of rs2020938 in P2 might lead to decreased expression levels and the risk allele to the opposite effect. Studies are currently ongoing comparing expression analysis in different subregions of the gut, including jejunum, ileum and colon to confirm our findings. We will report on latest replication and expression data.

P-Compl-165

Effects of Alzheimer's disease-associated genetic factors on the aging brain: a pathway-based polygenic scoring analysis of cortical thickness

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Late-onset Alzheimer's disease (LOAD) is the most common form of neurodegenerative dementia and pathologically defined by neuronal loss due to accumulation of neurofibrillary tangles and amyloid plaques in the brain. To identify individual common genetic variants (SNPs) with small effects on LOAD susceptibility, large genome-wide association studies (GWAS) have proven to be successful in recent years. To date, the

most promising GWAS findings beyond the strongest LOAD risk factor apolipoprotein E (APOE) consist of 19 further genome-wide significant gene loci from different biological pathways. So far, not much is known, how this framework of genes affect cortical atrophy in aging brains before LOAD manifestation.

Here, we aimed to characterize the summarized effects of these LOAD loci in 544 elderly from the general population (1000BRAINS) using polygenic scoring (PS) analyses on cortical thickness (CT), a heritable imaging endophenotype of the neuron-rich gray matter of the cerebral cortex. CT was determined on T1-weighted images (FreeSurfer) from a 3T scanner. Subjects were screened for mild cognitive impairment and dementia (DemTect). PS values were calculated for each subject (PLINK 1.9) on imputed SNPs (1000Genomes): six SNP sets targeted pathway-specific effects and a seventh set targeted effects across all pathways (overall). Each analysis tested if the correlation between PS and CT, accounting for gender, differed from zero; DemTect and age were used as nuissance factors. To evaluate effects of APOE, we used e4-status as covariate in secondary PS analyses and a single APOE analysis.

We observed negative correlations between PS and CT for overall, cholesterol (chol), beta-amyloid precursor (bap), endocytosis (endo), epigenetics (epi), and APOE: the higher the genetic risk for LOAD, the lower the CT in the affected cortical region. For overall, the findings mapped to the inferior frontal, posterior temporal, and medial occipital cortex. This basic pattern was mainly influenced by APOE. For chol and bap, the pattern was extended by findings in the left inferior frontal and right posterior temporal cortex. Bap showed an additional finding in the superior parietal cortex that was independent from APOE. One finding was specific for endo (anterior cingulate) and another was specific for epi (inferior parietal).

Our study provides evidence that cortical atrophy in elderly with an average age of less than 70 years is significantly influenced by polygenic risk for LOAD that can be assigned to individual pathways. As expected, APOE exhibited a pronounced effect on gray matter in the context of other LOAD loci. However, the finding in the superior parietal cortex suggest a differential role of APOE in the bap pathway. The variability of our findings beyond the basic pattern (app, endo, epi) supports the view that LOAD is heterogenous and that genetically guided definitions of anatomical subtypes may be useful as early markers of the disease.

P-Compl-166

The Genome and Microbiome are nested in Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is highly prevalent disorder affecting up to 15% of EU citizens and represents a serious burden to the healthcare system, recently estimated to amount €41 bio annually in the EU. Patients suffer from a reduced quality of life, no specific diagnostics and treatment is available owing to its poor understanding leading to poor management of the disease. Besides gastrointestinal symptoms (abdominal pain, abnormal bowel habits), patients present with a comorbid conditions such as psychiatric disorders (anxiety, depression, chronic fatigue) and pain syndromes (migraine, fibromyalgia).To date, IBS research has been challenging due to the marked symptom heterogeneity coupled with the multifactorial pathogenesis of the disease. One of the shortcomings of contemporaneous research in IBS is the lack of integrative approaches to investigate the different pathophysiological features using unified phenotyping tools.

The COST Action BM1106 GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe, www.genieur.eu) aims at significantly accelerating the establishment of a pan-European, interdisciplinary network of clinicians and basic scientists with the major goal to study epi-/genetic factors and microbiota in the etiology of IBS. To date, 21 European countries as well as the three overseas countries Australia, Chile and the USA are engaged in GENIEUR and established unified guidelines for patient / control recruitment as well as phenotypic characterization (deep phenotyping using a detailed case report form in addition to questionnaires assessing the gastrointestinal complaints and comorbid psychiatric conditions and somatization). For that purpose, a basic study protocol was established for harmonized phenotyping and standardized collection of blood, stool and tissue samples of patients and control individuals for molecular studies.

We are aiming at the first systems medicine approach integrating environmental (stress, infection, nutrition), intrinsic (genomics, epigenomics, microbiota) and detailed phenotypic data to advance the understanding of the pathogenesis of IBS and its comorbidities. Our major hypothesis is that specific combinations of intermediate traits and intrinsic factors (epi-/genome, microbiota) will allow definition of distinct patient entities, thereby facilitating diagnosis, individualizing therapy, and may serve as biomarkers to stratify patients entering clinical trials. Our ultimate goals are systems and gender specific approaches to examine large data sets in a central repository to (i) Identify biomarkers to define subgroups of IBS patients, (ii) establish subtype specific disease models in order to better understand their pathophysiological molecular basis, (iii) identify factors which modify the response to environmental exposures.

P-Compl-167

In-silico Prediction of Causal Coronary Artery Disease Genes

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Background: To date, we have identified 202 significant and suggestive coronary artery disease loci (CAD) through genome-wide association studies (GWAS). Here, we report an extensive bioinformatics analysis to predict the causal genes underlying the reported CAD loci.

Methods and results: We annotated each GWAS locus with respect to protein-altering SNPs, association with gene expression and altered miRNA binding sites. In addition, we used the publicly available ENCODE dataset to identify SNPs within regulatory regions of the genome, such as enhancer and promoter sites. Consistent with previous findings, we found that most CAD-loci lie in non-coding regions. Around half of the loci affect gene expression robustly, 2/3 overlap promoter regions and nearly all loci can be linked to other regulatory regions of the genome. In contrast, only a small percentage (5%) of SNPs affects protein coding. Comparing our in-silico gene annotation with the genes traditionally assigned to the loci through proximity mapping, we found that a substantial number of genes differs. Indeed, we identify around 100 genes not linked with CAD before.

Conclusion: Our results significantly revise the list of potential causal CAD genes underlying the genome-wide association signal and might help to shed new light on the genetic mechanisms of CAD.

P-Compl-168

SNPs in TLR2 and TLR4 genes as prognosis factors for coronary events

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Background: Infection and inflammation are considered to be major risk factors for cardiovascular diseases. Toll-like receptors (TLRs), including TLR2 and TLR4, play an important role in initiating signal transduction cascades leading to stimulation of inflammatory response. Functional relevant polymorphic changes in TLR2 and TLR4 were associated with cardiovascular diseases. In this longitudinal cohort study (ClinicalTrials.gov Identifier: NCT01045070) the impact of SNPs in TLR2 (rs5743708) and TLR4 (rs4986790) on the prognosis after cardiovascular events (CHD) was evaluated.

Patients and methods: At baseline a total of 1002 consecutive patients with angio¬graphically proven CHD of the Martin-Luther-University Halle-Wittenberg (Germany), Department of Medicine III, were prospectively included in the study. The three-year cardiovascular outcome of the patients was evaluated considering the predefined, combined, primary endpoint (cardiovascular death, death due to stroke, myocardial infarction, and stroke/TIA (transient ischemic attack)). Genotyping was performed using PCR and RFLP analyses. For statistical evaluation SPSS 22.0 was applied.

Results: The drop-out rate after three years was 5%. 16% of the patients achieved the primary endpoint (myocardial infarction: 3.4%, stroke/TIA: 2.4%, cardiovascular death: 9.5%, death due stroke: 0.7%). In Kaplan-Meier survival curves and Breslow tests, the TLR2 and TLR4-polymorphisms could not be proven as significant predictors for adverse cardiovascular events regarding the three-year outcome. However, evaluating the impact of TLR2 and TLRP4 SNPs on cardiovascular death the AG+GG genotype of rs4986790 (TLR4) was shown to be a significant prognosis factor (Breslow test p=0.042). AG+GG genotype carrier suffered 1.7 times more frequently from cardiovascular death than AA carrier. In multivariate cox regression analysis this association could not be confirmed.

Conclusions: AG+GG genotype of rs4986790 was shown to be a prognostic, but not independently, marker for further cardiovascular death among in-patients with CHD.

P-CompI-169

Role of miRNAs in the etiology of alopecia areata: A genome-wide miRNA association analysis

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Background: Alopecia areata (AA) is a common hair loss disorder characterized by a sudden onset of patchy areas of hair loss, which can occur on the scalp or elsewhere on the body. Immunological and genetic association studies support the hypothesis that AA is autoimmune in nature. Recent evidence points to a significant role of miRNAs in autoimmune diseases; but the role of miRNAs in AA has not been investigated so far.

Methods: We performed a systematic analysis to investigate whether common variants among all known autosomal microRNAs loci contribute to AA development. Gene-based analyses were performed by VEGAS for all miRNAs listed in miRBase and their flanking sequences using the largest GWAS data set of 3,253 patients and 7,543 controls.

Result: As a result, 78 of the 617 investigated microRNAs showed nominally significant p-values. After correction for multiple testing, three microRNAs (miR-1237, miR-30b/d, miR-548h-2) showed significant association with AA. The most promising one was miR-30B. Target gene analyses for 3 disease associated microRNAs revealed 2,072 nominally significant predicted target genes. Gene based p-values were calculated for the predicted target genes revealing 42 of them to be significantly associated with AA after correction for multiple testing, including IL2RA, ERBB3 as genome-wide significant loci from former AA GWA-studies. By luciferase assays, we validated the site-specific regulation of IL2RA, STX17 and TNXB of miR-30B.

Conclusion: Our study is the first to suggest the importance of microRNAs in the pathogenesis of AA which could be of interest for development of therapies in the future.

P-CompI-170

In demented oldest old, TREM2 is not associated with accelerated cognitive decline

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Compelling genetic evidence supports the involvement of rare genetic variants (minor allele frequency <1%) in the etiology of complex diseases such as Alzheimer's disease (AD). Novel sequencing technologies, such as whole exome sequencing (WES), have successfully identified rare coding variants in the triggering receptor expressed on myeloid cells 2 (TREM2) gene increasing the risk of AD, in particular p.R47H. Interestingly, research has also shown that the burden of rare TREM2 variants is significantly higher in AD patients compared to healthy individuals suggesting that additional variants may also contribute to the risk of AD. This study aims to evaluate the impact of rare TREM2 variants (A105Rfs*84, D87N, Q33*, R47H, R62C, R62H, T85I) on cognitive decline and time to Alzheimer's disease dementia. WES was performed on 269 patients from a general practice registry-based, prospective longitudinal study (AgeCoDe). 269 subjects were included in two groups: demented TREM2 carriers (n=10, mean age carriers: 83 years, female: 80%), and demented TREM2 non-carriers (n=259, mean age non-carriers: 81 years, females 71%). Using growth curve modeling of decline, group trajectories in mini-mental state examination (MMSE) for demented TREM2 carriers and demented TREM2 non-carriers were calculated. Confidence intervals for the trajectories of demented TREM2 non-carrier group were overlayed with observed MMSE group trajectories of the demented TREM2 carriers. Time to dementia was compared for demented non-carriers and carriers. The

TREM2 carrier group did not differ in MMSE decline from the demented TREM2 non-carriers. Demented TREM2 carriers and non-carriers did not differ in the time to dementia. In higher age, rare TREM2 variants have an effect on MMSE decline that is not as pronounced as previous studies suggested. In addition, the present variants did not have effect on the age of disease onset. Two alternatives may explain our results. First, rare variants in TREM2 have reduced effect sizes and they do not modify the age of onset in elderly population. This may be explained by additional, yet unknown, protective factors which become important in carriers of TREM2 variants in older ages. Alternatively, the AD phenotype produced by TREM2 rare variants is indistinguishable from other AD causes as suggested by previous studies on early-onset AD patients.

P-Compl-171

CNV Analysis in Panic Disorder

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INTRODUCTION

Copy number variants (CNVs) are a type of gene variation in which large parts of the DNA (up to several millions of base pairs) are duplicated or deleted. In the past, a number of rare CNVs associated with multifactorial psychiatric diseases such as schizophrenia or bipolar disorder were identified in overlapping genomic regions. These findings imply the contribution of CNVs in the same loci to highly variable phenotypes and lead to the idea of their influence on other psychiatric disorders.

With a prevalence of 3.4 - 4.7%, panic disorder is the most common of all anxiety disorders and severely affects the lives of patients. Clinical symptoms include sudden panic attacks in situations without imminent danger which present through extreme physical experiences of fear. The heritability of panic disorder is estimated to be up to 48%. However, till today the genetic and also environmental causes of this multifactorial disease are mainly unknown. Given the overrepresentation of CNVs in schizophrenia and bipolar disorder we aimed to examine the role of CNVs in panic disorder.

METHODS

For our study we used genome-wide SNP array data of a GWAS which was carried out with patients from Sweden, Denmark and Germany. All individuals were genotyped on Illumina HumanCoreExome BeadChips covering over 500K SNPs. Those samples are part of the greatest DNA collection of patients with panic disorder worldwide, which belongs to the research network Panic International Consortium (PAN*I*C).

QuantiSNP and PennCNV were applied for CNV calling. We applied a two-step analysis strategy to (i) focus on 18 previously described loci of CNVs in neuropsychiatric disorders and (ii) perform a genome-wide CNV association analysis between panic disorder patients and controls.

RESULTS

After obtaining 70,448 CNVs in 924 panic disorder patients and >140K CNVs in 1,470 controls by CNV calling, we conducted further stringent filtering concerning the quality of called CNVs. This resulted in 1,202 CNVs (mean 2.14 per sample) in 560 panic disorder patients and 3,467 CNVs (mean 3.28 per sample) in 1,047 controls. In the first analysis step, we identified two patients with a duplication in one of the 18 known regions (1q21.1) which could also be visually confirmed, while only one control had a duplication in this genomic region (p=0.1402). Technical validation of these CNVs using quantitative PCR is ongoing. Currently, we focus on the genome-wide CNV analysis and will present results of associated CNVs.

CONCLUSION

In respect of the size of our cohort, we assume that our study will provide novel insights into the role of rare CNVs in panic disorder, which might contribute to the understanding of pathologic pathways in the development of anxiety disorders.

P-CYTOGENETICS AND CNVS

P-CytoG-172

Two further patients with a de novo deletion 6q21q22.1 narrow down the region responsible for acro-cardio-facial syndrome

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The aetiology of acro-cardio-facial syndrome (MIM 600460) is unclear. Due to the observation of affected sibs born to unaffected consanguineous parents autosomal recessive inheritance was suggested [1]. Recently it was proposed that acro-cardio-facial syndrome is caused by microdeletions of the 6q21q22 region (minimal region of overlap 107,098,111-117,483,097; hg19)[2, 3].

Herein we present two patients who both were diagnosed with a deletion in 6q21q22.2. Patient 1 is a 3 year old boy who was evaluated for global developmental delay (walking at 27 months, single words at three years). On examination relative microcephaly (height 1.22 SDS, head circumference -1.8 SDS), hypertelorism, left sided epicanthal fold, downslanting palpebral fissures, and difference in ear size were noted. Fingers and toes were normal, though finger nails appeared small. At the age of three years testes were undescended. MRI of the cranium gave normal results. There is no suspicion of cardiac defects. Patient 2 is a 12 year old girl. She is affected by psychomotor retardation (walking at 4 years, speech just about 10 words). Currently her height is 167 cm (1,76 SDS), weight 40 kg (-0,36 SDS), and head circumference 51 cm (-2,09 SDS). Besides relative microcephaly, hypertelorism and prominent large ears (left more than right) are present. Ocular apraxia was only noticed during the first 3 years whereas seizures require continuous antiepileptic drug treatment. Since last year additional vagus nerve stimulation reduced number and duration of seizures. Patient 1 was diagnosed by microarray analysis with a de novo 8,5 Mb deletion of 6q21q22.1 (109,372,188-117,846,598x1; hg19). Patient 2 was diagnosed with a de novo 6,4 Mb deletion (110,606,987-117,0750,937x1; hg19). For both deletions the proximal breakpoint is about 2.3 Mb respectively 3.5 Mb telomeric to the proximal breakpoint of the minimal region of overlap which has been reported [2, 3].

Both patients do not present with symptoms typical of acro-cardio-facial syndrome. Therefore we propose that the region in 6q21 between 107,098,111-109,372,188 contains a gene region which is responsible for the phenotype. This could especially strengthen the assumption that *SNX*3 haploinsufficiency causes ectrodactyly [4].

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P-CytoG-173

Fetus with intrauterine growth retardation and congenital abnormalities due to maternal inherited cryptic translocation t(10;11)(q26.1;p15.3) detected by Subtelomere FISH

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Cryptic terminal chromosomal rearrangements are emerging reason of human genetic diseases. Often they are undetectable by conventional chromosome analysis even of the highest resolution. Subtelomere FISH is still a method to detect balanced cryptic translocation. Indications for this application are: 1) conspicuous familial medical history, 2) ultrasound abnormalities in a previous pregnancy, 3) late abortion/IUFT/still birth.

Here we present a healthy 28 years old woman, gravida 3, para 0. Family history was unremarkable. First she had a miscarriage at six weeks gestation. The second pregnancy was terminated at 21 weeks gestation because of severe intrauterine retardation and premature rupture of the membranes. Fetal

karyotyping in an external laboratory showed a normal karyotype. The third pregnancy following ICSI showed an abnormal fetus with IUGR, oligohydramnion, mild hydrothorax and pericardial effusion as well as placental insufficiency at 16 gestational weeks. No further abnormalities could detect due to difficult ultrasound conditions. Instead of amniocentesis a parental chromosome analysis including subtelomere FISH was performed in our lab. Karyotype of mother and father were normal. But FISH with subtelomeric probes revealed a cryptic reciprocal translocation between 10q and 11p in the mother. It was strongly suspected that the fetus inherited an unbalanced chromosomal situation. At 18+4 gestational weeks IUFT was observed. Array-CGH on fetal tissue proved our hypothesis, revealing an 8,9Mb deletion for 10q26.13->pter and an 8,3Mb duplication 11p15.4->pter.

Post mortem examination confirmed a severely hypotrophic female fetus with growth retardation, mild pleura and pericardial effusions and placental insufficiency. In addition a hydrocephaly, a large left sided posterolateral diaphragmatic hernia, complex cardiovascular malformations and adrenal, anal and urogenital anomalies and also distinct craniofacial dysmorphias were found.

Terminal 10q deletions have rarely been described in the literature. Characteristic features include growth retardation, psychomotor delay, facial dysmorphia, congenital heart disease, anogenital/urinary tract anomalies. Duplications in 11p15 are well known due to paternal inherited Wiedemann-Beckwith syndrome. But maternal duplications have not been described so far.

Our case illustrates that the subtelomeric screening method still represents a promising diagnostic tool, especially in absence of major chromosome rearrangements.

P-CytoG-174

Copy number variations in radiation-induced chromosomally unstable human fibroblast clones

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A central dogma in radiobiology for many years was that biological effects from ionizing radiation occur directly in the irradiated cell. However, in the last two decades, studies investigating animal models and tissue culture have disclosed that radiation-induced DNA damage can occur in cells not directly exposed to radiation. Radiation-induced genomic instability (RIGI) is a term used to describe the occurrence of *de novo* DNA damage in the clonal progeny of cells surviving the original radiation exposure. This effect can be observed many cell cycles after irradiation. RIGI is characterized by diverse endpoints at the cellular level including delayed cell death, chromosomal aberrations, gene mutations and amplifications, formation of micronuclei and changes in chromosome number. Genomic instability is also thought to play a role in radiation-induced carcinogenesis, but the underlying mechanisms remain to be elucidated.

To study RIGI, we clonally expanded normal human diploid fibroblasts after 2 Gray X-ray exposure and analyzed them by conventional chromosome banding. Depending on the stability of their genome, the cultured clones were classified as stable or unstable. Non-irradiated cells were cloned as controls. Altogether, 48 clones were established.

Copy number variations (CNVs), defined as duplications and deletions, are important for genetic variation, but also are linked to various human diseases including cancer. Therefore, we hypothesized that RIGI could be associated with the occurrence of CNVs and expected to find structural alterations in the progeny of irradiated cells. The HumanCytoSNP-12 DNA BeadChip (Illumina) was used to assess structural variations in the genomes of fibroblast clones that were already categorized according to their stability via chromosome analysis. This array-based approach allows the detection of microdeletions and microduplications in a higher resolution than standard G-banding.

De novo CNV induction was higher in irradiated clones compared to non-irradiated control clones. CNVs were distributed throughout the genome affecting, amongst others, genes associated with cancers and sites of increased genomic instability. Our findings indicate that structural variations occur in the progeny of irradiated cells displaying possible risk factors for radiation-induced cancer.

P-CytoG-175

A boy with partial trisomy 9p23->q22 and partial tetrasomy 9pter due to a maternal pericentric inversion of chromosome 9 [inv(9)(p23q22)]

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We report on a boy with a complex chromosomal rearrangement involving chromosome 9 who initially presented with anhydramnios, intrauterine growth retardation, enlarged cisterna magna and suspected dandy walker malformation at gestational age of 33 weeks. Chromosome analysis on cultivated amnion fluid cells revealed a supernumerary aberrant chromosome 9 with deletion of the segment q32 >qter in all cells analyzed. The boy was delivered in the 40th week of gestation and died at the first day of life. He was affected by a complex brain malformation with enlarged ventricles as well as agenesis of the corpus callosum and cerebellar vermis. Furthermore, he showed dysmorphic features like a sloping forehead, deep set malformed ears, a large nose with a low columella, simian palmar creases and a marked hypoplasia of the mandible. Chromosome analysis was performed for both parents from cultivated lymphocytes and from cultivated umbilical cord fibroblasts of the boy. Karyotype of the father was normal but in the mother´s lymphocytes we detected an apparently balanced pericentric inversion of chromosome 9 [inv(9)(p24q32)]. In the boy´s fibroblasts we again found an additional aberrant chromosome 9. Taken in account the mother´s karyotype and also FISH analysis with subtelomeric probes for 9pter and 9qter the finding in the boy could be described as: 47,XY,+rec(9)dup(9p)inv(9)(p24q32)mat. For further characterization of the boy's chromosomal aberration we performed microarray analysis which showed the result of partial tetrasomy pter->p23, a segmental trisomy 9p23q22.33 and copy number neutral LOH for the distal segment 9q22.33q34.3. Additional investigations for the further delineation of the rearrangement are described and hypotheses about the underlying mechanism of formation are discussed.

P-CytoG-176

Interstitial deletion in 12p12.3p12.1 in combination with a reciprocal translocation of the distal long arm of chromosome 11 and the distal short arm of chromosome 12 in a patient with global developmental delay

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We report on a male patient referred at the age of 2 years for evaluation due to global developmental delay particularly concerning mental and speech deficits. Molecular karyotyping using microarray analysis revealed a de novo deletion of approximately 6 Mb in 12p12.3p12.1. Conventional karyotyping of lymphocyte cultures showed a conspicuous short arm of chromosome 12 and a doubtfully conspicuous terminal long arm of chromosome 11. Subsequent FISH-analysis with a probe out of the chromosomal region 12p12.2 confirmed the deletion in the region 12p12.3p12.1 and further probes out of the region 12p13.33 and the long arm of chromosome 11, respectively, displayed a reciprocal translocation of the terminal long arm of chromosome 11 and the short arm of chromosome 12.

Conclusion:

Microarray analysis is a powerful tool to clarify genomic imbalances in patients but because it neither provides any information about the chromosomal position of the imbalance nor can detect balanced rearrangements, FISH as a complementary technique should be performed.

P-CytoG-177

Asymmetrical pseudoisodicentric chromosome 18 in a girl with multiple congenital anomalies

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Although Edwards syndrome (trisomy 18) and De-Grouchy syndrome Type 2 (terminal deletion 18q) are well-known chromosomopathies, asymmetrical dicentric or pseudoisodicentric chromosome 18 is a very rare finding. To the best of our knowledge, only nine patients with breakpoints in 18q have been reported so far. Breakpoints were narrowed down to the molecular level in only two of these cases. Here, we describe the first child of healthy parents of German origin with an unremarkable family history born at gestational age 38 weeks with weight of 2175g (<P3), length of 44cm (<P3) and OFC of 30cm (<P3). Using prenatal ultrasound, growth retardation, hypertrophic cardiomyopathy (HCM) and right side pelvic kidney were noted in 30th week of gestation; chromosome analysis had not been performed during pregnancy. Postnatal cardiac ultrasound confirmed HCM and pulmonic stenosis, additionally. Clinical evaluation at six months of age revealed decreased body weight (4600g; <P3) and short stature (46cm;<P3) consistent with dystrophy (BMI 13,7 [<P3]). Umbilical hernia, sacral dimple, fisted hands and minor facial and acral dysmorphisms including upslanted palpebral fissures and nail hypoplasia of fingers and toes were noted.

Array CGH analyses (Cytochip Oligo 4x180K v1.0/Fa. Illumina/Bluegnome) revealed a complex imbalance on chromosome 18. The array CGH formula according to ISCN 2013 was arr[hg19] 18p11.32q12.2(85,454-34,110,052)x3, 18q12.3-18q22.3(38,274,667-72,607,052)x3, 18q22.3q23(72,1626,522-78,012,800)x1 consistent with gain of chromosomal material from 18pter to 18q12.2 and from 18q12.3 to 18q22.3 and a loss of material from 18q22.3 to 18qter. A chromosomal section of ca. 4 Mb in bands 18q12.2 to 18q12.3 showed no imbalance. Conventional chromosome analysis on TPA-stimulated peripheral blood lymphocytes confirmed the suspicion of a pseudoisodicentric chromosome 18 (46,XX, psu idic(18)(pter□q22.3::q22.3□pter) in all 15 metaphases analyzed. Metaphase FISH with the BAC probe RP11-108G3 located in 18q12.2 showed loss of one signal on the pseudoisodicentric chromosome 18 consistent with asymmetrical pseudoisodicentric chromosome 18. Both parents were confirmed to have a normal karyotype and normal metaphase FISH results for the BAC RP11-108G3.

Clinical findings in the few patients reported so far as well as in the patient presented here were in part overlapping with the clinical phenotypes of trisomy 18 and partial monosomy 18q.

P-CytoG-178

Novel reciprocal Y;autosome translocation involving the short arm of the Y chromosome and the long arm of chromosome 10 in a male with non-obstructive azoospermia.

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Chromosomal anomalies are a well-documented cause of male subfertility and are more frequently observed in the population of azoo- and /or oligozoospermic males than in the general population. Y;autosomal (Y;A) translocations, however, are rare structural rearrangements with a frequency of approximately 1 in 2000 in the general population. The most common forms of Y;A translocations are balanced translocations between the short arm of any acrocentric chromosome and the heterochromatic region of the long arm of the Y chromosome (Yq12), that often are familial and have no effect on fertility. A rare group of unbalanced Y;A translocations can be seen in children with malformations and /or sexual ambiguities. Another form consists of balanced reciprocal Y;A translocations (between the Y chromosome and any autosome excluding the short arm of the acrocentric chromosomes). It arises almost exclusively *de novo* and can be detected in normal adult males with primary infertility. While in the majority of all reported cases with Y;A translocation the Y breakpoint is located in the long arm either in the euchromatic region distal to Yq11 that contains the azoospermia factor (AZF) or in the genetically inert heterochromatin block at Yq12, only few reports of Y;A translocations exist describing a breakpoint in the short arm of the Y chromosome. To the best of our knowledge, we describe here the first case of an apparently balanced reciprocal Y;A translocation involving the short arm of the Y chromosome (breakpoint in Yp11.31) and the long arm of chromosome 10 (breakpoint in 10q24.1) associated with non-obstructive azoospermia (NOA). A 36 year-old male clinically diagnosed with NOA was referred to our laboratory for cytogenetic

investigations and microdeletion screening at the azoospermia locus (Yq11.2). Chromosome analysis of cultured peripheral lymphocytes revealed an apparently balanced reciprocal translocation 46,X,t(Y;10)(p11.31;q24.1). The combined use of different probes in metaphase fluorescence *in situ* hybridisation (FISH) confirmed the reciprocal translocation (Yp;10q) and allowed the assignment of the Y chromosome breakpoint in the short arm proximal to the SRY locus (Yp11.31). Molecular studies performed on blood DNA confirmed the intactness of the SRY on Yp and demonstrated absence of microdeletions in the Yq11.2-AZF-regions. We discuss meiotic arrest as one possible cause for spermatocyte failure. However, if sperm are found in the testes of NOA male carriers of translocations, the application of intracytoplasmic sperm injection (ICSI) as therapy for infertility is associated with the risk of transmitting the chromosomal abnormality - balanced or unbalanced - into the next generation. Male carriers of translocations also have found an increased risk with respect to chromosomally unbalanced sperm and spontaneous abortus. In some cases preimplantation diagnosis (PGD) could be employed to improve the chances of a normal, healthy pregnancy.

P-CytoG-179

Constitutional inverted insertion in a healthy parent as a pathogenetic cause for recurrent duplications in offsprings.

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In two daughters of a healthy Chechen couple various dysmorphic features and anomalies including bilateral hexadactyly of hands and feet, epicanthus, as well as central paresis, a relative microcephaly, psychomotor retardation, several additional mandibular teeth and a general dystrophy were found. By cytogenetic analysis an identical wcp2 positive elongation of the long arm of a derivative chromosome 2 was recognized, but GAG-banding pattern did not univocally allow ruling out a more complex chromosomal rearrangement. By array CGH analysis in both sisters an identical 24.06 Mb duplication of 2q22.1-q24.2 was identified and confirmed by QF-PCR. Subsequent cytogenetic and QF-PCR-analysis of both parents showed that an obviously balanced structural aberration of the long arm of one chromosome 2 in the father is present. Since a paracentric inversion should lead to unstable dicentric or acentric chromosomal structures following meiosis, high resolution fluorescence in situ hybridization (FISH) studies using various multicolor (MCB-FISH) and locus specific probes were applied to characterize the balanced paternal chromosome 2 aberration in more details. Based on FISH analysis including probes RP11-64O2 (in 2q22.3), RP11-58K7 (in 2q23.3) and RP11-26B22 it was possible to show that the father carries an inverted insertion of a chromosomal segment containing probes RP11-64O2 and RP11-58K7. This chromosomal segment was found to be duplicated in the derivative chromosome 2 in both daughters. Inverted insertions have just been described very rarely especially in constitutional chromosome aberrations and even in malignancies such aberrations were reported in a rather low number. Further investigations of the father could be considered to find out if there might be evidence for a transposon mediated mechanism or if chromotrypsis could be involved in formation of the inverted insertion. In addition by extended phenotype characterization in both sisters and comparison with reported patients with similar duplications a better insight in the role of gene dosage of this chromosomal segment should be obtained. Finally although inverted insertions seem to be a rare event in particular in constitutional chromosomal aberrations it should be investigated if the observation described here could represent a more general principle in CNVs- and genomic unbalance formation.

P-CytoG-180

Assessing genetic stability of human induced pluripotent stem cells (iPSCs)

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The generation of patient-specific induced pluripotent stem cells (iPSCs) by reprogramming of adult somatic cells (e.g. skin fibroblasts) represents a novel technology for studying disease mechanisms. IPSCs can be differentiated into all three lineages including neural cells, which can finally be used as human cellular models for analyzing cell- and tissue-specific consequences of specific patient derived alleles. The process of reprogramming, though, is prone to somatic genetic alterations, including chromosomal aberrations, which

are normally screened for with conventional chromosome analyses. However it is unclear whether this level of resolution is sufficient to ensure reliable downstream experiments.

We now studied iPSCs derived from patients with Parkinson's disease (PD), the second most common neurodegenerative disease worldwide. The underlying molecular and cellular mechanisms, though, currently remain largely unknown. To monitor the genetic stability of iPSCs, we investigated copy number variants (CNVs) in addition to conventional chromosome analyses in a group of ten PD and six healthy control individuals. Using high-resolution chromosomal microarray analysis (CMA), we monitored the formation of relevant CNVs during reprogramming to pluripotency by comparing fibroblasts and iPSCs. To date, fibroblast cultures of all 16 selected probands as well as 40 generated iPSC clones were analyzed. Aneuploidies were detected in three fibroblast cultures (19%) and in five iPSC clones (12%). Interestingly, these affected only chromosomes 9, 12, 18, and Y. *De novo* CNVs were identified in 27 iPSCs (68%) – 15 clones showed one (38%), 9 clones showed two (23%), and three clones showed three newly arisen CNVs (8%). The CNVs were between 106 kb and 6.4 Mb in length encompassing up to 40 genes. Frequently affected genes are involved in cell-cycle regulation and cancer. Only two CNVs contained no genes. All these CNVs frequently occur as mosaics. Although most underlie negative selection due to growth or survival disadvantage to the cells, they were not selected for downstream experiments as a selective advantage of cells and changes in iPSC phenotype cannot be excluded.

Furthermore, discrepant patterns of CN polymorphisms and apparent LOH regions indicated a possible cell culture mix-up in one clone, which could be confirmed by DNA-fingerprinting. Hence, we introduced genetic fingerprinting of all cultures to validate their identity and exclude handling errors during the numerous culturing steps.

Our results indicate that iPSCs and cells derived thereof often contain aberrations not detected by standard chromosomal analysis thus requiring high resolution CMA. Both, detailed genetic characterization of iPSCs and derived clones as well as genetic fingerprinting are necessary to avoid cellular heterogeneity affecting the validity and reproducibility of results and for accurate tracking of cells.

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P-CytoG-181

Molecular Karyotyping of Abortion Samples with Normal Karyotypes and Cell-Free Fetal DNA from Amniotic Fluid Supernatant

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Many different factors are involved in healthy in utero development of a fetus. In addition to aneuploidies and other large-scale chromosomal aberrations, submicroscopic genetic changes may lead to pregnancy loss. DNA microarrays enable the detection of small-scale copy number variations (CNVs) in the fetal genome. In our experience, SNP-microarray outperforms common array-CGH by requiring lower amounts of DNA input and permitting the detection of copy-neutral loss of heterozygosity (LOH), polyploidy, and mosaicism. Out of 11 abortion samples with normal karyotypes (by conventional chromosome analysis), SNP-microarray analysis yielded findings with pathological significance in three (27%) cases, namely one mosaic tetraploidy, one mosaic monosomy X and one heterozygous duplication of the *laeverin* gene on chromosome 5q23.1, which plays an essential role in human placentation.

SNP-microarrays also represent a useful supplement to prenatal chromosome analysis. Cell-free fetal DNA (cffDNA) can be extracted directly from amniotic fluid supernatant, avoiding time-consuming cell culture. Altogether, 19 cffDNA samples were examined, including ten with aberrant karyotypes, eight with normal karyotypes but pathological ultrasound findings and one control. All ten aberrations which previously had been detected by conventional chromosome analysis could be confirmed by SNP-microarray analysis. Two (25%) of eight samples with normal karyotypes showed calls that could relate to their observed ultrasound abnormalities: One sample, which was collected from a fetus with congenital heart defects, showed a 2.8Mb heterozygous duplication on chromosome 1q21.1-q21.2 including the *GJA5*-gene, which is associated with tetralogy of Fallot. The second sample came from a fetus with multiple malformations and displayed a 13Mb LOH region on chromosome 6q22.3-q23.3, comprising ten OMIM genes.

Overall, our results argue in favour of the notion that SNP-microarrays can improve abortion- and prenatal diagnostics.

P-CytoG-182

A further case of fetoplacental mosaicism: a pure terminal 6q deletion in amniotic fluid cells and a complex add(6) in CVS short term culture

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Mosaic structural rearrangements detected by conventional chromosome analysis have a low incidence in chorionic villus sampling (CVS) and are often confined to the placenta. Here, we report on a 23-year-old healthy woman referred to CVS because of increased nuchal translucency (3.8 mm) and an echogenic intracardiac focus in the 13th week of pregnancy. Conventional chromosome analysis of the short term culture revealed a male karyotype with additional material on the long arm of chromosome 6q. Microarray analysis of DNA isolated from the mesenchymal core after enzymatic digestion showed two adjacent 6q duplications in chromosome bands 6q24.1q25.2 (~11 Mb) and 6q25.2q25.3 (~5 Mb), a duplicated region on 8q (8q21.3q24.3, ~51 Mb) as well as a terminal deletion of 6q (6q25.3q27, ~13 Mb). While duplications 6q24.1q25.2 and 8q21.3q24.3 were in mosaic, duplication 6q25.2q25.3 and deletion 6q25.3q27 appeared to be present in all cells. In order to clarify the fetal karyotype amniocentesis was performed. Remarkably, conventional chromosome analysis of cultured amniocytes did not show any additional material on 6q, however, in metaphases with a higher resolution the small terminal 6q-deletion was suspected. This finding of a pure terminal deletion 6q was confirmed by microarray analysis. Autopsy after termination of pregnancy revealed agenesis of the bulbus and tractus olfactorius, retrognathia, muscular pulmonary infundibular stenosis and a very small incipient tail at the os sacrum. We conclude that rearrangements seemingly confined to the placenta might refer to a small potentially cryptic chromosomal imbalance in the fetus. In these situations subsequent microarray analysis on amniotic fluid cells may be appropriate. In addition, analysis of the different placental and fetal cell lines may also shed light on the mechanisms of chromosome repair during early embryogenesis.

P-CytoG-183

Fine mapping of complex chromosome rearrangement: Mechanism of recurrent expansions of a small deletion in a family with dystonia

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DNA rearrangements are increasingly recognized as disease causes and their detection has been facilitated by the application of next generation sequencing (NGS). Thus, long-standing genetic “mysteries” can finally be solved as illustrated by the following example: We previously reported a patient with dopa-responsive dystonia (DRD) who carried a heterozygous deletion of the GTP cyclohydrolase 1 (GCH1) gene on chromosome 14q22 explaining the phenotype. The deletion not only comprised the GCH1 gene but a region of 3,370kb with additional genes. Mutational analysis in his family members revealed the same mutation in three affected relatives (paternal aunt and two of her sons). Surprisingly, the father of the index patient who was considered an obligate carrier of the deletion did not carry it, despite confirmed paternity. Haplotype analysis revealed that this person and his mother carried a smaller 670kb deletion downstream of GCH1. Notably, this deletion independently expanded in this family twice to the exact same deletion of 3,370kb. All deletions were confirmed by quantitative PCR, by array comparative genomic hybridization (aCGH), and by fluorescence in situ hybridization (FISH). The expansion was mediated by a 13kb duplication at the centromeric breakpoints of both deletions. Large-insert fragment libraries, so called “Jumping libraries” and their analysis by NGS finally revealed even more complex chromosomal rearrangements on chromosome 14q21.1-q22.3 in this family including an additional 29kb-deletion, a 327kb-duplication, and two inversions which were all lost by the large deletion. Our data demonstrate that NGS in conjunction with “old-fashioned” genetic methods are powerful tools to elucidate seemingly impossible inheritance patterns.

P-CytoG-184

Pseudoautosomal Region 1 Length Polymorphism

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The human sex chromosomes differ in sequence, except for the pseudoautosomal regions (PAR) at the terminus of the short and the long arms, denoted as PAR1 and PAR2. The boundary between PAR1 and the unique X and Y sequences was established during the divergence of the great apes. During a copy number variation screen, we noted a paternally inherited chromosome X duplication in 15 independent families. Subsequent genomic analysis demonstrated that an insertional translocation of X chromosomal sequence into theMa Y chromosome generates an extended PAR. The insertion is generated by non-allelic homologous recombination between a 548 bp LTR6B repeat within the Y chromosome PAR1 and a second LTR6B repeat located 105 kb from the PAR boundary on the X chromosome. The identification of the reciprocal deletion on the X chromosome in one family and the occurrence of the variant in different chromosome Y haplogroups demonstrate this is a recurrent genomic rearrangement in the human population. This finding represents a novel mechanism shaping sex chromosomal evolution.

P-CytoG-185

Fanconi anemia as a model for the characterization of low frequency common fragile sites on human chromosome 1.

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Within cytogenetic studies in healthy individuals chromosomal breaks occur at so-called common fragile sites (CFSs). CFSs are regions of genomic instability, which are in some cases associated with cancer-related breakpoints. Hence comes the understanding of the mechanisms of chromosomal instability as a focus of studies on neoplastic transformation. While about 230 CFS are mapped cytogenetically, only a small number is characterized on the molecular level. Especially the characterization of CFSs, which are expressed in low frequencies, is challenging. Given that about 50% of chromosomal breaks in material of Fanconi anemia (FA) patients correlate with CFSs and that low frequency CFSs are often observed in this material, FA could serve as a suitable model for their characterization.

Chromosomal breaks in mitomycin C-treated lymphocytes of FA patients were cytogenetically mapped and correlated in about 62% with CFSs, which occur in aphidicolin-treated lymphocytes of healthy individuals. Here we characterize selected low frequency CFSs of chromosome 1 (FRA1B, FRA1D, FRA1F, FRA1G und FRA1K) on the molecular level by using bacterial artificial chromosomes (BAC) as fluorescence in situ hybridization (FISH) probes in FA patients. To verify the results of the molecular characterization the breakpoint-specific BAC probes were hybridized on CFSs from aphidicolin-treated healthy individuals. The molecular characterization of the selected CFSs enabled the analysis of their DNA regarding the level of repetitive elements and flexibility to further clarify the genomic instability.

FA is suitable model system for the molecular characterization of the selected CFSs. This approach is highly recommended for the examination of low frequency CFSs. The collected data of the selected characterized CFSs were compared with data of CFSs available so far. The level of repetitive elements of DNA varies from CFS to CFS. The flexibility of the DNA was elevated in most of the selected CFSs. In summary, the instability of each CFS seems to be determined by an individual combination of fragility modulating features.

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P-CytoG-186

Strategies for dealing with incidental findings in diagnostic microarray-based copy number analysis

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During the past decade, chromosomal microarray-based copy number analysis has emerged as an important diagnostic tool for the evaluation of developmental delay, intellectual disability, autism spectrum disorders, and structural malformations in children. The benefit of the high-resolution detection of copy number variants offered by microarray applications comes along with an increased likelihood for the detection of unsolicited findings. These include copy number variants concerning genes not related to the clinical phenotype of the respective patient that could potentially implicate a higher disease risk for offspring or other family members or present risk factors for additional diseases.

In our lab, we processed more than 400 Affymetrix Cytoscan HD arrays for diagnostic purposes over the last two years. We mainly analyzed patients with developmental delay, intellectual disability, structural malformations and dysmorphic features and detected pathogenic copy number variations explaining the disease pattern in 16% of the samples. In 74 % of the patients, we found no evidence for a genomic imbalance causative for the clinical phenotype. Incidental findings were reported in 3 % of the cases (fourteen patients): Eleven patients were found to be carriers of a recessive genetic disorder such as Cohen syndrome, Joubert syndrome or Usher syndrome due to copy number variants in the respective genes. An increased disease risk was discovered in two cases due to copy number variants in genes associated with cardiac disorders. In a girl with Williams syndrome (7q11.23 deletion), a partial mosaic isodisomy of chromosome 21 (elevating the risk for an additional autosomal recessive disorder) was found.

Here we present selected cases of our incidental findings. We discuss strategies taking pre-and postanalytical counselling, severity and prevalence of the respective disorder, characteristics of the copy number variant and the challenges of medical reporting into account, based on our experience.

P-CytoG-187

A boy with microcephaly, developmental delay and contractures of hands and feet with tetrasomy 18p

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Isochromosome 18p or Tetrasomy 18p is one of the most commonly observed isochromosomes. The most common clinical findings included neonatal feeding problems, growth retardation, developmental delay, microcephaly, strabismus, abnormalities in muscle tone, scoliosis/kyphosis, dysmorphic features and variable MRI findings of the brain.

We report on a 7 4/12 year old boy with delay of psychomotor development born to healthy non-consanguineous parents. The maternal age at birth was 39 and the paternal age 48 years. The boy was born at 37 weeks of gestation with a birth weight of 2,400 g (-1,6 SD) and a length of 49 cm (-0,5 SD). The head circumference at birth is unknown. He suffered from chronic constipation and required tube feeding due to severe feeding problems. Current clinical examination revealed a normal height of 120 cm (-1,1 SD), low weight of 16,9 kg (-3,2 SD; BMI: 11,7 kg/m2 [-3,7 SD]) and microcephaly of 46 cm (-3,2 SD). Furthermore, he presented with intellectual disability, muscular hypotonia, mild dystonic cerebral palsy, flexion contractures of fingers and toes and facial dysmorphic features.

Conventional chromosome analysis and FISH analysis revealed an additional isochromosome consisting of two p-arms of chromosome 18 resulting in a karyotype of 47,XY,+i(18)(p10). Parental karyotypes were normal.

This unusual cytogenetic finding is likely the consequence of nondisjunction and centromeric misdivision leading to monocentric isochromosomes as in all cases reported so far. According to several reports, 21 of 22 comparable isochromosomes were of maternal origin. Nondisjunction and centromeric misdivision likely occur during maternal meiosis II, suggesting an influence of maternal age, which remains to be investigated in our case.

P-CytoG-188

Genetic analyses of chondrocytes for potential cell-based therapy using GTG, FISH, SKY, and SNP array

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In the development of (stem) cell-based therapies, it is crucial to guarantee that the application of (stem) cell-based products to humans is safe. Consensus of the Medicines Agencies in Europe is: “In conclusion, on the basis of the state of art, conventional karyotyping can be considered a valuable and useful technique to analyze chromosomal stability during preclinical studies “ (Barkholdt et al., 2013). Therefore genetic analysis of cells is recommended at several times during cultivation process to minimize tumorigenic risks.

Analyses of 408 chondrocyte samples (100 adherent cultures and 308 spheroids) from seven donors were performed using Trypsin-Giemsa staining (GTG-banding), spectral karyotyping (SKY), and locus-specific fluorescence in situ hybridization (FISH) in a preclinical study. SNP array analyses were done from spheroids of five donors (passage 2, 4, and 10).

Applying these techniques, our genetic analyses revealed no significant genetic instability for at least 3 passages [e.g. fra(4)(q31)]- only single event in passage 3 of one donor. We identified clonal occurrence of polyploid metaphases and endomitoses with prolonged cultivation time (passage 4-10). Furthermore, we observed gonosomal losses with increasing cultivation time. Interestingly, in one donor we were able to identify trisomy of chromosomes 1,7,8,12, and translocation of chromosomes 7, 9, and 12, which has also been described in extraskeletal myxoid chondrosarcoma (Sjögren et al., 2003). Using SKY we could confirm previously detected chromosomal aberrations by GTG and identify additional translocations (e.g. translocation of chromosomes 2 and 13). Applying the SNP-array analyses we detected chromosomal aberrations in two donors at passage 10 (gain 7/10: donor 2; loss X/gain 8: donor 5).

Our results showed the necessity of genetic analyses at certain cultivation times in preclinical studies. A combination of different (molecular) cytogenetic techniques is useful to increase the knowledge and experience of potential cell therapeutics.

P-CytoG-189

The fragile secret of 28 new molecularly mapped aphidicolin induced fragile sites shed light on gene and genome evolution

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Common fragile sites (CFS) are thought to be a feature of the normal chromosome structure and in contrast to other chromosomal rearrangements they are not recurrent within a specific sequence context but within a chromosome region that can span more than 10 Mb. A striking characteristic of these sites are their different intra and interindividual frequencies. The three most frequently observed CFS are FRA3B in 3p14 followed by FRA16D in 16q23 and FRAXB in Xp22. Therefore it is not surprising that these CFS are are among the so far molecularly defined 41sites.

There are reports for the involvement of CFS in chromosome rearrangements like translocations or deletions, gene amplification, viral integration or sister chromatide exchange and a cytogenetic co localization with cancer breakpoints as well as with evolutionary conserved breakpoints reflecting an indifferent picture for their functional and evolutionary role. Thus it is highly important to characterize these sites fully to get deeper insight at the molecular basis for the understanding of chromosome fragility.

In a former study we performed a global screening of CFS and mapped 230 aphidicolin inducible CFS cytogenetically. In the current study we mapped 10 CFS new and redefined 18 CFS in the human reference sequence. Together with 41 already published defined CFS we did a full sequence analysis of these breakage prone regions and draw conclusions for genome architecture and evolutionary traits.

P-MONOGENIC DISEASES - FROM GENE IDENTIFICATION TO MOLECULAR MECHANISMS

P-MonoG-190

A familial case of Gordon syndrome is associated with a PIEZO2 mutation

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Gordon syndrome (MIM #114300) or distal arthrogryposis multiplex congenita type 3 is a rare autosomal dominant disorder characterized by contractures of upper and lower limbs. It is distinguishable from other forms of distal arthrogyproses by cleft palate and short stature.

Very recently it has been shown, that Gordon syndrome is caused by mutations in the piezo-type mechanosensitive ion channel component 2 gene (PIEZO2). Malfunction of this ion channel provides pleiotropic effects on joints, ocular muscles, and bone development. In our case we identified the PIEZO2 mutation c.8057G>A (p.Arg2686His) to be causative. The diagnosis was detected through Sanger Sequencing.

We present a family with three affected individuals with multiple contractures (proximal finger joints, elbow, shoulder, knee and ankle joints), clubfeet, short stature, bifid uvula and a distinct facial phenotype including ptosis.

This family represents the multigenerational phenotypic spectrum of Gordon syndrome consisting of the affected 37-year-old father, his 4-year-old son and an affected male fetus showing distinct signs of arthrogryposis in the prenatal ultrasound examination already seen in the 13th week of gestation.

P-MonoG-191

Resilience formation against chronic stress: the Tsc2KO mouse model to study the involvement of the mTOR pathway

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Characteristic for autistic children is their reliability on well-structured days and predictable procedures. Deviations of these cause stress in them and result in behavioural abnormalities. We therefore hypothesize that autistic children have reduced resilience for chronic stress.

In order to follow up this hypothesize and analyse mechanism of stress resilience in autism we are using a heterozygous Tsc2 knock-out mouse model. Mutations in Tsc2 in patients cause tuberous sclerosis, a syndromic form of intellectual disability paired with autistic features. Tsc2 (together with Tsc1) is part of a complex that inhibits the mechanistic target of rapamycin in (mTOR) kinase. Mutations in either of the two genes interfere with the formation of this complex and result in increased mTOR activity and upregulated downstream signalling.

mTOR signalling plays an important role in memory formation. It also is supposed to be a key player in stress resilience. For example has increased phosphorylation of mTOR referring to increased mTOR activity been demonstrated after exposure of wildtype mice to chronic social defeat. According to these studies we have established a behaviour battery to analyse the consequences of chronic social defeat. In Tsc2 heterozygous animal. After a 14-days' period of chronic stress exposure mice are being analysed in this battery, which consists of an object recognition test, an elevated plus-maze test, a test of nest building, an evaluation of social interaction, sucrose preference, spontaneous alternation in a Y-maze test, a tail suspension test and the analysing of prepulse inhibition of startle response.

First results show that Tsc2KO animals do show alterations particularly in social behaviour after stress exposure, which reflects to autistic features in patients. These data will give us closer insight into specific problems in autism and will also gain our understanding of mechanisms underlying stress resilience and susceptibility.

P-MonoG-192

Profound developmental delay, congenital heart disease, epilepsy and dysmorphisms in a one-year-old girl caused by a novel heterozygous de novo missense variant in MED13L

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Heterozygous *MED13L* variants in 12q24 have been reported to cause a spectrum of phenotypes ranging from isolated congenital heart disease to a syndromic form of intellectual disability (ID) described recently (Adegbola et al. 2015). *MED13L* (MIM #608771), previously known as *THRAP2, PROSIT240, TRAP240L, KIAA1025* is a subunit of the CDK8-associated mediator complex that functions in transcriptional regulation through DNA-binding transcription factors and RNA polymerase II.

While missense mutations in *MED13L* have early been associated with the cyanotic form of non-syndromic congenital heart diseases including dextro-looped transposition of the great arteries (MIM#608880), a *MED13L* haploinsufficiency syndrome has recently been described in two patients with moderate ID, conotruncal heart defects, facial abnormalities and hypotonia (Asadollahi et al. 2013). Ten further patients with severe syndromic ID, delay in motor and speech development but without cardiac phenotypes have been reported, mainly associated with dosage changes

(van Healst et al., 2015, Adegbola et al. 2015). One report exists about a homozygous missense variant in *MED13L* in a patient with mild ID (Najmabadi et al. 2011).

Here we present the detailed phenotype of a one-year-old girl with coarctation of the aorta, profound developmental delay, epilepsy, hearing impairment and with additional dysmorphisms that fit into the syndromic spectrum found in patients with *MED13L* haploinsufficiency. The developmental delay in the girl apparent to date seems to be more profound than in any other patient described yet.

Whole exome sequencing and molecular karyotyping revealed a novel *de novo* heterozygous missense mutation in *MED13L* as the likely cause of the condition in this girl.

The accumulation of clinical signs and symptoms, dysmorphisms as well as the severity of the profound developmental delay rises the hypothesis that the novel missense mutation in *MED13L* in this girl could exert a dominant negative effect.

P-MonoG-193

Hereditary sensory autonomic neuropathy with hypoglycemia: Disentangling a distinct phenotype

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Hereditary sensory and autonomic neuropathies (HSAN) are a group of clinically and genetically heterogeneous disorders. These disorders affect the peripheral nervous system and are associated with loss of pain perception. Mutations in different genes have been identified to cause subtypes of HSAN, still the aetiology of a substantial number of cases remains unknown. We here report a young patient with complex and severe symptoms in part reflecting an HSAN phenotype. The clinical course was complicated by apnea and hypopnea and abnormal insulin, proinsulin and glucagon levels. Using whole-exome sequencing we identified a homozygous truncating mutation in a gene previously related to insulin metabolism, but not to HSAN. Further analyses have to substantiate a causal role of the mutation in the pathology of sensory neuropathies.

P-MonoG-194

BOD1 is essential for cognitive functions in drosophila and man

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Here we report on functional aspects of a stop mutation in the *BOD1 (Biorientation Defective 1)* gene which co-segregates with intellectual disability in a large consanguineous Iranian family. The BOD1 gene product plays a role in chromosome segregation as well as the regulation of phosphorylation of substrates of the cell cycle kinase PLK1 during mitosis, by influencing mitotic Protein Phosphatase 2A (PP2A) activity. In this study we could demonstrate that fibroblasts derived from homozygous carriers of the mutation show aberrant localisation of PLK1 as well as PP2A at mitotic kinetochores. While BOD1-siRNA treated HeLa cells showed mitotic arrest, patient-derived cells passed through mitosis without any apparent defects in segregation and did so at an accelerated rate as compared to controls. This comparatively normal progression through the cell cycle is in keeping with the absence of gross structural brain abnormalities in homozygous mutation carriers. Furthermore, we observed that in contrast to PLK1 expression, BOD1 expression remains at comparatively high levels in normal adult brain tissues. These findings point towards a possible cycle-independent role of the BOD1 protein in the nervous system. Following this lead, we established two *drosophila* models. In both, neuron-specific knockdown of the *drosophila* ortholog of BOD1 caused marked impairment in learning capability as well as synaptic abnormalities.

Thus, our findings reveal new postmitotic functions of BOD1 protein and pathogenic mechanisms which corroborate a causative role for BOD1 deficiency in the aetiology of intellectual disability. In addition, we provide evidence for a conserved role of BOD1 in maintenance and development of cognitive features by showing its necessity for cognitive functions in *drosophila* as well as humans.

P-MonoG-195

Association of CTG18.1 trinucleotide repeat expansion in intron 2 of the TCF4 gene with Fuchs Dystrophy

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Background: Fuchs endothelial corneal dystrophy (FECD) is a genetic disorder of the corneal endothelium. The late onset form of this disorder affects as much as 4% of the population in the USA over the age of 40. Symptoms are corneal edema and visual acuity decreases up to complete blindness. Therefore, FECD is a significant cause for many corneal transplantations performed every year. The FECD is genetically heterogeneous. CTG18.1 repeat expansion and variations (e.g. rs613872) in the transcription factor 4 (TCF4) have been identified as a major association with the disease (Mootha et al. 2014). Our aim was to studying these genetic loci in the German populations and to calculate their odds ratios as an indicator for the chance to suffer from FECD.

Method: Up to now, we tested an association between the intronic TGC trinucletiode repeat expansion in TCF4 and FECD in 42 affected German patients and 113 unrelated controls. The investigation was done by bidirectional triplet-primed (TP)-PCR. The TP-PCR is a method to analyze the presence or absence of repeat expansions. TP-PCR is a PCR with three primers and is easier and faster than southern blotting.

Results: We found an CTG18.1 repeat expansion >50 in 33 German FECD patients (79%) and in 13 (11,5%) of healthy volunteers. 9 patients (21%) had a normal repeat length <40 and 100 (88,5%) of unaffected controls showed a normal CTG18.1 repeats length < 40.

Further, we showed that the lengths of CTG18.1 repeat expansions correlated in blood and affected endothelial cornea cells.

Analysis of the polymorphism rs613872 in intron 3 of the TCF4 gene revealed in 33 of 42 unrelated patients (78.6%) the heterozygous genotype TG and in four homozygous GG (9.5%). 65 of 93 controls were homozygous TT (69.9%) and only 21 heterozygous TG (22.6%).

The odds ratio as indicator for being affected by FECD in our data for the expanded CTG18.1 allele is 30. The chance of being affected is thus 30 times higher when someone exhibits the expanded allele. For carriers of the risk allele G of rs613872 the chance is 16.5 times higher.

Comments: In summary, TP PCR is a fast and sensitive method for the detection of the presence or absence of CTG18.1 repeat expansion. An expanded CTG18.1 allele with more than 50 trinucleotide repeats in intron 2 and the risk allele G of the polymorphism rs613872 in intron 3 of the TCF4 gene are associated with FECD. The chance to be affected by FECD is up to 30 times higher when someone exhibits the expanded allele.

Literature:

Mootha VV, Gong X, Ku HC, Xing C: Association and familial segregation of CTG18.1 trinucleotide repeat expansion of TCF4 gene in Fuchs endothelial corneal dystrophy. Invest Ophthalmol Vis Sci 2014:55(1):33-42

P-MonoG-196

Functional characterization of human NSDHL-mutants

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CHILD (congenital hemidysplasia with ichthyosiform nevus and limb defects) is an X-linked dominant disorder affecting predominantly females since it is usually lethal for males in gestation stage. The syndrome is characterized by unilateral ichthyosiform skin lesions, ipsilateral anomalies of the limbs and organs as well as punctate cartilage calcification. The condition is associated with a mutation of the NSDHL gene, coding for a NAD(P)H steroid dehydrogenase involved in the cholesterol biosynthesis.

We characterized the expression of h (human)-NSDHL transgenes in cos7 cells, localization of the protein in the cell and possible interaction with neighboring components of the dehydrogenase-complex in the cholesterol biosynthesis pathway. In comparison to the wildtype, we used nine h-NSDHL-mutants observed in patients as well as seven h-NSDHL-expression vectors with artificial mutations localized in presumably functionally important regions of the gene.

We were able to confirm the arrangement of the h-NSDHL protein in lipid droplets for the wildtype as well as for some mutants. Other h-NSDHL-mutants show a distribution in the cytoplasm without any droplet formation preferentially overlapping with the Golgi-apparatus suggesting a disturbed cellular transport of selected h-NSDHL mutants in patients. We did not see any expression in the ER membranes nor in the Golgi for h-NSDHL-wildtype or for the mutants showing the lipid droplet phenotype.

Yeast NSDHL (ERG26) has been shown to interact in a dehydrogenase protein complex with other ERG-proteins to catalyze the same cholesterol biosynthesis step as h-NSDHL. The human homologues, h-SC4MOL (ERG25), h-HSD17B7 (ERG27), and h-C14ORF1 (ERG28) transgenes were all expressed relatively ubiquitous in the cytoplasm of cos7 cells. Coexpression of h-NSDHL and h-C14ORF1 did not change the localization of either protein in the cell. NSDHL-mutants show the same distribution as if expressed alone in the cell. In contrast, we could show a complete colocalization and change of expression for some h-NSDHL-WT/-mutants and h-HSD17B7 when transfected together. Presumably, HSD17B7 might recruit NSDHL for interaction in the cell. A colocalization of h-SC4MOL and NSDHL-wildtype can also be seen, but some mutants of NSDHL show less overlap in expression with this ERG25-homologue pointing towards a disturbed interaction due to mutations affecting NSDHL.

Our results suggest that mutations observed in CHILD patients affect the cholesterol synthesis by various molecular mechanisms, in some cases related to the interactions of the protein with other enzymes of the dehydrogenase complex.

P-MonoG-197

Brain-specific Foxp1 deletion impairs neuronal development and causes autistic-like behaviour

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Neurodevelopmental disorders are multi-faceted and can lead to intellectual disability, autism spectrum disorder and language impairment. Mutations in the Forkhead box FOXP1 gene have been linked to all these disorders, suggesting that it may play a central role in various cognitive and social processes. To understand the role of Foxp1 in the context of neurodevelopment leading to alterations in cognition and behaviour, we generated mice with a brain-specific Foxp1 deletion (Nestin-Cre(*Foxp1*$^{-/-}$)mice). The mutant mice were viable and allowed for the first time the analysis of pre- and postnatal neurodevelopmental phenotypes, which included a pronounced disruption of the developing striatum and more subtle alterations in the hippocampus. More detailed analysis in the CA1 region revealed abnormal neuronal morphogenesis that was associated with reduced excitability and an imbalance of excitatory to inhibitory input in CA1 hippocampal neurons in Nestin-Cre(*Foxp1*$^{-/-}$) mice. Foxp1 ablation was also associated with various cognitive and social deficits, providing new insights into its behavioural importance.

P-MonoG-198

Gender-dimorphic expression of Foxp1 in the developing mouse brain and its impact on sex-specific communication

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Autism spectrum disorders (ASD) are four times more prevalent in males and speech and language disorder are about two times more prevalent in males than females. The reason for this is still unknown, although it has been suggested that sex-specific regulation of distinct genes may promote the development of ASD. The human *FOXP1* gene represents an interesting candidate as *FOXP1* mutations have been associated with ASD, intellectual disability and speech and language deficits in predominantly male patients.

The *FOXP1* gene belongs to the family of forkhead box (FOX) transcription factors and is widely expressed in the developing and mature brain. Homozygous loss of *Foxp1* is embryonically lethal in mouse due to cardiac defects. To investigate the role of *Foxp1* in neurodevelopmental processes, we generated Nestin-Cre(*Foxp1*$^{-/-}$) mice with conditional loss of *Foxp1* in the brain. We could demonstrate that Foxp1 is crucial for the development of the striatum and hippocampus but also for normal learning,memory and social behaviours.

We also observed that male *Foxp1-KO* animals exhibit a more severe phenotype and die earlier than female *Foxp1-KO* mice. To investigate whether this sexual dimorphic phenotype of *Foxp1-KO* animals is caused by gender-specific expression and function of Foxp1, we explored the differences in the expression of Foxp1 in male and female WT animals at six different stages of brain development in the cortex, striatum, hippocampus and cerebellum. We also examined the expression level of Foxp2, the closest relative of Foxp1, which is associated with language disorder. Foxp2 is known to form heterodimers in those tissues where it is coexpressed with Foxp1, such as the striatum. Our study revealed sexually dimorphic expression of *Foxp1* and *Foxp2* in the striatum and cortex at E 17.5 and P 7.5. Both the cortex and striatum are known to be crucial for language and communication. Interestingly, at about E 17.5 testosterone levels peak in male embryos suggesting that the observed sex-dimorphic expression may be caused by a gender-specific androgen or estrogen signalling.

To further test whether the detected sex-specific expression of *Foxp1* at E 17.5 and P 7.5 has an impact on communication at early postnatal stages, we examined the isolation-induced ultrasonic vocalisation in WT and *Foxp1-KO* animals with a special focus on sex. WT pups exhibit a significantly higher calling rate than *Foxp1-KO*. Strikingly, WT animals in addition show a gender-dimorphic vocalisation with a higher number of calls in males at P 4 and P 8 which is completely missing in *Foxp1-KO* pups.

Our findings may have important implications for neuropsychiatric developmental disorders involving impairments in communication such as ASD.

P-MonoG-199

Launching systematic whole exome sequencing in patients from Jordan with autosomal recessive Intellectual disability

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Intellectual disability (ID) is characterized by IQ ≤ 70 and adaptive functioning problems. Most cases are due to genetic mutations. The prevalence of ID is 1 to 3 % which tends to be higher in low-income countries and in communities with high consanguinity. Although the genetic background of ID is highly heterogeneous, some of the ID cases are syndromic, and though associated with clinical symptoms that enable putting a differential diagnosis. However, in many other cases with no clear specific pattern of associated symptoms, setting a diagnosis is a major difficulty. High throughput molecular genetic techniques, i.e. Next Generation Sequencing (NGS), are more and more used to identify the causes of ID.

We have used these techniques to identify the causative variants of ID in 8 Jordanian families. The families are characterized by consanguineous mating and two or more affected children, suggesting an autosomal recessive inheritance pattern. Whole genome mapping was run for the families, followed by whole exome sequencing for the index patient in each family.

In 6 families (63%) we have identified the causative mutation in a previous reported gene. A homozygous variant in the gene ADCY1 was identified in family MR201 where the parents are first cousins with two affected daughters having mild ID and speaking and expression difficulties. We have identified a previously described homozygous pathogenic variant in GPR56 (bilateral frontoparietal polymicrogyria) in the family MR203 where the parents are second cousins with two affected sons characterized by ID with motor and speech delay and epilepsy. A homozygous variant in the gene RASAL2 was identified in family MR205 where the parents are double first cousins with three affected sons characterized by ID and epilepsy. Two compound heterozygous variants in SEC24C were identified in family MR204 where the parents are second cousins. In family MR207, a repeat expansion in FMR1 was found, and thus the diagnosis Fragile X syndrome was set in the two affected sons.

In three of the families (38%), we have identified candidate mutations. A homozygous variant was identified in gene MBOAT7 in family MR206 where the parents are third cousins. In families MR202 and MR208, two homozygous candidate mutations in DRD4 and NANP as well as in CHRNA9 and TCEAL5, respectively, were identified. Further investigations are needed to reveal the most candidate gene in these two families.

This research is a pilot project between Germany and Jordan funded by the DFG. The methodology of the field work in Jordan and the results are very promising for further future cooperation identifying further genes and variants for intellectual disability, but also other genetic disorders.

P-MonoG-200

Gene Panel Sequencing of 1387 Patients with Epilepsy Disorders

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The field of epilepsy genetics has been constantly evolving. Next Generation Sequencing techniques, such as whole exome sequencing (WES) or targeted panels significantly facilitated the identification of underlying genetic causes. Panel sequencing targets a specific range of genes enabling a better coverage than WES at an overall cheaper price. Consequently, panel sequencing has evolved to an important diagnostic tool, not only in epilepsy diagnostics. We are currently analyzing gene panel data of a cohort of 1387 patients with different epilepsy disorders. The cohort comprises different panel designs containing approximately 300 genes on average. Patients were divided into broad phenotypic categories. With the analysis of our cohort we aim to identify the most commonly affected genes and mutational spectra across different epilepsy phenotypes. We intend to generate confirming or refuting evidence on genes with yet uncertain or questionable association to epilepsy.

In our study we will be the first to address the diagnostic yield of panel diagnostics in a large cohort of epilepsy patients. Additionally, we will gain insight into the distribution of pathogenic and rare variants in different epilepsy phenotypes.

P-MonoG-201

Skewed X-inactivation as a possible cause for phenotypic variability in female patients with Alport syndrome

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Background:

Alport syndrome (ATS) is characterized by a hereditary nephropathy with progression to renal insufficiency, high-tone sensorineural hearing loss, and ocular abnormalities. Mutations in *COL4A3* and *COL4A4* (both autosomal recessive and autosomal dominant; 15% of the cases) and *COL4A5* (X-chromosomal; 85% of the cases) are responsible for the disease. Whereas in male patients with a hemizygous *COL4A5* mutation genotype-phenotype correlation is known, female carriers of a heterozygous *COL4A5* mutation show an intrafamilial and interindividual variability concerning the phenotype.

Methods:

In this study 13 female patients out of 6 families with ATS were analyzed. In addition to a clinical questionnaire, mutational analysis of the genes *COL4A3*, *COL4A4*, *COL4A5*, *MYH9*, *NPHS2*, and the analysis of the X-inactivation was performed in blood (in one patient additionally in kidney tissue).

Results:

The median age at diagnosis was 12.5 years. Nine patients (69%) showed hematuria, six patients (46%) proteinuria. One patient suffered from renal insufficiency at the age of 10 years, one patient had end-stage renal disease (ESRD) at 9 years of age. Both patients additionally showed hearing loss. No patient had ocular abnormalities.

All patients were carrier of a heterozygous mutation in *COL4A5*. Further mutations in other genes were not observed. A genotype-phenotype correlation was not obvious, but a distinct intrafamilial variability of the phenotype.

Analysis of the X-inactivation showed in four patients a symmetrical, in four patients a moderately skewed and in two patients an extremely skewed pattern. In three patients the X-inactivation pattern could not be determined because of non-informative markers. The patient with analysis of the X-inactivation in blood and kidney tissue showed nearly identical results in both samples. Both patients with a severe phenotype of ATS had a skewed X-inactivation pattern with a predominant inactivation of the normal allele. This X-inactivation pattern was different compared to other female relatives that were up to now not or only slightly affected.

Discussion:

The prognosis for girls/women with X-linked ATS is benign according to the literature. The risk for developing ESRD until the age of 10 years is around 2%. The data of this single center study give evidence for an increased risk for renal insufficiency and ESRD in females. X-linked ATS seems to be less benign than described in the literature. The observed intrafamilial variability concerning the severity of the phenotype in our patients is in correlation to the X-inactivation pattern. A regular nephrological check-up of female patients is indicated. The examination of larger patient cohorts should be performed next.

P-MonoG-202

A nine-year-old boy with CEDNIK syndrome (Cerebral Dysgenesis, Neuropathy, Ichthyosis and Keratoderma)

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CEDNIK (Cerebral Dysgenesis, Neuropathy, Ichthyosis and Keratoderma) syndrome is caused by homozygous or compound heterozygous mutations in the SNAP29 gene, located in 22q11.2. Eleven patients from four families and two sporadic cases have been described until now. The clinical features include microcephaly, severe neurological impairment with severe developmental delay, failure to thrive, impairment of vision and hearing, palmoplantar keratoderma, ichthyosis and hypohidrosis. Brain MRI findings are polymicrogyria, pachygyria, absence of corpus callosum and cortical dysplasia.

We report on a nine-year-old boy, the first child of consanguineous parents of Turkish origin, with severe psychomotor retardation, microcephaly, short stature, optic nerve hypoplasia, hearing impairment, ichthyosis and keratoderma. Pachygyria and corpus callosum dysgenesis were seen in brain MRI.

We performed next generation sequencing analysis with sequencing of 31 genes known to be associated with neuronal migration disorders, including the SNAP29 gene. The patient carries a homozygous 4-bp-deletion in exon 2 of the SNAP29 gene which confirms the clinical diagnosis CEDNIK syndrome. The parents are heterozygous carriers. At the time of diagnosis the mother was pregnant again. The fetus was found to be a carrier of the familial heterozygous mutation in the SNAP29 gene.

To our knowledge, this is the fifth consanguineous family with CEDNIK syndrome described. Interestingly two sporadic patients with CEDNIK syndrome from the literature carry a heterozygous mutation in the SNAP29 gene and a 22q11.2 microdeletion on the second allele.

We provide detailed information on the phenotype of CEDNIK syndrome, based on the clinical report of our patient and a review of the literature. The specific combination of the neurological and dermatological features represents a highly recognizable phenotype.

P-MonoG-203

Characterization of a mouse model for intellectual disability caused by FTSJ1 mutations suggests expansion of the clinical spectrum in hemizygous mutation carriers

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Severe X-linked intellectual disability (ID) is a genetically heterogeneous disorder. Mutations in each gene are rare, making it difficult to define syndromic features, especially when the phenotype among affected individuals within a family varies. Truncating mutations in the X-chromosomal gene *FTSJ1* have been found in five families with ID and duplications and microdeletions involving *FTSJ1* as well as a missense change in this gene have also been reported to be associated with this phenotype. FTSJ1 is a methyltransferase and as such one of several proteins with similar functions that have been implicated in hereditary forms of ID. To study the role of FTSJ1 in the aetiology of this disorder we generated an Ftsj1 deficient mouse line and report here the results of a thorough characterization of hemizygous mutant mice in comparison with heterozygous and wildtype animals. We found statistically significant changes in parameters pertaining to behavior, dysmorphology, clinical chemistry and the immune system. Re-examination of individuals affected with ID in two previously reported families showed that several features observed in the mouse model were recapitulated in some of the patients, all of whom were previously diagnosed with non-syndromic ID. Although the clinical spectrum related to Ftsj1 deficiency in mouse and man is variable, our findings suggest that additional clinical features may be shared by patients with FTSJ1 deficiency.

P-MonoG-204

Identification of deep-intronic variants in CCM1, CCM2 and CCM3

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Cerebral cavernous malformations (CCM; OMIM 116860, 603284, 603285) are vascular lesions of the central nervous system that occur in familial (autosomal dominant) and isolated forms. CCMs are characterized by dysfunctional cell-cell junctions of the vascular endothelium. Clinical symptoms include recurrent headaches, seizures and hemorrhagic stroke. Loss-of-function mutations have been identified in three genes: *CCM1*, *CCM2* and *CCM3*.

Despite stringent inclusion criteria, standard genetic screening of the coding regions and the adjacent exon-intron boundaries fails to identify causative mutations in 13 % of familial and 43 % of isolated cases. Therefore, twenty mutation-negative index cases were selected for NGS-based resequencing of the entire genomic regions of *CCM1-3* to detect deep-intronic variants.

A long-range PCR approach for target enrichment and library preparation using Nextera XT kit (Illumina®) was applied. By paired-end-sequencing on a MiSeq benchtop sequencer (2x250 cycles; Illumina®), 98% of the target region were covered by a minimum of 50x. A total of 862 variants were detected of which 51 were unique. Splice site predictions and global scores like CADD (Combined Annotation Dependent Depletion) indicated functional relevance for 14 rare or previously not identified variants. Transcript analyses are ongoing to verify the suspected effect on splicing.

This approach proves to be an efficient and cost effective method to detect probably disease causing mutations not only in exonic but also in deep-intronic and regulatory regions of the known *CCM* genes and might be an alternative to Sanger sequencing in CCM diagnostics.

P-MonoG-205

Synergistic activity of the DYT6-associated THAP1 protein and HCFC1 in regulating gene expression

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THAP1, the gene mutated in DYT6 dystonia, codes for a transcription factor protein. While the THAP domain within the N-terminal part of the protein allows specific DNA-binding the functional relevance of the remaining part of THAP1 is largely unknown. Beside a coiled-coil domain and a nuclear localization signal at its C-terminus a stretch of four amino acids (134-137) within the 218 amino acids spanning protein was shown to be relevant for the interaction with the transcription regulator HCFC1. Interestingly, three mutations (Asn136Ser, Asn136Lys, Y137C) have been reported within this motif in DYT6 patients. By protein-protein interaction analyses we demonstrated that these substitutions abolish HCFC1-THAP1 complex formation. Notably, HCFC1 co-localization was observed in >90% of the >3,500 chromatin regions loaded with THAP1 based on genome-wide ChIP data. To further characterize the interaction between HCFC1 and THAP1 in the regulation of gene expression, we performed siRNA-mediated depletion of HCFC1 or THAP1. By this, we detected similar dysregulation of THAP1 target gene expression in cells with HCFC1- or THAP1 downregulation which indicates synergistic activity of both transcription factor proteins. To examine whether THAP1 mutations that abolish HCFC1-binding do affect THAP1 or HCFC1 recruitment to gene promoters, we used quantitative ChIP-seq on selected promoters. While none of the three THAP1 mutations significantly modified DNA-binding ability of THAP1, HCFC1 was strongly reduced at THAP1-target promoters. These findings suggest a THAP1-mediated recruitment of HCFC1 to THAP1 target sites further supporting an interplay of both transcription factors. In a final step, we investigated whether mutations in the THAP1 interacting domains of HCFC1 are a cause of dystonia. For this, we screened Exons 1-9 and 17 in 160 dystonia patients but did not detect any clear mutation in *HCFC1*. Of note, mutations in *HCFC1* have been reported in X-linked intellectual disability (XLID) and Cobalamin type X (CblX), a Vitamin B12 metabolic disorder with severe neurodevelopmental impairment. In conclusion, we demonstrate that THAP1 recruits HCFC1 to THAP1 target promoters and that both proteins are necessary for the transcriptional regulation. Importantly, DYT6-causing mutations within the 4-amino-acid interaction domain in THAP1 abolish the interaction. Thus, we highlight disturbed HCFC1 interaction as the consequence of three different DYT6-causing mutations.

P-MonoG-206

IPS cell based human models for neuronal dysfunction in Opitz BBB/G syndrome

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Opitz BBB/G Syndrome is an X-linked monogenic disorder that is caused by mutations in the MID1 gene. It is characterized by a broad variety of ventral midline defects. Of note, 30% of OS patients present with intellectual disability (ID). MID1 is a microtubule-associated ubiquitin ligase that regulates the ubiquitin

dependent degradation of protein phosphatase 2A (PP2A) thereby upregulating mechanistic target of rapamycin (mTOR) signaling. mTOR signaling is important for long-term potentiation and learning and memory and is dysregulated in many forms of ID including fragile X syndrome, RETT syndrome, tuberous sclerosis and Down syndrome. Because MID1 is closely linked to mTOR and PP2A activities, OS is an ideal model for ID caused by mTOR dysregulation.

Mutations in MID1 have been shown to result in a decrease in cell size in patients' fibroblasts reflecting to disrupted mTOR activity. In the mouse, loss of MID1 function could be connected to abnormal axonal outgrowth. We are establishing iPS cell based models to study neuronal phenotypes of OS in patients and be able to set up assays for compound screening. Such assays will possibly allow us to identify small molecules that interfere with mTOR signaling in humans, which are interesting candidate compounds for a drug therapy of ID caused by mTOR dysregulation.

We have established iPS cells from 5 different OS patients with mutations at different parts of the MID1 gene. To generate isogenic controls we are collecting fibroblasts of patients' mothers and have generated iPSCs from the mother of one of our patients carrying a heterozygous MID1 mutation on one of her two X chromosomes. By analyzing the X inactivation status and using allele specific RT-PCR of several iPS clones, we have identified clones that express either only the mutated or only the wildtype MID1 allele. By comparing the mutation with the wildtype MID1 expressing iPSCs we have found a smaller cell size in iPSCs expressing only the mutated MID1, which is in line with data from fibroblast cell lines and might reflect to mTOR dysregulation in the mutation expressing clones. Currently, we are testing for changes in the phosphorylation of mTOR downstream targets p70S6 kinase and S6. Here we expect to see a reduction in phosphorylation of these targets in the iPSCs expressing the mutant MID1.

In a next step we have successfully generated neuronal precursor cells (NPCs) and neuronal progenitors expressing the forebrain markers NFKX2.1 and FOXG1 from mutation and wildtype MID1 expressing iPSCs. We are now differentiating these neuronal progenitors further to generate fully functioning GABAergic interneurons and 3D neuron cultures. We will use these neurons to study the function of the MID1 protein in regulating axon length, mTOR signaling and local translation at the synapse.

P-MonoG-207

Exome sequencing reveals AGBL5 as novel candidate gene and additional variants for retinitis pigmentosa in five Turkish families.

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Retinitis pigmentosa (RP) is the most common inherited retinal disease with high genetic heterogeneity and variable phenotypes. Characteristic symptoms include night blindness and progressive loss of visual field, leading to blindness. Mutations in >60 genes have been identified to date as causative for RP, and additional candidate genes are assumed. To find the disease-causing mutations in the affected members of five Turkish families, we sequenced whole exomes using an Illumina platform. Among all candidate genes for retinal degeneration we found two previously known sequence variations: a 4 bp deletion in the RPGR gene (c.1662_1665delAGAA; p.Glu555Glyfs*14) and a recently described USH1-causing missense mutation in MYO7A (c.472G>A, p.Gly158Arg). Furthermore, a novel 1 bp deletion in the VCAN gene (c.5118delA; p.Ser1707Valfs*44) was detected as well as a large deletion in EYS, spanning ~400kb and comprising exons 16-26 (p.fs*). In one family, exome analyses of two affected individuals revealed a homozygous missense mutation (c.883G>A; p.Asp295Asn) in the AGBL5 (Agbl5; CCP5) gene, previously not reported to be associated with RP. RNA and protein analyses showed expression in human retina, as well as in mouse retina, brain and testis. Furthermore, cDNA analyses indicate the existence of tissue-specific AGBL5 splice variations in humans. AGBL5/CCP5 immunoreactivity was also visualized in human and mouse retinae. Due to the characteristic RP phenotype in patients carrying the AGBL5 missense mutation we suggest this gene as a candidate for a new form of autosomal recessively inherited RP and recommend further investigation to confirm this hypothesis.

P-MonoG-208

Branchio-otic (BO) syndrome: first description of an association of an EYA1 mutation with esophageal atresia

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Branchio-oto-renal (BOR) syndrome is an allelic and rare autosomal-dominant disorder with variable expressivity and a prevalence of 1:40.000. Patients show branchial arch anomalies, hearing loss and renal anomalies. In case of absence of any renal anomalies the term branchio-otic (BO) syndrome (BOS) has been coined. Esophageal atresia (EA) with or without trachea-esophageal fistula (TEF) has a birth prevalence of 1 in 3.000 live births. It occurs isolated or in combination with other congenital anomalies e.g. renal anomalies. Here, we performed whole-exome sequencing in a patient with bilateral branchial cleft fistulas, bilateral preauricular pits and EA/TEF type IIIb which revealed a mutation in EYA1 (c.966+5G>A). This mutation is known to affect EYA1 splicing leading to exon skipping and a premature termination and was described before in association with BOR syndrome. Sanger sequencing confirmed the c.966+5G>A mutation in the patient and in a further two affected family members, presenting solely BO syndrome (mother and sister of the index patient) who all showed at least one major BO criterion. The co-occurrence of BOS and EA/TEF suggests a rare but inherent association, although we cannot exclude a purely coincidental finding in our patient. Further analysis of EYA1 in EA/TEF patients with typical BOR/BO syndrome associated anomalies is warranted.

P-MonoG-209

Multi gene panel analysis in patients with mitochondrial disorders

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Mitochondrial disorders have emerged as a common cause of inherited disease, but their diagnosis remains challenging. Next generation sequencing (NGS) enables development of high-quality clinical tests for disease diagnosis with fast turnaround times. We report the results of a mitochondrial multi gene panel analysis in 55 patients, presenting with various clinical phenotypes. These included pediatric mitochondrial encephalopathy, encephalomyopathy, leukoencephalopathy and adult onset external ophthalmoplegia. Target enrichment with the Agilent SureSelectXT Kit was followed by massive parallel sequencing (Illumina NextSeq500). Data analysis was performed with a bioinformatics pipeline consisting of BWA, SAMtools, snpEff and Alamut-Batch. Diagnostic quality criteria assured a coverage depth of more than 30 sequences per base pair in at least 98% of the analyzed regions with 99,92% sensitivity and specificity in variant calling.

Likely causal variants were identified in 20% of cases involving 10 different genes. These included 5 novel mutations in the genes C12orf65, RARS2, SARS2, SDHD and SLC25A12. Our findings show the clinical and genetic heterogeneity of mitochondrial disorders which often do not present with classical phenotypes. However, multi gene panel analysis rapidly identifies the likely causal variants and prevents the need for invasive and complex biochemical testing. Next generation sequencing should be implemented early in the investigation of suspected mitochondrial disease.

P-MonoG-210

Diagnosis and classification of patients with inherited platelet disorders by targeted NGS

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Inherited platelet disorders (IPD) constitute a heterogeneous group of disorders which may occur in combination with other symptoms (syndromic) or as an isolated clinical feature. Typically about half of the patients with a platelet-based bleeding disorder remain without a substantiated diagnosis despite extensive diagnostic analyses. In addition to the clinical phenotype it is helpful to identify the underlying genetic defect in order to come to a substantiated diagnosis and provide the best possible patient care/therapy. We thus aimed to develop a targeted next generation sequencing (NGS) approach to identify genetic variants in patient suspected to have an inherited platelet disorders.

We developed a customized NGS gene panel encompassing 58 genes previously reported to be associated with IPD. We excluded genes that can easily be verified by determining plasmatic coagulation factors and that typically show defects in secondary hemostasis. Target enrichment was performed by the Nextera Rapid Capture Enrichment kit (Illumina) and sequencing was done on the Illumina MiSeq platform. Sequencing data were analyzed with the NextGENe software. In a proof-of-principle study known pathogenic variants in six patients were correctly identified by this approach. Besides point mutations we accurately detected copy number variants (CNVs) i.e. a microdeletion on chromosome 1q21.1 in a patient with TAR syndrome.

Up to now we analyzed 30 patients with phenotypically well-characterized platelet disorders of unknown genetic cause. Four samples did not fulfill the quality requirements and were thus excluded from further data analysis. Within the remaining cohort (26 samples) we identified potential pathogenic variants in 14 samples (54%). Variants have been confirmed by Sanger sequencing. None of the variants has been previously described in IPD cases. Segregation was so far shown in two index patients and co-segregation analysis is on-going in the remaining cases. This approach allows for detection of rare genetic causes i.e. *ACTN1* [MIM 102575; platelet-type bleeding disorder 15] or *GFI1B* [MIM 604383; platelet-type bleeding disorder 17/gray platelet syndrome]. In addition, we present genotype-phenotype correlations in our cases aiming to define a novel classification of inherited platelet disorders based on the genetic/molecular defect in the future.

In summary, we established a targeted NGS panel to study 58 platelet-specific genes in parallel which yielded detection of potential pathogenic variants in 54% of cases with IPD of unknown genetic cause. Implementing this approach in the diagnostic algorithm of inherited platelet disorders is currently discussed within the platelet community.

P-MonoG-211

Rare non-coding mutations extend the mutational spectrum in the PGAP3 subtype of Hyperphosphatasia with Mental Retardation Syndrome

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HPMRS or Mabry syndrome is a heterogeneous glycosylphosphatidylinositol (GPI) anchor deficiency that is caused by an impairment of synthesis or maturation of the GPI-anchor. The expressivity of the clinical features in HPMRS varies from severe syndromic forms with multiple organ malformations to mild non-syndromic intellectual disability. In about half of the patients with the clinical diagnosis of HPMRS pathogenic mutations can be identified in the coding region in one of the six genes, among them PGAP3.

In this work we describe a screening approach with sequence specific baits for transcripts of genes of the GPI pathway that allows the detection of functionally relevant mutations also including introns and the 5' and 3' UTR. By this means we increased the diagnostic yield and also identified pathogenic non-coding mutations. In 8 affected individuals from different ethnicities we found 7 novel pathogenic mutations in PGAP3. Besides 5 missense mutations we identified an intronic mutation, c.558-10G>A, that causes an aberrant splice product and a mutation in the 3'UTR, c.*559C>T, that is associated with substantially lower mRNA levels. We show that our novel screening approach is a useful rapid detection tool for alterations in genes coding for key components of the GPI pathway.

P-MonoG-212

Classification of novel FANCI mutations and the existence of biallelic null mutations in contrast to FANCD2

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Known as a rare heterogenic disease Fanconi Anemia (FA) is characterized by various anomalies, like radial ray defects, pigmentation abnormalities, growth retardation, microcephaly and malformations of inner organs e.g. kidney and liver. In addition, FA patients have an increased risk developing cancer, especially squamous cell carcinomas and hematological problems resulting in bone marrow failure. FA is genetically based on 19 known gene products, all involved in the FA/BRCA pathway (FA-A, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, -P, -Q, -R, –S and -T). These genes share key functions during cell maintenance by preserving the genomic stability after DNA damaging interstrand crosslinks.

An important part of this pathway is the ID complex, consisting of FANC-D2 and FANC-I which become monoubiquitinated by the upstream core complex. For FANCD2 a lot of clinical data is published, while FANCI patients have been already described in 2007 (Dorsman, Levitus et al. 2007; Sims, Spiteri et al. 2007; Smogorzewska, Matsuoka et al. 2007) only sparse molecular and clinical data is available until today. FANCI is a paralog of FANCD2 but shares only weak sequence similarity (20%) over the entire protein. Although there are no biallelic null mutations reported in FANCD2 so far, we could assign one of six new patients with p.1303* and p.1317* as a part of the FANCI complementation group. Interestingly, he had a mild phenotype and was primarily diagnosed FA-positive at the age of four. At the age of 18, the patient developed bone marrow failure and succumbed multiple infections in the same year. Furthermore both mutations are located at the C-terminus of FANCI containing the important EDGE and NLS motifs. (Colnaghi, Jones et al. 2011). Thus both null mutations are interesting variants since p.1303* destroys the EDGE motif and p.1317* is located upstream of the NLS resulting in loss of this essential motif.

All six new FA-I patients could be verified via Sanger sequencing or Next Generation Sequencing (NGS) and immunoblotting. We found eight not yet described variations in these patients with probably one founder mutation of Finnish origin.

The identification of new FANCI patients and their genetic variants is important to gain a more detailed insight into the FA-pathway and to correctly classify their clinical relevance to a possible genotype-phenotype correlation.

P-MonoG-213

Targeted NGS for analysis of craniosynostosis identifies a novel mutation in MEGF8

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Craniosynostosis is a frequent craniofacial malformation affecting 1 in 2500 newborns and is defined as the premature fusion of one or more cranial sutures. Premature fusion of the cranial sutures can occur either as isolated malformation in non-syndromic craniosynostoses or as part of a syndrome. So far genetic causes have been identified mainly for syndromic craniosynostoses, i.e. mutations in *FGFR2*, *FGFR3*, *TWIST1*, and *EFNB1*. High phenotypic variability and clinical overlap of the different syndromes often hamper straight forward genetic diagnostics, thus, necessitating conventional sequencing of more than one gene. Furthermore, in more than 50% of cases the underlying genetic cause remains unknown.

We compiled a next generation sequencing (NGS) gene panel comprising 68 genes. In addition to known and candidate craniosynostosis genes of the syndromic and isolated type, the panel includes downstream targets of participating signaling pathways and genes associated with bone development. Target enrichment was performed by the Nextera Rapid Capture Enrichment kit of Illumina and sequencing

was done on the Illumina MiSeq platform. Sequencing data were analysed with the NextGENe software. Performance of the NGS gene panel was validated by sequencing five control patients with known mutations. All of these mutations were detected correctly. Subsequently, we sequenced genomic DNA of 36 patients with syndromic as well as isolated craniosynostosis. Hot spot sequencing of *FGFR1-3* and *TWIST1* was performed previously for most of the cases, but did not reveal a pathogenic mutation. Until now sequencing of craniosynostosis patients with our NGS gene panel enabled the identification of potential pathogenic variants in 33% of the cases. Two patients are siblings and children of consanguineous parents. Both patients show an atypical Carpenter phenotype with sagittal craniosynostosis. We identified a novel homozygous splice site mutation (c.828G>A) in *MEGF8* leading to a predicted loss of the splice donor of exon 5 in both patients. The variant was confirmed by Sanger sequencing. Parents were shown to be heterozygous. An in-vitro minigene assay was performed to further analyse the effect on splicing. Indeed the c.828G>A variant led to skipping of exon 5 which is predicted to result in frameshift and subsequently in a premature stop codon (p.Gln248Cysfs*18). Mutations in *MEGF8* have previously been shown to be associated with Carpenter syndrome 2 (MIM 614976). Our patients share clinical features with Carpenter syndrome 2, i.e. craniosynostosis, hypertelorism, low-set ears, polysyndactyly as well as cryptorchidism. In summary, our data convincingly show the diagnostic benefit in applying NGS gene panel analysis in syndromic as well as non-syndromic craniosynostosis cases.

P-MonoG-214

De novo KDM1A missense mutation in a male case with severe intellectual disability

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Herein we report the phenotype of an 8-year-old boy displaying severe intellectual disability who was revealed to harbour a *de novo KDM1A* missense variant of hitherto unknown significance within a previously published exome sequencing study by our own (Rauch et al., Lancet 2012). This sequence alteration presumptively results in a substitution of a highly conserved aspartic acid residue (CADD v.1.0 phred-like 27.2; GERP 5.72) in the FAD-binding subdomain of the carboxyterminal Amine Oxidase Domain (AOD) of the KDM1A protein. As a component of several histone deacetylase complexes, Lysine (K)-Specific Demethylase 1A (KDM1A) plays a major role in regulating gene expression during ontogenesis by functioning as epigenetic coactivator or corepressor mediated by histone demethylation of specific lysine residues. The murine ortholog Kdm1a has been reported to be involved in cell lineage identity of non-neuronal cells by transcriptional repression of neuronal genes, and during the perinatal period, alternating splicing of KDM1A results in the expression of two neuron-specific isoforms regulating neurite-specific differentiation. In the meanwhile, further evidence from two other cases with overlapping phenotype suggest that *KDM1A de novo* variants are indeed phenocritical for intellectual disability and distinct facial dysmorphisms including prominent forehead, slighty arched eyebrows, elongated palpebral fissures, a wide nasal bridge, thick lips, and abnormal dentition. While one of these cases is so far unpublished, the other was published under the suspicion of a combined phenotype due to de novo mutations in the *ANKRD11* and *KDM1A* genes. Among other clinical findings being further presented, our propositus also shares musculosceletal hypotonia, ocular anomalies and constipation with the both other *KDM1A de novo* variant carriers. Unique phenotypical features include a supernumerary nipple, hypertrichosis and synophrys. We describe this child representing the first patient habouring an obviously deleterious sequence variant in *KDM1A* causing a distinct syndrome accompanied by extensive intellectual disability.

P-MonoG-215

Delineating the GRIN1 phenotypic spectrum – a distinct genetic NMDA receptor encephalopathy

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Objectives: We aimed to determine the phenotypic spectrum caused by mutations in GRIN1 encoding the NMDA receptor subunit GluN1 and to investigate their underlying functional pathophysiology.

Methods: We collected molecular and clinical data from several diagnostic and research cohorts. Functional consequences of GRIN1 mutations were investigated in Xenopus laevis oocytes.

Results: We identified heterozygous de novo GRIN1 mutations in 14 novel individuals and reviewed the phenotypes of all nine previously reported patients. These 23 individuals presented with a distinct phenotype

of profound developmental delay, severe intellectual disability with absent speech, muscular hypotonia, hyperkinetic movement disorder, oculogyric crises, cortical blindness, and epilepsy. Mutations cluster within transmembrane segments and resulted in loss of channel function of varying severity with a dominant-negative effect. In addition, we describe two homozygous GRIN1 mutations (1 missense, 1 truncation), each segregating with severe neurodevelopmental phenotypes in consanguineous families.

Conclusions: De novo GRIN1 mutations are associated with severe intellectual disability with cortical visual impairment and movement disorders being discriminating phenotypic features. Loss of NMDA receptor function appears to be the underlying disease mechanism for pathogenic GRIN1 mutations. The identification of both heterozygous and homozygous mutations blurs the borders of dominant and recessive inheritance of GRIN1-associated disorders.

P-MonoG-216

Detection of a KCNC3 promotor variant in an ataxia patient: Rare polymorphism, or disease-causing mutation?

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To date, at least 32 different loci for spinocerebellar ataxias (SCAs) have been mapped. Among those, 26 disease-causing genes have been identified. Common SCAs are caused by a repeat-expansion-mechanism. The emerging group of rare SCAs is characterized by point mutations in the respective disease genes. Although the large group of SCAs is genetically heterogeneous, signs and symptoms of the various types overlap. In particular, gait and limb ataxia, abnormal eye movements and dysarthria occur in most SCAs.

Among the rare SCAs affecting proper channel function, SCA13 is caused by point mutations in the *KCNC3* gene encoding a voltage-gated potassium channel (Kv3.3). Up to now, only a limited number of four amino acid changes, affecting voltage gated signaling were detected in single, or few families, respectively.

To learn more about the mutation spectrum in SCA13, we investigated 46 ataxia patients with a proven family history of ataxia for mutations in the *KCNC3* gene, especially in exon 1 overlapping with a large CpG island. Known SCA loci (SCA1-3, 6-8, 10, 12, 14, 17, 19, 23, 27, and 28) had previously been excluded. No putatively pathogenic base change was detected within the coding region of *KCNC3* in any of the ataxia patients. In one patient, a C to A transversion was observed in the exon 1 noncoding region of *KCNC3* in close proximity to the Kozak sequence. The variant had been described previously in 1 out of 6448 alleles resulting in a MAF(A) of 0.00016. Since no information concerning age and health status of the proband listed in the Exome Aggregation Consortium (ExAC) database is available, it might be a disease-causing mutation. Expression analysis of the differentially spliced *KCNC3* transcripts is being performed to examine whether the variant detected is pathogenic.

P-MonoG-217

A novel splice mutation in RTTN gene causes Seckel syndrome

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Seckel syndrome is a rare autosomal recessive disorder characterized by intrauterine growth retardation, postnatal dwarfism, severe microcephaly with mental retardation, and a typical 'bird-headed' facial appearance. Seckel syndrome is a heterogeneous disorder. Mutations in seven genes including ATR, RBBP8, CENPJ, CEP152, CEP63, NIN, and DNA2 have been reported to cause Seckel syndrome. We studied a large consanguineous family with three individuals with Seckel syndrome from two different branches of the family. A genome-wide search for homozygosity using a DNA microarray was performed in the pedigree. Four homozygous regions were identified and none of these overlapped with known Seckel loci. Next, we performed whole exome sequencing (WES) with two affected individuals. Deep WES analysis combined with homozygosity mapping, we found a variant in RTTN, c.5648 -5T>A, which was present in homozygous status in the two patients. This variant was confirmed by Sanger sequencing. Familial analysis revealed that the third patient also carried the homozygous variant, whereas health parents were heterozygote carriers. The variant is not annotated in the current databases of normal genetic variation (EVS, ExAC, dbSNP). Splice prediction programs predicted that the c.5648 -5T>A variant could influences or disrupt the splice acceptor site. Exom trapping conformed the splicing disruption. The variant caused completely skipping of exon 42 of the RTTN gene. RTTN (rotatin) encodes a large protein that is required for

the early developmental processes of left-right (L-R) specification and axial rotation and may play a role in notochord development. Two homozygous missense mutations in RTTN have been reported to be responsible for polymicrogyria with seizures (PMGYS), a developmental malformation demonstrated to have some phenotypes overlapped with Seckel syndrome. Very recently, recessive mutations in this gene have been also reported to cause microcephalic primordial dwarfism confirming our data and establishing RTTN as novel disease-associated gene for Seckel syndrome.

P-MonoG-218

The role of mutations in RAB39B in patients with Parkinson's disease

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Background: The identification of monogenic forms of Parkinson disease (PD) over the past 20 years has raised interest in genetic testing in patients with PD. With the advent of next generation sequencing (NGS), many novel putative PD genes have been reported but not all disease-linked genes proved valid after replication studies and thus should not be included in genetic testing schemes. *RAB39B* (member RAS oncogene family) is the latest putative PD gene, located on the X chromosome. Initially, *RAB39B* mutations were found in male patients with intellectual disability (ID), epilepsy and autism disorders. Later, mutations were reported in patients with ID and PD and most recently in a family with PD without marked ID. In our study, we aimed to investigate the role of *RAB39B* mutations in PD patients.

Methods: We Sanger-sequenced RAB39B exons and exon-intron boundaries in 552 mostly German PD patients (mean age at onset [AAO] 50.9±15.3 years, 59.8% male, 22% with positive family history). None of the patients had marked, premorbid PD. Since *RAB39B* is located on the X chromosome, we also examined 91 Filipino patients with X-linked dystonia parkinsonism (XDP, AAO 42.3±7.6). In addition, we analyzed 186 German controls for variants in RAB39B.

Results: Among the PD patients, we detected three different changes without obvious impact on the encoded protein. This included a synonymous variant in two unrelated individuals (rs369970931; c.543A>G; p.Thr181=, reported frequency <0.001). cDNA sequencing did not reveal activation of an alternative splice site. In addition, we detected two novel, intronic variants (c.215+61G>A and c.215+39C>G, Table) in two males. All three variants are predicted to be disease causing by Mutation Taster but none received a CADD score >15. RAB39B variants were neither found among the XDP patients nor in the 186 controls. The number of carriers of rare variants in RAB39B in patients seems to be comparable to that in controls (4/552 [0.7%] vs. 0/186 vs. 169/60.706 [0.3%] in the Exome Aggregation Consortium [ExAC]).

Conclusions: Although the number of reports pointing towards a pathogenic role of *RAB39B* mutations in PD grows, our screen of more than 600 patients did not reveal disease-causing variants. This finding is consistent with another negative screening in a cohort of 504 Chinese Han PD patients. Combining this and our data, no mutation in *RAB39B* was found among >1.000 PD patients. We therefore conclude that *RAB39B* mutations in classic PD patients without ID are rather rare, and do not need to be included in genetic testing.

P-MonoG-219

Mutations of FOXI3 may contribute to development of the oculo-auriculo-vertebral syndrome

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Congenital aural atresia (CAA) occurs as a result of abnormal development of the first and second branchial arches about once in 10,000 to 20,000 live births, and unilateral CAA occurs more often than bilateral CAA (Abdel-Aziz M, 2013). CAA can occur sporadically or in a syndromic context with other craniofacial malformations like in the oculo-auriculo-vertebral spectrum (OAVS), a disorder of the first and second branchial arches with anomalies of the internal and external ear structures. Recently, a heterozygous deletion of the *FOXI3* gene encoding a forkhead transcription factor has been proposed to be responsible for internal and external ear anomalies in a girl with unilateral microtia, ipsilateral CAA and mild facial asymmetry (Tassano E *et al*, 2015). Therefore, we considered the *FOXI3* gene as one possible candidate gene for OAVS, and screened the entire *FOXI3* gene for mutations in 152 individuals with at least unilateral microtia. Eighty-five persons from this cohort had either bilateral (n=16) or unilateral (n=69) CAA, 34 were

described of having no CAA, and for 33 probands no clear information was available regarding their ear canal.

We found no nonsense, frameshift or splice site mutation, and more than 50% of probands was heterozygous for a frequent intronic SNP arguing against a high frequency of deletions of this 2-exon gene. However, nine different heterozygous missense variants were present in eleven unrelated individuals. Eight variants cause amino acids changes, and one is an in frame insertion (ala-pro-ala) into a small repetitive stretch of alanines and prolines. In *silico tools* predicted seven of these changes including the in frame insertion to affect protein function and as probably deleterious. Interestingly, the p.(A50P) variant, that is listed in the 1000 genomes database as a rare SNP and predicted as a benign exchange, was found in one familial case with bilateral CAA in the index and his unilaterally affected father, one sporadic case with unilateral CAA and in one individual with unilateral microtia but without CAA. The second missense mutation with a 'benign' prediction was found in a boy with unilateral CAA. In all cases where DNA of parents was available (n= 8), the variant was inherited either from the father (n=6) or the mother (n=2), but only two fathers and one paternal grandfather were described as being variably affected.

Five of the eleven patients with *FOXI3* variants are affected with bilateral CAA. This means that nearly one third of the total of 16 patients with bilateral CAA carry heterozygous *FOXI3* variants. We conclude that variants of the FOXI3 transcription factor are not the exclusive reason but may well contribute to the abnormal inner and outer ear development as seen in the OAVS. But in contrast to the proposed haploinsufficiency for the whole gene deletion, a missense mutation may only be incompletely penetrant or require further as yet unknown triggers to produce the CAA/OAVS phenotype.

P-MonoG-220

Mendeliome sequencing increases the diagnostic yield in patients with unexplained intellectual disability by 30% (a single center experience).

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Introduction: Advances in deciphering the genetic causes for impaired development have always been accompanied by advances in technology. In the past, array-CGH technology has boosted the detection rate significantly; but up to 50% of children with developmental delay still remain undiagnosed. Since the latest technological advance, next generation sequencing (NGS), has the power to improve the diagnostic yield tremendously, we applied this method to 54 index patients with developmental delay or intellectual disability (ID) and pre-excluded genomic imbalances.

Method: Genomic DNA samples of 25 parent-child trios plus 29 individuals were analyzed for mutations in 4813 genes, using the TruSightOne gene panel on the MiSeq platform (Illumina, San Diego, CA). After sequencing with median target coverage of 80-fold and mapping to hg19, sequence variants were called by two independent platforms: the GATK pipeline installed on the MiSeq and the CLC Biomedical Genomics platform (Qiagen, Hilden, Germany). All de novo variants, homozygous variants with heterozygosity in both parents and compound heterozygous variants were analyzed as well as hemizygous variants in male patients. These variants were screened for clinical und molecular concordance (i.e. disease-association of the gene, published mutation). In addition, all splice-relevant variants, all frameshift and nonsense-mutations were screened for clinical concordance. The results were discussed in a team of clinicians and molecular geneticists, relevant variants were validated by Sanger-sequencing.

Results: Using the mendeliome in a diagnostic setting, we established a diagnosis in 15 of the 54 index patients (28%). For seven further patients, we found one or two possibly causative candidates (13%). 5 patients (9%) showed incidental findings which either made treatment or surveillance necessary (homozygous MUTYH-mutations, SDHA-mutation) or led to an increased risk for a recessive disease in children (PAH- or CFTR-mutations).

Conclusion: Mendeliome sequencing can significantly increase the diagnostic yield in patients with ID unsolved by previous routine testing (array-CGH, conventional karyotyping). However, variant interpretation remains challenging and requires standardized procedures to implement the new technology in a standard diagnostic setting.

P-MonoG-221

Identification of three genes involved in recessive disorders with dystonic features by exome sequencing in Pakistani families

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Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements and postures. Dystonia can be the sole clinical sign (isolated dystonia), can occur along with other movement disorders (combined dystonia), or other neurological signs including for instance ataxia (complex dystonia). Whereas a number of genetic causes of autosomal dominant dystonias have been identified, genes for recessive forms remain largely unknown. Recessive causes can best be elucidated in consanguineous families using next generation sequencing.

To unravel the genetic cause of recessive disorders with dystonic features, we collected six consanguineous families with multiple affected individuals from Pakistan. Patients were clinically examined and videotaped. We performed exome sequencing in 4-5 samples per family. Variants were filtered based on their effect on the encoded protein and their frequency in public databases. We only considered variants that were homozygous in the affected offspring and heterozygous in the parents. Segregation of candidate variants was tested in all available family members by Sanger sequencing.

To date, we identified the disease-causing mutation in three of the six families. In Family RDHM-01, all patients had an onset of about 1.5 years. The disease was characterized by ataxia of gait, bradykinesia including hypomimia, mild dystonic postures of upper limbs, supranuclear gaze palsy, and spasticity. We identified a novel, homozygous 1-basepair duplication (c.9119dup; p.Asn3040fs) in all four patients in the SACS gene encoding sacsin. SACS mutations have previously been described in spastic ataxia of the Charlevoix-Saguenay type. In Family RDHR-04, a homozygous 7-basepair deletion (c.599_605del; p.Pro200fs) was found in all five patients in exon 6 of ATCAY encoding caytaxin. ATCAY mutations have so far only been reported in a few individuals with Cayman cerebellar ataxia. Our patients had severe gait ataxia and mild bibrachial dystonia. They also had strabism and apraxia, as well as a suspicion of cognitive impairment. Onset was at the age of about 4 months. Finally, in Family RDHM-03, a homozygous missense mutation (c.551T>C; p.Ile184Thr) was found in exon 4 of MCOLN1 encoding mucolipin 1. Both patients presented with generalized dystonia in adolescence. Of note, MCOLN1 mutations have been reported as a cause of mucolipidosis IV, a neurodegenerative lysosomal storage disorder characterized by psychomotor retardation and ophthalmologic abnormalities. None of the three mutations was found in 200 ethnically matched controls.

In conclusion, exome sequencing is a powerful tool to detect disease-causing variants also in genes that would not have been chosen for diagnostic testing by Sanger sequencing. So far, we have solved the genetic cause in half of our families and further investigations are underway to also uncover the likely novel disease genes in the remaining families.

P-MonoG-222

Next-generation sequencing diagnostics of inherited arrhythmogenic cardiac disorders

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Inherited arrhythmogenic cardiac disorders are caused by cardiac channelopathies such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and Brugada syndrome (BrS) and the cardiomyopathies leading to a secondary arrhythmia risk such as hypertrophic cardiomyopathy (HCM), dilatative cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). All these diseases are associated with significant morbidity and mortality and are a known risk factor for sudden cardiac death. The presymptomatic diagnosis is essential as for most of the cases a therapy is available. Mutation detection in the index cases followed by cascade screening of the family members could be life saving. As some cardiomyopathies may present with only little structural symptoms, differential diagnosis of arrhythmogenic disorders may be difficult. Moreover, some arrhythmogenic disorders are clinically and genetically extremely heterogeneous. An Agilent SureSelect Target Enrichment assay was designed for the capture of coding regions including splice sites of 72 genes known to be associated with arrhythmogenic disorders. Target enrichment followed by re-sequencing on the Illumina NextSeq platform was used for mutation detection. We screened 100 clinical samples with a suspected inherited arrhythmogenic disorder with a minimum coverage of 20-fold. Data analysis was performed with the CLC Genomics Workbench and

custom developed Perl scripts. Potentially disease causing variants and regions with an insufficient coverage were re-analyzed with Sanger sequencing. Disease causing mutations were detected in about 40% of cases. Additional 15% of cases showed variants of unclassified significance.

P-MonoG-223

Unraveling the genetic cause of hereditary ophthalmic diseases in Arab societies originating from Israel and the Palestinian Authority applying Next Generation Sequencing

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The prevalence of hereditary recessive diseases is high in Middle Eastern Arab societies, mainly due to the high rate of consanguineous marriages. In an ongoing collaboration between physicians and scientists from Israel, the Palestinian Authority and Germany, blood samples of consanguineous families with rare neurogenetic disorders are collected in Arab villages of Israel and the Palestinian Authority. The present study aims to identify the molecular basis of rare ophthalmic diseases applying next generation sequencing (NGS) in order to provide the families with a molecular diagnosis and genetic counseling.

Nine affected patients out of eight consanguineous families with ophthalmic disorders underwent whole exome sequencing. In an additional family, SNP genotyping using Affymetrix CytoScan HD arrays was done prior to mutation screening in >4,800 genes applying the Illumina TruSight One Sequencing Panel ("clinical exome") on an Illumina MiSeq sequencer (2 X 300bp paired-end). For whole exome sequencing, exonic regions were enriched applying Agilent's Sure Select XT Human All Exon V5 system. Massively parallel sequencing was performed on an Illumina HiSeq 2500 (2 X 100bp paired-end) or NextSeq 500 instrument (2 X 150bp paired-end). Mapping, variant calling and annotation of the identified variants was accomplished using an in-house bioinformatics pipeline based on tools that are freely available. Segregation analysis in the extensive families was done by PCR and Sanger sequencing. Informed written consent was signed by all study participants or by the parents in case of minor study subjects.

Applying NGS technology, we were able to discover the disease causing mutations in eight out of nine families. All these mutations could be identified by focusing on homozygous variants in genes that have already been associated with autosomal recessively inherited ocular diseases. Four out of seven mutations are novel. The affected genes are *CRB1* (n=3), *ABCA4*, *CNGA3*, *RLBP1*, in inherited retinal dystrophies, *GALK1* in a family with congenital cataract, and *PXDN* in a family with congenital nanophthalmos and microsclerocornea. In addition, one promising candidate gene is currently under investigation in the ninth family. In rural or underdeveloped areas of the world, unraveling the molecular basis of hereditary diseases can be the easier way to provide a family with a precise diagnosis, since comprehensive clinical testing is often not available for such families.

In summary, NGS is a fast tool to identify mutations underlying a large spectrum of rare ophthalmic disorders even with limited prior clinical workup. The consanguineous background of our patients with different ophthalmic disorders enabled us to identify the disease causing mutations in eight out of nine families.

P-MonoG-224

The Small GTPase RIT1: Expansion of the Molecular Pathomechanism in Noonan Syndrome

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Noonan syndrome (NS) is characterized by distinct craniofacial dysmorphism, postnatal growth retardation, congenital heart defects, learning difficulties and a predisposition to malignancies. It belongs to a group of phenotypically overlapping syndromes caused by heterozygous germline mutations in genes encoding components of the RAS-MAPK signaling pathway and therefore are referred to as RASopathies. Enhanced signal flux through the RAS-MAPK cascade is considered the common molecular pathomechanism underlying these syndromes. Recently, heterozygous germline mutations in *RIT1*, encoding a small GTPase out of the *RAS* family, have been described in individuals with classical NS. RIT1 is highly homologous to RAS, however, it shows a unique effector domain and a C-terminal polybasic motif

instead of the classical CAAX membrane targeting motif. Although controversially discussed, RIT1 has been proposed to act in functional contexts apart from RAF-MEK-ERK signaling.

To study whether RIT1 can activate the RAS-MAPK signaling module and bind to classical downstream effectors of RAS, we performed GTPase pulldown assays and found that wild-type RIT1 efficiently co-precipitated with PIK3CA effector peptide in HEK293T cells cultivated under steady state conditions (10% serum), but not in serum-deprived cells; this indicates serum factor dependency for RIT1 activation and binding to PIK3CA. We could not observe serum-dependent binding of RIT1 to the RAS effectors RAF1, RALGDS and PLCE1 suggesting that these three proteins are no physiological interactors of RIT1. Notably, we could pulldown wild-type RIT1 by using the CRIB domain of PAK1, a central effector of the Rho GTPases CDC42 and RAC1 which are master regulators of the actin cytoskeleton. Moreover, co-immunoprecipitation experiments revealed RIT1 in complex with CDC42. These data may indicate the existence of the novel RIT1-CDC42-PAK1 signaling axis.

Next, we studied the functional consequences of the NS-associated RIT1 amino acid changes p.K23N, p.G31R, p.A57G, p.F82L, p.M90V and p.G95A. In line with data from our binding studies, we could not detect a clear impact of the mutations on ERK phosphorylation. Similarly but unexpectedly, our data on Akt phosphorylation in HEK293T cells expressing different RIT1 mutants were inconsistent and/or not significant. Most interestingly, our preliminary data indicate that NS-associated RIT1 amino acid changes result in increased binding of the respective RIT1 mutant to both CDC42 and PAK1.

Taken together, our data raise the exciting possibility that a regulatory crosstalk between RIT1 and RHO GTPases exists. Moreover, the current view that an increased signal flux through the RAS-MEK-ERK cascade is the exclusive pathomechanistic basis for RASopathies should be reconsidered as other pathways such as dysregulated CDC42-PAK1 signaling may also contribute to the pathophysiology of NS.

P-MonoG-225

Case report: Splice site mutation of TCF4 gene in a patient with suspected Pitt-Hopkins syndrome

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Pitt-Hopkins syndrome (PHS) is a rare neurodevelopmental genetic disorder, remaining underdiagnosed due to similarities with other known genetic syndromes like Angelman or Rett Syndrome. It is mainly characterized by extensive developmental delay, severe intellectual disability (ID), and a typical facial gestalt. Other features include episodic hyperventilation, seizures, eye abnormalities and constipation. Mutations of the TCF4 gene were identified as disease-causing and haplo-insufficiency of TCF4 has been reported to cause classical PHS.

We report on a 20-year old girl, born as a second child to healthy, non-consanguineous parents, presenting with severe developmental delay, severe speech delay, facial dysmorphism and movement disorder. Initial genetic investigations at age 4 including molecular genetic diagnostics of Angelman-Syndrome (Methylation assay, Deletion-/UPD-testing, sequencing of UBE3A-gene) and Fragile X syndrome (Southern Blot analysis), classical karyotyping and array-based comparative genomic hybridization (array-CGH) revealed negative results.

We performed molecular genetic diagnostics of TCF4 gene consisting of Sanger sequencing followed by MLPA (multiplex ligation-dependent probe amplification) analysis. By sequence analysis we were able to identify the splice donor variant c.1486+3A>C of the TCF4 gene (NM_001083962.1; chr18:52901776(hg19)). This substitution is localized at position +3 of intron 16 (IVS16+3 A>C) and was not reported in relevant databases (ExAC Browser (Exome Aggregation Consortium); dbSNP; EVS) or the literature to date. By the result of parental analysis the variant had occurred de novo in the patient. Bioinformatic algorithms (MaxEntScan, Human Splicing Finder a.o.) predicted not to significantly alter the splice site activity.

In order to determine the in vivo effect of this substitution on the splice donor site of intron 16 in RNA extracted from the patients' platelets and leukocytes we performed functional RNA studies to elucidate the true effect of the variation detected in this individual.

P-MonoG-226

Mutation spectrum in patients with suspected isolated ectopia lentis

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Ectopia lentis (EL) frequently is a symptom of connective tissue diseases such as Marfan syndrome where it is a major clinical diagnostic criterion. However, it can also be observed as an isolated finding. The correct differentiation between syndromic and isolated EL is crucial for the management and surveillance of the affected patients, especially regarding cardiovascular complications. Main causes for isolated EL are mutations in ADAMTSL4 (autosomal recessive) and FBN1 (autosomal dominant). The latter also are the major cause for syndromic EL, i.e. Marfan syndrome. Rarely, mutations in other genes, such as LTBP2, can cause an isolated ocular phenotype including EL.

We analyzed 20 patients with clinically suspected isolated EL for the underlying genetic cause. 19 patients were primarily screened for mutations in ADAMTSL4 and FBN1. In patients without identifiable mutation in ADAMTSL4 and FBN1, an NGS based gene panel containing 11 genes associated with EL was performed. One additional patient was primarily analyzed with the gene panel.

The most common cause for EL identified in this cohort were mutations in ADAMTSL4 (12/20); of these, 9 carried the common founder mutation p.(Gln256Profs*38) on at least one allele. Three patients carried FBN1 mutations (two missense mutations and one single exon deletion). Both missense mutations have already been described as causative for Marfan syndrome. Other molecular causes identified included mutations in LTBP2, COL2A1, and ASPH.

Molecular genetic testing in patients with ectopia lentis can facilitate the differentiation between isolated and syndromic EL. This can significantly influence the management, especially in young children, where a discrimination often is not possible clinically. Also, it can show up possible ocular complications such as glaucoma in LTBP2 mutation carriers.

P-MonoG-227

The mutation p.E113K in the rhodopsin Schiff base counterion causes two distinct phenotypes within the same family.

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The diagnoses of retinitis pigmentosa (RP) and night blindness belong to a group of clinically and genetically heterogeneous retinal dysfunctions. Although night blindness is frequently reported as the first sign of progressive RP, forms of congenital stationary night blindness (CSNB) are known to be non-progressive disorders, i.e. lacking signs of retinal degeneration.

The current study focused on the identification of causative mutations in the RP-affected index patient and in several older members of the same family diagnosed with CSNB. Ophthalmological examinations of the index patient confirmed a typical form of RP, including midperipheral loss of choriocapillaris, constricted retinal vessels, isolated retinal vessel-associated pigmentary clumping and foveal thinning. The electroretinogram (ERG) showed pathologically reduced minor amplitudes in both eyes. Clinical characterizations and ERGs of the CSNB-affected family members suggested an incomplete form of CSNB (Schubert–Bornschein type). Applying whole exome sequencing we detected the non-synonymous substitution c.337G>A, p.E113K in the rhodopsin (*RHO*) gene. The identification of the pathogenic variant p.E113K is the first description of a naturally-occurring mutation in the Schiff base counterion of RHO in human patients. The five exons of *RHO* were re-sequenced in the index patient and in available family members in order to exclude other genetic variants in this gene. The heterozygous mutation c.337G>A in exon 1 was confirmed in the RP-affected index patient and in five CSNB-affected relatives. This mutation

was excluded in a healthy family member and in 199 ethnically matched controls. Furthermore, exome sequencing analysis suggested genetic modifiers, which may explain the more severe phenotype of the RP-affected index patient.

Our findings demonstrate that a mutation in the biochemically well-characterized counterion p.E113 in RHO can independently cause RP and CSNB in the same family.

P-MonoG-228

Molecular Mechanism of CHRDL1-mediated X-linked Megalocornea in Humans and in Xenopus Model

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Chordin-Like 1 (*CHRDL1*) mutations cause non-syndromic X-linked megalocornea (XMC) characterized by enlarged anterior eye segments. Mosaic corneal degeneration, presenile cataract and secondary glaucoma are associated with XMC. Besides that *CHRDL1* encodes Ventroptin, a secreted Bone Morphogenetic Protein (BMP) antagonist, the molecular mechanism of XMC is not well understood yet.

In a family with broad phenotypic variability of XMC we identified the novel *CHRDL1* frameshift mutation c.807_808delTC [p.H270Wfs*22] presumably causing *CHRDL1* loss-of-function. Using *Xenopus laevis* as model organism we demonstrate that *chrdl1* is specifically expressed in the ocular tissue at late developmental stages. The *chrdl1* knockdown directly resembles the human XMC phenotype and confirms CHRDL1 deficiency to cause XMC. Interestingly, secondary to this *bmp4* is down regulated in the *Xenopus* eyes. Moreover, phospho-SMAD1/5 is altered and BMP receptor 1A is reduced in a XMC patient. Together, we classify these observations as negative-feedback regulation due to the deficient BMP antagonism in XMC. As *CHRDL1* is preferentially expressed in the limbal stem cell niche of adult human cornea, we assume that *CHRDL1* plays a key role in cornea homeostasis.

In conclusion, we provide novel insights into the molecular mechanism of XMC as well as into the specific role of *CHRDL1* during cornea organogenesis, amongst others by establishment of the first XMC *in vivo* model. We show that unravelling monogenic cornea disorders like XMC - with presumably disturbed cornea growth and differentiation - contributes to the identification of potential limbal stem cell niche factors that are promising targets for regenerative therapies of corneal injuries.

P-MonoG-229

Expanding the phenotype associated with Naa10 related N-terminal acetylation deficiency

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Background: N-terminal acetylation is a common protein modification in eukaryotes associated with numerous cellular processes. Inherited mutations in *NAA10*, encoding the catalytic subunit of the major N-terminal acetylation complex NatA have been associated with diverse, syndromic X-linked recessive disorders, while de novo missense mutations have been reported in one male and one female individual with severe intellectual disability but otherwise unspecific phenotypes. Thus, the full genetic and clinical spectrum of *NAA10* deficiency is yet to be delineated.

Methods and Results: Whole exome or panel sequencing identified three different novel and one known missense mutation in *NAA10*, de novo in eleven females, and due to maternal germ line mosaicism in another girl and her more severely affected and deceased brother. Common phenotypes in the affected females included severe intellectual disability and postnatal growth failure with pronounced microcephaly. In vitro enzymatic assays for the novel, recurrent mutations p.(Arg83Cys) and p.(Phe128Leu) revealed a reduced catalytic activity. X-inactivation was random in four of five tested females.

Conclusions: We report on 12 females with mutations in *NAA10* and thus further expand the mutational and clinical spectrum. The core phenotype of X-linked *NAA10* related N-terminal-acetyltransferase deficiency in both males and females includes developmental delay, severe intellectual disability, postnatal growth failure with severe microcephaly and skeletal or cardiac anomalies. Genotype-phenotype correlations within and between both genders are complex and may include various factors such as location and nature of mutations, enzymatic stability and activity, and X-inactivation in females.

P-MonoG-230

Homozygous missense mutation in the LMAN2L gene segregates with intellectual disability in a large consanguineous Pakistani family

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Intellectual disability (ID) is a neurodevelopmental disorder affecting 1 – 3 % of the population world-wide. It is characterized by high phenotypic and genetic heterogeneity and in most cases the underlying cause of the disorder is unknown. In our study we investigated a large consanguineous family from Baluchistan, Pakistan, comprising seven affected individuals with a severe form of autosomal recessive ID (ARID) and epilepsy, to elucidate a putative genetic cause.

Whole Exome Sequencing (WES) of a trio, including a child with ID and epilepsy and its healthy parents that were part of this large family, revealed a homozygous missense variant p.R53Q in the LMAN2L gene. This homozygous variant was co-segregating in the family with the phenotype of severe ID and infantile epilepsy; unaffected family members were heterozygous variant carriers. The variant was predicted to be pathogenic by five different in silico programs and further 3D structure modeling of the protein suggests that variant p.R53Q may impair protein-protein interaction. LMAN2L (OMIM: 609552) encodes for the lectin, mannose-binding 2-like protein which is a cargo receptor in the endoplasmic reticulum important for glycoprotein transport. Genome wide association studies have identified an association of LMAN2L to different neuropsychiatric disorders. This is the first report linking LMAN2L to a phenotype of severe ARID and seizures, indicating that the deleterious homozygous p.R53Q variant very likely causes the disorder.

P-MonoG-231

Impaired Notch signaling in Dowling-Degos disease: Novel mutations in POGLUT1 and further insight into disease pathogenesis

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Pigmentation disorders (PDs) comprise a large group of rare and heterogeneous disorders that are mainly characterized by various pigmentation abnormalities affecting single parts of the body or the complete integument. PDs can either be congenital or acquired later in life. Among the large group of PDs resides a subgroup of individuals affected with the hyperpigmentation disorder Dowling-Degos disease (DDD), which is an inherited condition following an autosomal-dominant pattern of inheritance. Affected individuals from both sexes develop post pubertal a progressive and disfiguring hyperpigmentation in a reticulate pattern and small hyperkeratotic, dark-brown papules that predominantly affect the flexures, large skin folds, trunk, face and extremities. In 2006, we identified loss-of-function mutations in *KRT5* as the genetic cause for DDD. A recent study of two Chinese families with DDD led to the elucidation of mutations in *POFUT1*, which encodes O-fucosyltransferase 1; while our group identified mutations in *POGLUT1*, encoding protein O-glucosyltransferase 1. Both enzymes are involved in posttranslational modification of Notch receptor proteins respectively, highlighting the notch signaling pathway as a key player in pathogenesis of DDD.

After publication of POGLUT1, we screened 30 additional patients, whose DNA had been referred to us for molecular genetic confirmation of presumed DDD, for pathogenic mutations in the known disease genes by usage of Sanger sequencing. Analyzing the sequencing data, we identified not only known, but also nine novel causative mutations in *POGLUT1*. The effects of the identified mutations on the resultant protein are examined on the basis of a homology model and by mammalian cell culture based experiments. Additionally, further experiments, like gene expression analysis and immunofluorescence assays, in POGLUT1-deficient HaCaT cells are ongoing to elucidate the role of POGLUT1 in epidermal notch signaling. These experiments will help us to further understand the pathogenesis of DDD and thereby paving the way for the future development of potential targeted therapies.

P-MonoG-232

Identification of binding partners of the LPAR6 protein involved in hypotricosis

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Hypotrichosis simplex (HS) is a genetically heterogeneous hair loss disorder characterized by progressive hair loss. While the hair is usually normally developed at birth, the first symptoms appear at pre-school age, and hair loss is diffuse and progressive. This involves the partial or total loss of scalp hair, while body hair, eyebrows, eyelashes and beard can be either affected or not. Mutations in more than 10 genes have been identified for HS, including the LPAR6 gene encoding for a G-protein coupled receptor, the

human lysophosphatidic acid receptor 6 (LPAR6). This protein belongs to the same signaling network of lipase H (LIPH), which is also a causative gene for HS. Considering the importance of the pathway involving LPAR6 and LIPH in the determination of proper hair growth, we speculated that more genes related to the same pathway could be a cause of HS. Therefore, our goal was to identify the interactors of LPAR6 to shed light on the signaling pathways in which this protein is involved. Membrane yeast two hybrid (MYTH) assay and bait-dependency test were performed to spot putative binding partners (BPs) of this receptor. The results revealed 72 putative interactors from the skin/hair follicle library. The last step was to further assess the interaction by performing pull-down assay, to discard false positive results and, at the same time, confirm the interaction between LPAR6 and the candidate proteins. Using this methodology, five true BPs have already been confirmed, including the U11/U12 small nuclear ribonucleoprotein 35 kDa protein (SNRNP35), the 40S ribosomal protein S28 (RPS28), the emopamil-binding protein (EBP), the transcription factor AP-1 (JUN) and the lymphocyte antigen 6 complex locus D (LY6D). Many more putative BPs have been cloned and await to be studied. Considering that LPAR6 is the first known G protein-coupled receptor that plays a specific and essential role in hair growth, further studies on this protein, its ligands and signaling pathways could lead to better understand the etiopathogenesis of HS and provide some new ideas for the development of new drugs.

P-MonoG-233

hSNM1B/Apollo and FANCP/SLX4 do not function epistatically in the response to acetaldehyde-induced DNA damage

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OBJECTIVES: Fanconi Anemia (FA) is a genetic disorder manifesting in congenital defects, increased risk of cancer and bone marrow failure. To date, there are 19 known genes that encode FA proteins (FANCA-FANCT), all of which function in the FA/BRCA DNA-repair pathway. Recent research shows that this pathway counteracts acetaldehyde-induced genotoxicity. However, only a subset of FANC-deficient cells exhibit hypersensitivity towards this reactive aldehyde. We examined whether U2OS cells depleted for FANCP/SLX4 and for the nuclease hSNM1B/Apollo, which we have recently shown to be linked to the FA DNA-repair pathway through its interaction with FANCP/SLX4, share this phenotype. Additionally, we tested whether these genes function epistatically in response to acetaldehyde-induced DNA damage. METHODS: U2OS cells were depleted for FANCP/SLX4 and/or hSNM1B/Apollo using siRNAs and treated with 1mM acetaldehyde (24h). Subsequently, the activation of the FA/BRCA DNA-repair pathway was assessed by quantifying the protein band intensity of monoubiquitinated FANCD2 in Western Blot assays. Additionally, metaphase chromosomes were evaluated for abnormalities. Following an exposure to 20mM acetaldehyde (1h), the ability of FANCP/SLX4- and/or hSNM1B/Apollo-depleted cells to form colonies was examined. RESULTS: Acetaldehyde exposure induced an increased FANCD2 monoubiquitination in both FANCP/SLX4- and hSNM1B/Apollo-depleted U2OS when compared to untreated cells. There was no significant difference between control-cells and cells depleted for FANCP/SLX4 and/or hSNM1B/Apollo regarding the amount of chromosome breaks per cell following acetaldehyde treatment, with all cells showing significant increases in chromosomal abnormalities. The ability to form colonies was, however, significantly reduced in FANCP/SLX4-depleted cells and in FANCP/SLX4-hSNM1B/Apollo double knockdown cells when compared to control-cells. No significant increase in sensitivity towards acetaldehyde was found in hSNM1B/Apollo-depleted cells. CONCLUSION: Here we show that acetaldehyde activates the FA DNA-repair pathway in FANCP/SLX4- and hSNM1B/Apollo-depleted U2OS cells. Contrary to their role in the cellular response to DNA damage produced by ionizing radiation or by the DNA crosslinking agent mitomycin C, hSNM1B/Apollo and FANCP/SLX4 do not function epistatically in the response to acetaldehyde-induced DNA damage. Our results suggest that FANCP/SLX4 is involved in the repair of DNA lesions resulting from acetaldehyde exposure, while hSNM1B/Apollo is not. Since hSNM1B/Apollo is known to take part in the repair of DNA interstrand crosslinks, our findings raise the possibility that these particular DNA lesions may not be, as prior research suggests, the main type of DNA damage induced by acetaldehyde.

P-MonoG-234

The ASD-Rett syndrome protein MeCP2 regulates N-cadherin

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Rett syndrome (RTT) is an X-linked dominant disorder which is caused by mutations in the MECP2 gene, encoding methyl CpG-binding protein 2 (MeCP2). RTT mainly affects girls who carry a mutated MECP2 allele on one of her two X chromosomes. In contrast to girls boys carrying RTT causing MECP2 mutations suffer from severe neonatal encephalopathy suggesting that MeCP2 may be essential already during embryonic or perinatal brain development and function. This hypothesis is further supported by the fact that MeCP2 is highly expressed in migrating neurons and to a lesser extent also in neural progenitor cells during neocortical development.

Recent studies of our group suggest that MeCP2 positively regulates the protein levels of N-cadherin (Ncad). Ncad is a homophilic cell adhesion protein that maintains the architecture of the developing neocortex and is a key regulator of neocortical neuronal differentiation and migration. We have recently shown that several microRNAs of the miR379-410 cluster including miRNA-543 downregulate Ncad in the developing neocortex of the mouse. This regulation is essential for neurogenesis and neuronal migration.

Because MeCP2 inhibits the biogenesis of microRNAs by binding to DiGeorge syndrome critical region 8 (DGCR8) we hypothesized that it may also be indirectly involved in the posttranscriptional regulation of Ncad. A misregulation of Ncad in the brains of RTT patients could contribute to the pathogenesis of this disease. Indeed, we found Ncad to be downregulated at the protein but not at the RNA level in MeCP2 knockdown P19 and primary cortical neurons. To study the MeCP2 dependent regulation of Ncad during neocortical development in vivo we have established a MeCP2 knockdown in the developing neocortex using the in utero electroporation technique. At the moment we are analyzing the consequences of the MeCP2 knockdown on expression of Ncad and neuronal differentiation and migration.

P-MonoG-235

Mouse model for Molybdenum Cofactor Deficiency type B

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To date, 80 MoCo deficient patients have been diagnosed worldwide. The majority (50%) of the mutations leading to MoCo deficiency have been identified in the MOCS1 gene (MoCo deficiency type A), while 32.5% of mutations have been described in the MOCS2 gene (type B), and only 17.5% have been reported in the Gephyrin (GPHN) gene (type C). Mouse models for type A and type C diseases have already been established (Feng et al., 1998; Lee et al., 2002).

In the present study, the mouse model for MoCo deficiency type B has been developed. Similar to $Mocs1^{+/-}$ (Lee et al., 2002) and $Gphn^{+/-}$ (Feng et al., 1998) mice, heterozygous $Mocs2^{+/-}$ animals are phenotypically normal and fertile. Heterozygous mating lead to the birth of homozygous mutants in expected numbers (~25%). $Mocs2^{-/-}$ pups appear normal at birth and suckle; however, they failed to thrive and died between day of birth and day 12 after birth. $Mocs2^{-/-}$ neonates failed to grow due to an impaired gain of weight and present a poor skin condition and an overall problem with hair growth. All $Mocs2^{-/-}$ pups manifested urinary obstruction associated with a presence of deposits in the bladder and kidney. Additionally, we showed for the first time that the mouse model for MoCo deficiency resembles not only biochemical but also pathological features observed in MoCo-deficient patients, i.e. brain atrophy. Take advantage of created model we are trying to establish gene therapy for MoCo deficiency type B.

P-MonoG-236

Comparison of the DNA damage response in Mcph1-deficient mouse embryonic fibroblasts

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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 MCPH1 gene are the cause of autosomal recessive primary microcephaly (OMIM 251200). This disease condition is associated with a unique cellular phenotype marked by premature chromosome condensation (PCC) in G2 phase and delayed decondensation post mitosis. The MCPH1 gene has 14 Exons and is located on the short arm of

chromosome 8. Its product, microcephalin, is a multifunctional protein that was reported to be involved in brain development, DNA damage response and the regulation of chromosome condensation. Microcephalin contains three BRCT domains, which are predominantly found in proteins that are involved in cell cycle functions responsive to DNA damage.

In this study we investigated mouse embryonic fibroblasts (MEFs) from three different Mcph1 knockout mice. Two were produced by gene targeting strategy, where whole exons were deleted and early stop codons were generated. The third knockout mouse originated from a gene trapping experiment, which only deleted the C-terminal BRCT domain.

We failed to detect a delay in G2 checkpoint release after irradiation in these MEFs, as shown in earlier experiments of our group using patient derived MCPH1-deficient cells. However, we did observe a flatter slope of increase of the mitotic rate of MEFs from the Mcph1 knockout mice compared to control cell lines.

Furthermore, we compared the rates of DNA repair focus formations over a 2 hour period after exposure to hydroxyurea. Analysis of yH2AX, 53BP1, RPA, Rad51 and TopBP1 foci appeared at similar rates and with similar time courses in all investigated cell lines.

Finally, we prepared mitotic chromosomes to compare the levels of PCC. The rate of prophase-like cells (PLCs) in Mcph1-deficient MEFs was increased up to a level between 1.25-5.65%, whereas controls showed PLC rates only between 0.29-0.59%. Interestingly, MEFs from gene trapping knockouts showed much lower PLC rates (1.25%) than the gene targeting knockouts (4.12-5.65%). This cellular feature could be based on a remaining function of the Mcph1 protein lacking only the C-terminal BRCT domain.

Our results suggest that the presence of Mcph1 is necessary for the early DNA damage response after irradiation and that the effect of Mcph1 deficiency is confined to mitosis.

P-MonoG-237

Genome-wide LINKAGE analysis and targeted exome sequencing identifies GNB2 as a novel disease gene in congenital sinoatrial dysfunction

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Purposes. Inherited sinus node disease (SND) is a rare disorder that leads to dyspnoea, syncope and palpitations secondary to bradycardia and sinus arrest. Sometimes, the SND is accompanied by additional conductance disturbances such atrio-ventricular block (AVB) which is an unusual feature. The genetic cause of the disease is mostly unknown.

Methods. In the present study a large family with autosomal dominant SND and AVB (25 family members, 11 affected, 14 unaffected) was clinically evaluated. Using a combined strategy of genome-wide linkage analysis and targeted exome sequencing the disease gene was identified and functional effects were investigated in vitro.

Results. Multipoint linkage analysis identified a 272 genes encompassing region on chromosome 7 (7q21.1-q31.1a) with a maximum lod score of 4.6 at 0% recombination. Targeted exome sequencing revealed a missense mutation in the $ß_2$ subunit of the heterotrimeric G-protein, GNB2, c.155G>T, p.Arg52Leu. The mutation was co-segregating with the disease in the family in an autosomal dominant manner and absent in Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC). Gene expression of GNB2 was shown in human sinus node and atrioventricular node tissue. In heterologous expression systems (Xenopus laevis oocytes and HEK 293 cells) an enhanced activation of the G-protein activated K^+ channel (GIRK) by the expression of mutated $Gß_2$ was demonstrated.

Conclusions. GNB2 is a novel disease gene for sinoatrial dysfunction. The demonstrated sustained activation of the cardiac GIRK channel as the effector site may lead to a hyperpolarization of sinoatrial cells and ,thus, to slowing of the heart rate. Our findings demonstrate for the first time a role of a mutant G-protein subunit in the aetiology of an inherited cardiac arrhythmic disorder.

P-MonoG-238

Homozygosity for a factor XII mutation in one female and one male patient with hereditary angio-oedema

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Hereditary angio-oedema (HAE) with normal C1 inhibitor is associated with heterozygous mutations in the factor XII gene (FXII-HAE). Due to the dominant inheritance and the low population frequency of FXII mutations, patients with FXII-HAE are usually heterozygous mutation carriers. To date, no homozygous cases have been reported. We report two Brazilian FXII-HAE families segregating the mutation c.983 C>A (p.Thr328Lys). In each family, one patient with a homozygous mutation was found. The homozygous female patient in family 1 displayed a severe phenotype. However, this falls within the clinical phenotype spectrum reported for heterozygous female mutation carriers. The homozygous male patient in family 2 also showed a severe phenotype. This finding is intriguing, as to our knowledge, it is the first such report for a male FXII-HAE mutation carrier. In the rare instances in which male mutation carriers are affected, a mild phenotype is typical. The present findings therefore suggest that homozygous FXII-HAE mutation status leads to a severe phenotype in females and males, and to an increased risk of manifest symptoms in the latter.

P-MonoG-239

A familial case of Dopamine-responsive Dystonia with a complicated phenotype

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Dopamine-responsive dystonia (DRD, Segawa's disease) usually begins in childhood showing gait ataxia, local postural tremor and dystonia, later spreading to all limbs. Symptoms commonly worsen with fatigue and exercise. Currently three different types of DRD are known: DYT5a (OMIM# 128230; AD), DYT5b (OMIM #605407, AR), and DRD due to sepiapterin reductase deficiency (OMIM #612716, AR). We saw two brothers (sons of healthy German parents from rural Palatinate) with clinically confirmed generalized dopamine-responsive dystonia. In both of them absence seizures appeared from age 2 years and were treated by anticonvulsive medication. Tremor of both hands and the tongue became visible around age 18 and 15 respectively which worsened continually. At age 31 and 20 years respectively both patients displayed generalized dystonia of the whole body and were hardly able to move or speak. Administration of small dosages of levodopa showed a dramatic improvement of symptoms within several hours. Assessment of the family history revealed that the parents are most probably consanguineous. Metabolic findings of the cerebrospinal fluid in one affected brother pointed to tyrosine hydroxylase deficiency, yet analysis of the TH gene showed no mutation. Subsequent analysis of GCH1 and SPR proved similarly unproductive. As no other affected family members are known we suggest a recessive form of DRD with yet unknown genetic origin. By our methods we cannot rule out regulatory mutations in the three analyzed genes. To identify a possible new form of DRD we will perform whole exome sequencing of the two affected brothers and their parents.

P-MonoG-240

Nexilin deficient mice show a differential regulation of cardiac development transcription factors.

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Introduction: In patients with dilated as well as hypertrophic cardiomyopathy (DCM, HCM) mutations in Nexilin (Nexn) were identified. Nexn deficient mice develop a clear DCM phenotype and strong endocardial

fibroelastosis (EFE) in the left ventricle with progressive cardiomyopathy between day 4 and 6. Interestingly, all Nexn knock out (KO) mice die around postnatal day 8. This restricted time-frame as well as the very specific defects in heart development suggests a key role for nexilin in postnatal cardiac development. Thus we aimed to analyze the role of Nexn in the context of cardiac development transcription factors (TFs) during the initiation and development of DCM and EFE between Nexn KO and wildtype (WT) mice at different postnatal time points.

Methods: Total RNA was isolated from Nexn KO and WT hearts at days 0, 1, 4 and 6. Quantitative PCR were carried out for a panel of cardiac development transcription factors including GATA4, GATA6, Nkx2.5, TBX5, MEF2C, ANP, cTnC, NCX1, SCN, CaV1.2 and also for different Smad genes (1-7).

Results and Discussion: From the 17 tested cardiac TFs 7 TFs were differentially regulated. Interestingly, quantitative analysis revealed an 2.5– 3 fold up-regulation of the transcription factors Nkx 2.5, Tbx5, Mef2c, Smad 2 and 3 early before the initiation of DCM and EFE at day 0 and 1 postnatal in the KO mice compared to WT. Later on and at day 6 more than a 10 fold up-regulation of the transcription factor GATA6 and a 2 fold down regulation of GATA4 was observed. Previous studies could show that Nkx 2.5 and GATA4 interact with each other and together are responsible for the activation of other cardiac genes such as MHC, MEF-2 and MLC2v. Our data demonstrated also an up regulation of Mef2c. A link between the TF Nkx 2.5 and Nexn, which binds downstream of the Nexn transcriptional start site was previously reported. Based on the previous observation and our own finding we suggest a critical role of nexilin in the regulation of cardiac developmental TFs. We could also show that there is an up regulation of Smad 2 and 3 genes. Alteration in the expression of these factors is always related to deleterious development of cardiac failure and thus may explain the DCM and EFE phenotype observed in the Nexn deficient mice.

Conclusion and outlook: Based on our data Nexn deficiency leads to the dysregulation of the expression of several TFs, which are involved in cardiac development before the development of DCM and EFE. Thus it seems that Nexn plays an important role in early postnatal cardiac development by activating cardiac development TFs. Further interaction analyses are needed to confirm these results at protein level.

P-MonoG-241

Stapes ankylosis with broad thumbs and toes in a mother and daugther carrying a deletion of NOG

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The human *NOG* gene encodes for Noggin, a small peptide that is secreted as a homodimer and acts as an extracellular antagonist of bone morphogenic protein (BMP) signaling. Noggin is important for correct joint formation during embryonic development, and heterozygous mutations in *NOG* have been found to be causative of five different, but overlapping disorders: brachydactyly type 2 (BDB2; MIM#611377), Tarsal-carpal coalition syndrome (TCC; MIM# 186570), proximal symphalangism 1A (SYM1; MIM# 185800), stapes ankylosis with broad thumb and toes (SABTT; MIM# 184460) and multiple synostoses syndrome 1 (SYNS1; MIM# 186500). Due to the partly overlapping features and the variable phenotypic spectrum (both inter- and intrafamilial), it has recently been suggested to summarize all of these disorders under the collective term *NOG*-related-symphalangism spectrum disorder (*NOG*-SSD).

We report on a mother and daughter, both presenting with hearing defects, severe hyperopia, short fingers, broad thumbs and a characteristic facial gestalt including prognathism, a flat nasal bridge, a hemicylindric nose and a flat, short philtrum. The girl additionally had mild syndactyly of fingers II to V and mild developmental delay. In the mother, stapedectomy had been performed, and she had ptosis. The clinical presentation perfectly matched the phenotype of stapes ankyloses with broad thumbs and toes or Teunissen-Cremers syndrome. While sequencing of the *NOG* gene revealed no abnormalities, fluorescence in situ hybridization detected a deletion of *NOG* in both mother and daughter. Subsequent microarray analysis in the girl defined the deletion to a size of 358 kb containing *NOG* and two further genes (*C17orf67*, *DGKE*).

Apart from missense and truncating mutations within *NOG*, several deletions of the gene have been reported previously. However, they were of larger size, frequently affecting several other genes and being associated with additional phenotypes. The deletion observed in our patient is, to our knowledge, the smallest one identified so far and only includes two further genes. We therefore expand the clinical and mutational spectrum of *NOG*-related disorders.

P-MonoG-242

Identification of type 1 NF1 deletion breakpoints located between the NAHR hotspots PRS1 and PRS2

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Neurofibromatosis type 1 (NF1) is a hereditary cancer syndrome which occurs with an incidence of 1 in 3000. In 5 % of all patients with NF1, large deletions encompassing the *NF1* gene and its flanking regions at 17q11.2 are the cause of the disease. The majority of large *NF1* deletions are of type 1, they encompass 1.4-Mb and are caused by nonallelic homologous recombination (NAHR). The breakpoints of type 1 deletions are located in the low-copy repeats NF1-REPa and NF1-REPc, which exhibit high sequence homology to one another and are composed of different segmental duplicons. Previous studies indicated that type 1 *NF1* deletion breakpoints cluster within the paralogous recombination sites PRS1 and PRS2 which represent NAHR hotspots. PRS1 and PRS2 harbour the strand exchange regions (SERs) of all NAHR-mediated type 1 deletions reported so far. These SERs span 20 to 700-bp and are flanked by paralogous sequence variants (PSVs). PSVs are non-polymorphic nucleotides that differ between NF1-REPa and NF1-REPc. The NAHR-associated crossovers are located within the SERs and the proximal boundaries of the SERs demarcate the locations of the double Holliday junction resolution.

Multiplex Ligation-dependent Probe Amplification (MLPA) is a screening technique frequently used to detect type 1 *NF1* deletions. However, using this technique as a sole method of analysis, it is not possible to determine the precise deletion breakpoints due to the wide spacing of the MLPA probes. To identify the breakpoints and to localize the SERs, it is necessary to perform breakpoint-spanning PCRs with paralog-specific primers. Previous analyses of breakpoint-spanning products amplified from 118 patients with type 1 deletions, initially identified by MLPA, indicated that 80 (68%) of the SERs are located in PRS2 and 19 (16%) in PRS1. However, 19 type 1 *NF1* deletions did not exhibit SERs in PRS1 or PRS2.

The aim of this study presented here is to determine the breakpoint location of these 19 type 1 *NF1* deletions not mediated by NAHR within PRS1 or PRS2. Array CGH analysis suggested that several of these breakpoints are located within the 20-kb region separating PRS1 from PRS2. Four overlapping long-range PCRs with paralog-specific primers were established to identify the respective deletion breakpoints and to determine the precise location of the SERs. Preliminary results suggest that the majority of these unknown SERs are indeed located between PRS1 and PRS2 in close proximity to specific PRDM9 DNA-binding motifs. PRDM9 is an important regulator of recombination initiation and known to influence recombination hotspot location. Our findings imply that not only PRS1 and PRS2-mediated type 1 deletions but also those type 1 deletions with breakpoints located outside the recombination hotspots PRS1 and PRS2 are caused by PRMD9-regulated NAHR.

P-MonoG-243

Next-Generation Sequencing in the Molecular Diagnostics of Rare Diseases using a Gene Panel Approach

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The implementation of Next-Generation Sequencing in a clinical diagnostic setting opens vast opportunities through the ability to simultaneously sequence all genes contributing to a certain indication at a cost and speed that is superior to traditional sequencing approaches. Especially in the case of rare, heterogeneous disorders this may lead to a significant improvement in diagnostic yield. On the other hand, the practical implementation of NGS in a clinical diagnostic setting involves a variety of new challenges which need to be overcome. Among these are the generation, analysis and storage of unprecedented amounts of data, strict control of sequencing performance, validation of results, interpretation of detected variants and reporting.

We present a panel approach for the molecular diagnostics of rare, monogenic disorders. Exonic regions of more than 1200 custom selected genes are enriched in parallel by oligonucleotide hybridization and capture (Agilent QXT) and sequenced on the Illumina NextSeq500 platform. During analysis, only genes from the requested indication (organized in subpanels) are selected to limit analysis to relevant genes, while simultaneously minimizing the possibility of incidental findings. Data analysis is performed using the CLC Genomics Workbench (v.8.0.5, CLCbio / Quiagen) and custom developed software tools. Target regions which fail to reach the designated coverage threshold of 20X are re-analyzed by Sanger sequencing.

Additionally, identified candidate mutations are independently confirmed. All detected variants are imported into an in-house relational database scheme (MIDAS-database) which may be queried via a web interface for dynamic data analysis and filtering. Information from all 1200 genes is used in an anonymized way for internal variant frequency calculation, quality control and the detection of potential sequencing artifacts.

We have applied this approach to more than 450 samples from a variety of different disorders. In particular we use the outlined approach for the diagnostics of arrythmogenic cardiac disorders (LQTS, HCM, DCM), connective tissue disorders (EDS, TAAD), rare kidney disorders (Nephrotic Syndrome, CAKUT), hearing loss, RASopathies and metabolic disorders (MODY diabetes).

P-MonoG-244

Re-examination of reference ranges in molecular genetic analysis of repeat lengths for SCA 17

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Spinocerebellar ataxia type 17, a rare neurodegenerative disorder, is caused by a trinucleotide CAG/CAA repeat expansion in the TATA box-binding protein (TBP) gene, resulting in a polyglutamine expansion in the TBP protein. SCA17 patients share 49-66 repeats, and reduced penetrance is observed in individuals with 43-48 alleles as defined in the best practice guidelines for molecular genetic testing of spinocerebellar ataxias (Sequeiros, 2010). However, currently the repeat reference range is a matter of debate. Reports of clearly symptomatic patients with 41 and 42 repeats were recently published, so that even shorter repeats might account for SCA17 (Nanda, 2007; Nolte, 2010). Recently, it was reported that a 60-year-old Caucasian patient presented with marked hypointensities of basal ganglia and cerebellar atrophy reminiscent of neurodegeneration with brain iron accumulation (NBIA) and 44 CAG/CAA trinucleotide repeats in the TBP gene within the SCA17 repeat range, that is associated with reduced penetrance (Claassen, 2015). Because of neuroimaging and clinical features presented in this case, we screened a cohort of 34 adult patients clinically diagnosed with NBIA for repeat expansions in the TBP gene. Out of 34 patients, three NBIA patients fell into the range just below the threshold (one patient with 41 repeats, two patients with 42 repeats). Interestingly, revisiting an internal registry of patients with suspicion of SCA, we identified five individuals exhibiting 41 or 42 repeats in the TBP gene. The normal allele distribution in our patient group ranged from 27 to 42 repeats. The majority of individuals exhibited <39 repeats, according to earlier reports, where almost all normal TBP alleles comprise <39 repeats, including Caucasians (Koide, 1999; Silveira, 2002). Borderline expansions of the CAG/CAA block within TBP were reported earlier in family members as well as unrelated patients exhibiting a relatively benign phenotype and mild ataxia (Nolte, 2010). Thus, SCA repeat expansions near the reduced penetrance range (41 or 42 trinucleotide repeats) might account for mild forms of late onset ataxia and/or share features characteristic for NBIA. Therefore, careful evaluation of molecular genetic findings and the definition of the threshold in molecular genetic diagnostics of SCA 17 should be discussed, including an “uncertainty” or “reduced penetrance” range in the classification of allele lengths.

P-MonoG-245

Genetic determinants regulating early signs of atherosclerosis in different wild type strains of zebrafish (Danio rerio) using QTL analysis

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Zebrafish (Danio rerio) has been established as a brilliant model to study dyslipidemia or atherosclerosis due to the remarkable similarities with humans in lipid and lipoprotein metabolism. In contrast to mice, zebrafish show lipoprotein oxidation and dyslipidemia after feeding with a high cholesterol diet for as short as 15 days’ time period, even without any genetic intervention. This makes the zebrafish an attractive model for atherosclerosis research, along with its transparency for the first month of development, cost effective, fast breeding and ease in genetic manipulation.

Here we tested whether the genetic background from different Zebrafish strains affects lipid deposits after feeding a high cholesterol diet.

We tested five different wild type strains of zebrafish i.e. AB, TU, TL, WIK and LEO. Interestingly, after feeding a high cholesterol diet (standard zebrafish diet plus 4% cholesterol) we observed a strong variability in lipid accumulation among the tested strains. Our results demonstrate that AB strain was most susceptible

to lipid accumulation while TU strain was most resistant. TL, WIK and LEO strains demonstrated a moderate phenotype in terms of lipid deposits.

Now, we claim to identify genetic loci controlling this variability in lipid deposits using the classical e-QTL-analysis. For this reason F2 generation from the most susceptible and resistant strain i.e. AB and TU respectively is in progress. In addition, we will put new insights into the possible pathways leading to the observed lipid deposits variation between AB and TU strains on gene expression level by conducting gene expression profiling.

Identification of genetic loci by QTL-analysis controlling lipid accumulation and leading to an almost resistant phenotype for lipid accumulation in zebrafish might have important implications for humans as well. Lipid accumulation is considered as an early step initiating atherosclerosis. Thus our results will contribute considerably to advance the identification of novel targets for dyslipidaemia and therapeutic objectives for atherosclerosis.

P-MonoG-246

Gain-of-function mutation leads to hyperactive Na^+-channel and results in cold-aggravated pain

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Heterozygous gain-of-function mutations in the human *SCN11A* gene, encoding for the voltage-gated Na^+ channel $Na_V1.9$, lead to hyperactive $Na_V1.9$ channels. Surprisingly, the spectrum of the respective phenotypes ranges from congenital pain insensitivity on the one hand to severe on the other hand.

Applying whole-exome sequencing we here identified a yet unreported missense change (p.V1184A) in $Na_V1.9$, which leads to cold-aggravated peripheral episodic pain in a large family of Irish/Scottish descent. Electrophysiological analysis showed that p.V1184A shifts the voltage dependence of channel opening to hyperpolarized potentials thereby presenting gain-of-function characteristics. Mutated channels diminish the resting membrane potential of mouse primary sensory neurons and cause cold-resistant hyperexcitability of nociceptors, suggesting a mechanistic basis for the temperature dependence of the pain phenotype. On the basis of direct comparison of the mutations linked to either cold-aggravated pain or pain insensitivity, we propose a model in which the physiological consequence of a mutation, that is, augmented versus absent pain, is critically dependent on the type of $Na_V1.9$ hyperactivity.

P-MonoG-247

Identification of mutations in retinal dystrophies using a MIP-based targeted resequencing 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 non-syndromic RD can be attributed to mutations in more than 125 genes. Consequently, next generation sequencing (NGS) technologies are among the most promising approaches to identify mutations in RD. Molecular inversion probes (MIPs) have shown to be a cost-effective enrichment technology, particularly if used in large sample cohorts. We have developed a workflow of sequencing 108 genes associated with various forms of non-syndromic retinal degeneration using MIPs in combination with Illumina NextSeq 500 sequencing. Using this approach, we screened 250 probands with different forms of RD (autosomal recessive Retinitis Pigmentosa [n=110]), sporadic Retinitis Pigmentosa [n=98], cone-rod dystrophy [n=28], cone dystrophy [n=7], and Leber congenital amaurosis [n=7]).

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 total, we detected

mutations in 32 different RD genes. A total of 129 distinct mutations were identified in our study; 67 of them had not previously been reported. Mutations explaining the disease phenotype were identified in 95 (38%) cases. In the remaining 155 probands, we did not detect sequence alterations that explain the disease phenotype. Among these, we found 40 autosomal recessive cases that carry sequence alterations in one of the frequently affected genes (e.g. USH2A, EYS 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 probands harbor mutations in yet unknown disease-genes.

To summarize, we used a MIP-based targeted resequencing approach in a cohort of 250 patients with different forms of RD and were able to identify causative mutations in 38% of cases. The fact that more than half of the mutations we identified were novel clearly demonstrates the advantage of a panel-based sequencing approach over a mutation array: if a mutation has not been described at the time of its design, it will escape detection.

P-MonoG-248

A Homozygous Mutation in the Desert Hedgehog Gene in Two 46,XY Sisters associated with Partial Gonadal Dysgenesis and Late Onset Polyneuropathy

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Background: 46,XY disorders of sex development (DSD) comprise a heterogeneous group of congenital conditions. Mutations in a panel of genes can affect gonadal development, androgen biosynthesis or androgen action and thereby influence the development of the internal and external genital organs.

Objective: To elucidate the genetic cause of 46,XY DSD in two sisters of a consanguineous family

Methods: Mutation screening was performed by whole exome sequencing (WES). Associated signs of polyneuropathy were studied by electrophysiological and high resolution ultrasound examinations. Histopathological examination of the gonads was performed.

Results: We identified a novel homozygous R124Q mutation in the Desert Hedgehog gene (DHH). Residue R124 is conserved among the three mammalian hedgehog ligands SHh, IHh and DHh and lies in a conserved overlapping binding region and makes contacts to hedgehog co-receptors Cell adhesion molecule, down-regulated by oncogenes (CDO) and Brother of CDO (BOC) and the hedgehog interacting protein (HHIP). The elder sister developed a seminoma while the gonads of the younger sister showed partial gonadal dysgenesis with loss of Leydig cells in tubular areas with seminoma in situ. Immunohistological analysis showed a hyperplasia of Leydig-cell like cells expressing CYP17A1 in more dysgenetic parts of the gonad. In adulthood, both sisters developed a glove and stocking like polyneuropathy. Electrophysiological analysis revealed reduced compound muscle unit potentials, reduced motor nerve conduction velocity and reduced or absent sensory nerve action potentials. High resolution ultrasound revealed an abnormal echo texture of the peripheral nerves.

Conclusion: Mutations in DHH can lead to 46,XY gonadal dysgenesis and are associated with the development of germ cell cancer. Gonadal dysgenesis in these cases may be due to impairment of Sertoli cell – Leydig cell interaction during gonadal development. These are the second and third cases of homozygous mutations in DHH leading to partial gonadal dysgenesis that are additionally associated with a polyneuropathy. Like in the first described case development of the polyneuropathy with minifascicle formation occurred in adulthood and points to a role of DHh signalling in maintenance of the structural and functional integrity of the peripheral nerves in humans, as suggested by mouse models.

P-Techno-249

Improved bias correction for gene-set analysis in genome-wide methylation data applied to genetically determined CpG sites in human hippocampus

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INTRODUCTION

DNA methylation microarrays including Illuminas Methylation 450K array are a popular approach to studying the role of epigenetics across different phenotypes. Several methods have been developed to identify both single CpG sites and regions associated with a trait of interest. The application of gene-set analysis is a major component of data analysis and typical applications include pathway analysis to identify enriched gene-sets within a set of differentially methylated genes. It has been shown that gene-set analysis is severely biased as a result of differences in the numbers of CpG sites associated with different classes of genes and gene promoters (Geeleher et al. 2013). Here we propose an improved method to correct for this bias and applied it to identify enriched gene-sets in genetically determined CpG sites in human hippocampal tissue.

METHODS

For methylation gene-set enrichment analysis, we implemented a novel method to correct for the number of uncorrelated CpG sites per gene. The correction was done to avoid a potential bias as genes with a higher number of CpG sites are more likely to be associated and subsequently, gene sets containing a disproportional number of these genes are more likely to appear significantly enriched. To correct for this bias, our method sets the number of non-correlated CpG sites per gene based on the methylation profile ($r^2<0.8$) as the value of each gene's confounding variable to control for different a priori probabilities of each gene to appear in the foreground list of differentially methylated genes. The Bioconductor 'GOseq' package was then used to model the relationship between the number of independent CpG sites per gene and its probability to be associated to genetic variance. We applied our approach to identify enriched pathways in genetically determined CpG sites (methylation quantitative trait loci, meQTL) in 115 human hippocampal samples.

RESULTS

In total, 18,547 CpG sites showed a significant association to SNP genotypes located within ± 1 Mb around each CpG sites. While the mean number of CpGs in genes without a meQTL was 9, the number of CpGs within meQTL genes was significantly higher (mean 21). KEGG and GO pathway analysis using our novel correction identified 283 enriched pathways after correction for multiple testing for all genetically determined CpG. Enriched pathways include MAPK and Calcium signalling (KEGG) as well as calcium ion transmembrane transport and neuron development (GO) as well as an enrichment for a set of brain-disorder GWAS genes.

CONCLUSION

With our improved method for gene-set analysis in genome-wide methylation analysis, a balance between the correction for different numbers of CpGs per gene and correlated CpG sites is achieved and novel biological insights about the role of genetically determined CpG sites in human hippocampus is provided.

P-Techno-250

Integrative approach for quantitative trait loci mapping in human monocyte transcriptome and proteome

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Background

GWAS have detected multiple loci associated with different phenotypes. A subsequent functional annotation is necessary, and mapping expression quantitative trait loci (eQTL) has already provided a rich source of relevant information. In contrast, much less work has examined variation in protein sequence and abundance, and the genetic basis of proteomic variation remains largely unexplored.

An integrative analysis of expression and protein quantitative trait loci in human monocytes, perturbed with an inflammatory stimulus, has never been performed before and offers important new insight into the intracellular mechanisms involved in immune response.

Methods

Human monocytes are treated with lipopolysaccharide (LPS). RNA and proteins are extracted and currently investigated via microarray analysis and mass spectrometry. DNA will be isolated and used for genotyping of donors. QTLs will finally be mapped through integration of genotype and the quantitative trait, i.e. gene expression and mass spectrometry data, respectively.

Results

The objective of this project is to quantify protein abundance upon LPS stimulation and to characterize genetic variation that influence protein abundance under baseline and stimulation. In a first assessment, differentially expressed proteins will be identified and compared to genes measured in a large cohort (Kim et al. 2014). Finally, eQTLs and pQTLs will be mapped using genotypes, gene expression and relative protein quantification.

Conclusions

This project will produce the first large-scale characterization of the genetic basis of complex traits on the cellular immune response and provide a valuable resource for the interpretation of GWAS hits in complex traits. Furthermore, human monocytes in an inflammatory state are a suitable model system for the inflammatory response and allow the discovery of genetically determined immune processes.

P-Techno-251

Gene expression and electrophysiological profiling of single dopaminergic neurons

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According to array tomography, the human Central Nervous System (CNS) contains an average of 86 billion neurons and roughly the same number of non-neuronal cells. Neurodegenerative diseases like Alzheimer's and Parkinson's disease as well as autism spectrum disorders arise as a consequence of specific neuron subtype dysfunction. CNS neurons can be grouped into hundreds of different subtypes depending on their location, connectivity, neurotransmitter identity, passive and active electrophysiological properties and molecular markers. Such cell-specific information is diluted when pooling groups of neurons. It is therefore of major clinical interest to relate functional aspects of neuronal subtypes to an unbiased strategy that would allow an objective classification founded on unique transcriptional states.

We received electrophysiological profiles of murine dopaminergic (DA-) neurons from the ventral tegmental area (VTA) by patch clamp recordings. Given that DA VTA neurons are pacemakers i.e. generators of cell autonomous rhythmic activity, their integrated intrinsic excitability can be easily assessed by electrophysiology in in vitro brain slice preparations. Following electrophysiology recording from an individual neuron, total RNA was extracted by aspirating the cellular contents into a fine glass electrode tip and then transferred to a PCR tube. cDNA synthesis was performed with the CelluLyser™ Lysis and cDNA Synthesis Kit from TATAA-Biocenter according to the manufacturer's instructions. Preamplification was performed for 5 subtype specific neuronal markers and 3 target genes in a Multiplex-PCR approach with an exon-bridging RT-PCR assay design. Enzymatic PCR cleanup was followed by singleplex SYBRgreen based qPCR.

Molecular markers that are specific for DA neurons like Tyrosine hydroxylase (*TH*) and solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 (*Slc17a6*) could be reliably quantified from single DA neurons. In contrast, markers for glia cells that represent a potential source of contamination could not be detected in our setting. Glial fibrillary acidic protein (*Gfap*) was absent in all analyzed single DA neuron preparations. Furthermore we included a "mock patch clamp" sample to model the negative control as closely as possible and found no amplification at all.

We could clearly demonstrate the feasibility to combine both, electrophysiological and gene expression profiling of single DA neurons. We will continue our efforts towards a protocol that allows a comprehensive transcriptional profiling by single cell RNAseq.

P-Techno-252

Establishing a custom-designed "in house" MPSeq-Panel: technical and consent considerations

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Introduction

Massive-parallel sequencing (MPSeq) technology is replacing traditional technologies for the diagnosis of genetic disorders as the sensitivity, speed, and reduced cost make is superior to other sequencing modalities. Panel sequencing approaches that cover a limited number of genes are less expensive than exome sequencing approaches. However, in a regional medical genetics center which offers tests for a large number of genes with relatively infrequent occurrence of individual indications, analyzing single small genes by Sanger sequencing and/or MLPA may be more cost-efficient. In order to optimize speed and costs we introduced a custom-designed "in house" sequencing panel targeting genes from a large range of indications. The analysis pipeline was adapted for genes relevant for local indications and the validity of informed consent procedures.

Method

Approximately 100 genes were selected for a customized panel based on the indications normally requested in our molecular genetic laboratory (e.g. Charcot-Marie-Tooth disease types, inflammatory diseases, glycogen storage diseases, disorders in copper- and fatty acids metabolism). Target exonic regions and adjacent exon-flanking regions (±30 bp) are enriched from genomic DNA by Nextera® Rapid Capture (Illumina) and underwent massively parallel sequencing (MiSeq, Illumina). Sequences are aligned to the human reference sequence GRCh37 (HG19), and data analysis (qualitative and quantitative) is performed with NextGENe® and Geneticist AssistantTM software. In order to limit the investigation to consented indications, data analysis is restricted to regions of interests (ROIs) defined in individual bed.files. All relevant variants are confirmed by Sanger sequencing or MLPA. For validation of our custom-designed "in house" panel, Sanger sequencing and MLPA were performed in parallel.

Results

There was complete concordance of the results from MPSeq analysis and Sanger-Sequencing as all gene variants detected were recognized by both methods. In addition, MPSeq provided reliable quantitative information about deletions/duplications through the CNV tool, confirmed by MLPA. The selected targets had an average coverage of 200 and a minimum coverage of 30 was reached for all target regions.

Discussion

Use of the "in-house" MPSeq panel on a weekly basis allows cost-efficient diagnostic analyses with short reporting times for at least 12 to 24 patients with a wide range of indications in a regional laboratory. Restricting the data analysis to specific ROIs avoids the need to obtain extensive informed consent and reduces the likelihood of secondary (incidental) findings to a minimum. This strategy was agreed by the regulating Austrian institution at the Ministry of Health (formal legal implementation pending). With the option of revising the included genes on reordering panels, the approach is well suited to the needs of regional genetics centers that cover a wide range of indications for their communities.

P-Techno-253

QuantSeq 3' mRNA sequencing for fast and efficient gene expression quantification

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With the rapid development of NGS technologies, RNA-Seq has become the new standard for transcriptome analysis. Although the price per base has been substantially reduced, sample preparation, sequencing, and data processing are major cost factors in high-throughput screenings. The QuantSeq kit

provides an easy protocol to generate highly strand-specific NGS libraries close to the 3' end of polyadenylated RNAs within 4.5 h, requiring only 0.1–500 ng of total RNA input.

To evaluate differential gene expression with QuantSeq, FDA's Sequencing Quality Control (SEQC) standard samples A and B were used. The correct detection of differential gene expression (true-positive versus false-positive) as assessed on ERCC level showed better performance for QuantSeq than for standard RNA-Seq. The numbers of ERCC transcripts detected stayed constant, even when down-sampling from 10 M to 0.625 M reads. Comparing the coverages of QuantSeq and standard RNA-Seq reveals a possible 12-fold reduction in read depth due to the fact that QuantSeq only covers the 3' end of transcripts. Therefore, a higher degree of multiplexing is possible.

With a strand specificity of >99.9 %, antisense transcripts and overlapping genes can also be quantified. The input-output Spearman correlation of 0.973 and 0.986 for ERCC mix 1 and mix 2 emphasizes QuantSeq's very high gene count accuracy. Data analysis is simplified since no junction detection is necessary and transcript abundances are given by read counts. 6 QuantSeq data sets were aligned in only 35 min using Bowtie2 while the corresponding RNA-Seq alignment took 2 h 50 min requiring TopHat2. Therefore, QuantSeq is the method of choice for fast, affordable and accurate gene expression detection and quantification.

P-Techno-254

Implementation of Molecular Inversion Probes for routine diagnostics of BRCA1 and BRCA2

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Despite the great success of exome sequencing, there is still a need for improved (NGS-based) individual gene testing. Molecular inversion probes (MIPs) have shown to be a cost-effective enrichment technology, particularly if used in large sample cohorts. Single molecule (sm) tags thereby further enhance data quality, by removal of duplicate reads thereby constructing consensus sequences free of experimental artifacts. Single molecule molecular inversion probes (smMIPs) therefore proof excellent for usage in highly sensitive tests, such as breast cancer genetic testing. We have developed a workflow for re-sequencing BRCA1, BRCA2 and CHEK2 c.1100del (p.(Thr367fs)) using single molecule MIPs (smMIP) in combination with NextSeq500 sequencing. 527 overlapping smMIPs on both strands were designed, with every base being targeted by at least two independent smMIPs. More than 150 BRCA1 and BRCA2 mutation positive cases were sequenced for both genes with an average coverage of >130-fold (unique reads), yielding in 100% coverage of all targeted bases. Analysis of known pathogenic mutations and SNPs in 152 samples resulted in an analytical sensitivity and specificity of 100%, with no false positives and no false negatives. Based on manual protocols that produced these data, the workflow was completely automated. Next to automation of pre- and posthybridization pipetting, also file handling, data transfer and analysis of sequencing data was automated, delivering fast sequencing data of highest quality. In addition, our setup is such that each analysis is performed in duplicate to make the analysis more robust and to have an independent confirmation of mutation positive cases in the same run. Sequencing in duplicate leads to less loops in the workflow, to a reduction of rework, and accordingly reduces turnaround times. In conclusion, genetic testing of breast cancer susceptibility genes BRCA1 and BRCA2 works exceptionally well using smMIPs. Although ordering costs of smMIPs are relatively high, probes can be highly multiplexed, allowing time- and cost-efficient sample preparation. We have successfully replaced our former amplicon based diagnostic NGS-test for germline and formalin fixed paraffin embedded BRCA1 and BRCA2 testing. Together with automation of both library preparation and data analysis, this resulted in a reduction of 10-15 days in turnaround times which is now stable at ~10-12 days. Implementation of additional genes is ongoing.

P-Techno-255

Evaluation of the evenness score in next generation sequencing

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The evenness score (E) has been developed in the field of next generation nucleic acid sequencing (NGS) to quantify the homogeneity in coverage by sequencing reads of the NGS targets. As such it is a measure of the width of a distribution. Here, I first clarify the mathematical description of E which is 1 minus the integral from 0 to 1 over the cumulative distribution function F(x) of the normalized coverage x, where normalization means division by the mean. Then, I derive a computationally more efficient formula, i.e., 1 minus the integral from 0 to 1 over the probability density distribution f(x) times 1-x. An analogous version of

that formula for empirical coverage data is provided, as well as simple R command line scripts implementing both versions. The new formula allows for a general comparison of E with the standard deviation σ which is the conventional measure of the width of a distribution. For symmetrical distributions including the Gaussian distribution, E can be predicted closely as $1 - \sigma/2 \geq E \geq 1 - \sigma/2$ with $\sigma \leq 1$ due to the normalization. Without capturing, NGS coverage is distributed symmetrically about the mean whereas capturing might cause substantial positive skewness of the coverage. As a typical representative of positively skewed data, I therefore also analyzed the log-normal distribution, yielding $E \approx \exp(-\sigma^*/2)$ with $\sigma^{*2} = \ln(\sigma^2 + 1)$ for small to medium sized σ, and $E \approx 1 - F(\exp(-1), \sigma)$ for large σ. In the latter kind of rather uneven coverage, E can provide direct information on the fraction of well covered targets that is not immediately delivered by σ. Otherwise, E does not appear to have major advantages over the conventional σ or over a simple score exp(-σ) based on it.

P-Techno-256

Spike-In RNA Variants (SIRVs): External transcript isoform controls in RNA-Seq

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RNA spike-in controls are currently applied to assess sensitivity, input / output correlation, differential gene expression, etc. in RNA-Seq experiments. These control RNAs are monoexonic and do not represent transcript isoforms. However, the vast majority of genes in higher eukaryotes undergo alternative splicing, and transcript isoforms are present in concentrations spanning several orders of magnitude. To address this added complexity of transcriptomes, we have designed Spike-In RNA Variants (SIRVs) for the quantification of mRNA isoforms in Next Generation Sequencing (NGS). The initial consideration was to produce sets of transcripts which are variants of a given gene to provide for the training and evaluation of bioinformatics algorithms to accurately quantify, map and assemble isoforms. In detail, we have developed 7 transcript variant sets, based on human gene structures but with artificial sequences. For each of the genes, 6-18 transcript variants were derived either from known, annotated isoforms or additionally designed to comprehensively address alternative splicing, alternative transcription start and end sites, overlapping genes and antisense transcription. The SIRVs are designed to mimic human transcripts closely in terms of length (190-2500 nt) and GC content (30-51%), and the GT-AG exon-intron junction rule was observed. They do not show significant sequence similarities to any sequenced genome or transcriptome when searched against the NCBI database. Therefore, they can be spiked into total RNA from any sequenced organism – also alongside existing ERCC spike-in mixes – and are unambiguously identifiable in the resulting mRNA-Seq NGS data. In an initial experiment, the SIRVs were NGS-sequenced on their own or used as external standard by spiking reference RNAs. The ability of current and newly developed algorithms to identify these known input and to quantify transcript isoforms – also in dilutions – will be presented and discussed.

P-Techno-257

Optimization and evaluation of differential methylation analyses for RRBS data

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Background:

The transcriptional landscape is coordinated by the underlying genetic and epigenetic backbone. Among epigenetic regulatory mechanisms, DNA methylation is certainly one of the key modifications. Aberrant CpG methylation patterns in the expression-regulating promotor regions are associated to several diseases, e.g. Alzheimer's disease but also many types of cancer. Reduced Representation Bisulfite Sequencing (RRBS) is a NGS-based technique profiling DNA methylation on a genome wide scale, while the MspI-based enrichment for CpG sites achieves a high cost-efficiency. Although, there is a wide range of analysis methods for bisulfite sequencing data available, so far no golden standard has been established.

Results:

We extracted, isolated and purified neuronal and non-neuronal cells from three human and three chimpanzee prefrontal cortices and generated methylation profiles via RRBS. To exemplify the hurdles and strategies of the differential methylation analyses, we analyzed these datasets as well as several published datasets of different cell types and multiple species. We optimized the RRBS data analysis and evaluated the results after each step: (1) read mapping, (2) quantification of site-specific methylation levels, (3) identification of region methylation and (4) subsequent statistical testing for differential methylation. The

effects of several experimental parameters, i.e. read length, sequencing depth and cell separation could be identified via comparison of simulated as well as multiple biological RRBS datasets.

Conclusion:

Here we present a novel approach for RRBS data analysis to produce more robust results and hereby gather meaningful biological and medical insights.

P-Techno-258

GenCor - a tool for quality control of exome sequencing data through incorporation of microarray-based genotyping

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Background

Whole exome sequencing (WES) describes the targeted sequencing of the protein-coding regions of the human genome. It is now routinely used in human genetic studies and has proven to be a powerful and cost-efficient method for the identification of rare variants. Many bioinformatics pipelines have been developed for variant calling in WES data. The performance of these pipelines depends on the applied algorithms as well as the implemented filter strategies. Given the complexity of large scale sequencing projects sample mix-ups can also interfere with the final results. Here we present GenCor, a tool for validation of WES variant calling pipelines as well as improved quality control by incorporation of array-based genotyping data.

Methods

For assessing the quality of WES data and variant filtering, GenCor calculates the genotype concordance between WES called variants and array-based genotypes for variants located within exons. The concordance is defined as the percentage of identical genotypes of the total number of compared genotypes. GenCor extracts called variants from the respective VCF file on which different quality filters based on GATK best practices were applied, it then looks for regional overlap of variants. We assessed the quality of WES data from five human DNA samples which were sequenced on an Illumina HiSeq2500. Target enrichment was performed with the Agilent SureSelect Human All Exon V5 kit. Variant calling was performed using a standard BWA/GATK (Unified Genotyper) pipeline following the recommended best practices. The same set of samples was processed on Illumina's Infinium CoreExome-24 beadarray that comprises >269,000 exonic markers. The applied filters (read depth>10, mapping quality>60, genotype quality>20) showed a maximum number of overlap between array genotypes and WES variants. GenCor can also be applied for sample quality control to exclude sample mix-ups and low quality samples during preprocessing and can be applied to estimate samples gender as well as ethnicity by identity by state (IBS) analysis.

Results

In our example data set, no sample mix-up in WES preprocessing could be identified. Array-based IBS calculation defined each samples ethnicity and gender without any relationship inference. For variant quality control, an average agreement of 10,135 variants per sample between WES variants passing filter criteria and SNP array genotype was observed. In total, 99% concordance of WES variants and array-based genotypes were found for the applied filter criteria.

Conclusion

As SNP microarrays remain the gold standard for genotyping the GenCor tool can be used for quality control assessment of next generation sequencing projects and bioinformatics pipelines. We recommend the incorporation of SNP genotyping for improved quality control in WES projects. The GenCor pipeline can also be applied to whole genome sequencing and is in principle compatible with genotype data from other genotyping platforms.

P-Techno-259

Identification of Stem Cells marker genes using an unbiased data-driven approach

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Stem cells promise a precious cell source for the treatment of various degenerative diseases due to their capacity for self-renewal and ability to differentiate along multiple cell lineages. Gene expression analysis, both genome-wide and targeted at specific gene subsets, has played a key role in improving our understanding of the genetic attributes of stem cells through identification of molecular signatures that characterize normal stem cell function.

In this study, our goal has been to identify stem cell specific marker genes with an unbiased data-driven approach. We used the “Genevestigator” curated database gene expression platform to screen a compendium of 26,982 profiled samples on the Affymetrix Human 133 Plus2 chip to identify genes specifically expressed in embryonic stem cells as compared to over 1400 other tissues (including primary cells), cell types and cell lines.

Removing redundant transcripts yielded a list of 46 unique transcripts, of which several are well known in stem cell research (e.g. ESRG) but about half of the genes identified have not yet been associated with stem cell development or pluripotency in the literature, and about one fourth has not yet been characterized at all. And thus provided a short list of potentially novel stem cell specific marker genes to become further investigated.

From the 3032 experimental conditions tested, less than 100 caused significant changes of expression in any of these stem cell specific genes. To identify patterns of co-regulation, we created a new data matrix containing only conditions that significantly regulate the 46 genes and ran various bi-clustering and hierarchical clustering analyses. Interestingly, genes from one cluster identified were non-responsive to almost all 3032 conditions, except for the stem cell differentiation studies where they are strongly down-regulated. Most of the transcripts from this cluster are uncharacterized and thus represent potential novel targets.

“Genevestigator” allowed us to easily exploit over 25,000 profiled samples and to efficiently identify genes highly specific for a cell type and to study the regulation of these genes in response to perturbations. In fact, while all stem cell specific genes possessed the same profile by tissue/cell type, subsets of genes had very distinct patterns of regulation across perturbations and provide promising marker targets for further, also in-vivo, investigations.

P-Techno-260

Targeted next-generation sequencing of 32 genes in molecular diagnostics of patients with connective tissue disorders

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Inherited connective tissue disorders are a group of genetically heterogeneous diseases that primarily affect the skeletal and cardiovascular systems and may also involve the skin, joints, eyes and other systems. Diagnosis of these disorders may be challenging due to extensive variable expressivity, clinical heterogeneity as well as overlapping and atypical presentations. Establishing a specific diagnosis is of high importance since some of these disorders are associated with life-threatening symptoms, such as aortic dissection or rupture, demanding regular medical surveillance. To detect disease-causing mutations we designed a connective tissue disease Next-generation sequencing (NGS) panel covering the coding regions and splice sites of 32 genes associated with various phenotypic overlapping connective tissue disorders such as Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome, Stickler syndrome, Weill Marchesani syndrome, Shprintzen-Goldberg syndrome and familial thoracic aortic aneurysm. Target enrichment of all coding exons the patient's genomic DNA is performed using the HaloPlex Target Enrichment System (Agilent) followed by 2x250bp paired end sequencing on the MiSeq system with the MiSeq Reagent Kits v2 (Illumina). To evaluate the sequence data for the presence of nucleotide substitutions and small deletions and duplications, the SureCall Software (Agilent) is used and the patient's gene sequences are compared to the human reference genome (NCBI GRCh37.3). Pathogenicity of variants is assessed using several in silico predictive algorithms and comparison against population, disease-specific, and sequence databases. Sanger sequencing is used for confirmation of potentially causative variants and analysis of areas with low coverage in NGS analysis. NGS analysis is complemented by multiplex ligation-dependent probe amplification (MLPA) of the FBN1, TGFBR2 and TGFBR1 genes to detect pathogenic deletion or duplications of one or more exons. So far, we analyzed 50 patients with a suspected inherited connective tissue disorder and achieved 97% coverage for the coding sequences of all examined genes with

at least 40 individual reads per amplicon. We were able to detect 11 disease causing or likely disease causing variants in 11 patients including 6 different FBN1 variants in 6 patients with Marfan syndrome, 1 TGFBR1 variant in 1 patient with Loeys-Dietz syndrome, 1 COL1A1 variant in 1 patient with Ehlers-Danlos syndrome and 1 COL1A1 variant in 1 patient with Osteogenesis imperfecta, 1 COL2A1 variant in 1 patient with Stickler syndrome, and 1 COL11A1 variant in 1 patient with Stickler syndrome. Further efforts to complement the existing panel with genes associated with sudden cardiac death and to implement the bioinformatic detection of copy number alterations and single exon deletions are in progress. Our data show that targeted NGS is efficient and diagnostically useful at detecting variants implicated in connective tissue disorders.

P-THERAPY FOR GENETIC DISEASES

P-Therap-261

A novel gene therapeutic method combining vitrectomy and intravitreal AAV injection results in retina-wide transduction in mice

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Different genetic methods to treat eye disorders have been extensively studied over the past 20 years. Frequently, the therapeutic agent is transferred via an injection of adeno-associated virus (AAV) particles into the retina or vitreous. Such application methods typically transduce about 10-30% of retinal cells in mice.

We developed a novel intravitreal injection procedure that leads to transduction of more than 80% of the retina, an efficacy that significantly exceeds that of standard retinal injections. Over 90 adult C57Bl/6J mice have been evaluated. An aspiration of vitreous tissue (vitrectomy) prior to a single intravitreal injection of GFP-expressing AAV2/8 resulted in an almost complete transduction of the retina. Fluorescence laser scanning microscopy showed co-localization of GFP with markers of retinal cells. We found GFP signals in all retinal layers, thus, suggesting that the retina was fully penetrated by the AAV. The nerve fiber layers and the optic nerve were GFP positive. Electroretinographic measurements and analyses of retinal flatmounts suggested that the novel method does not lead to obvious side effects on the morphological and physiological level.

Taken together, we developed a novel method to transduce the vast majority of retinal cells with a single AAV injection. Our results will have implications on basic science applications and on virus-based gene therapies in mouse models. The new method may further provide the basis to advance AAV applications to the human retina.

P-Therap-262

A potential cancer prophylaxis in the hereditary cancer disorder Nijmegen Breakage Syndrome

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Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disease in which immunodeficiency and predisposition to cancer are the major life threatening symptoms. Average age at malignancy, typically lymphoma, is 9.7 years. The gene responsible for NBS is *NBN*, it codes for the 754 amino acid protein nibrin and is the human homologue of the yeast gene, *Xrs2*, involved in DNA double strand break repair, DNA replication and cell cycle checkpoints. Over 90% of patients affected by NBS are homoallelic for a founder mutation, a 5bp deletion in exon 6 of the *NBN* gene. After alternative translation from a cryptic start codon upstream of the deletion, this hypomorphic mutation leads to a partially functional 70kDa carboxy-terminal protein fragment termed p70-nibrin. Null mutation of the mouse gene *Nbn* is embryonically lethal. Variation in the amount of p70-nibrin in B-cell lines from homozygous patients correlates with cancer occurrence in those patients. Due to the DNA repair deficiency, NBS patients are hypersensitive to chemotherapeutic and radiotherapeutic treaments. Thus prevention of cancer development is of even greater importance for these

patients. Cells from NBS patients have slower DNA replication rates and larger numbers of active replication origins during S-phase, thus illustrating the role of nibrin in DNA replication also in human cells. In one NBS patient however, DNA replication and origin firing rates are comparable to control cells. This particular patient carries a unique mutation leading to alternative splicing and expression of an internally deleted nibrin protein, p80-nibrin, lacking the 104 amino acids coded for by exons 6 and 7. This protein, which, unlike p70-nibrin, contains both carboxy-terminal and amino-terminal functional domains, has previously been made responsible for the milder cellular phenotype and absence of cancer in the 63 year old NBS patient, the oldest known NBS patient. Since the founder mutation which the majority of NBS patients carry is located in exon 6, directed alternative splicing to remove exons 6 and 7, and generate p80-nibrin, could be the basis of cancer prophylaxis for NBS patients. Here we have used antisense oligonucleotides to mask critical splice signal sequences and enforce alternative splicing in NBS cells *in vitro* and a humanized NBS mouse model *in vivo*. Thus, we have demonstrated proof of principle for the use of antisense oligonucleotides as a potential cancer prophylaxis in the hereditary cancer syndrome NBS.

P-Therap-263

Patient-specific iPS cell-derived melanocytes for 3D skin modelling

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Induced pluripotent stem cells (iPSC) generated from dermal fibroblast are an excellent source for fate-specific differentiation of cells into any desired tissue. We have generated all our iPSC in-house from control and patient dermal fibroblasts using STEMCCA lentivirus (a kind gift from Prof. Mostoslavsky, CReM, Boston University).

3D skin model systems reached international acclaim to further patient-specific drug development and to minimize the use of animals in applied medical research. Lately we developed 3D skin models for various monogenic severe genodermatoses with and without changes in pigmentation. Only model systems which reflect the patient skin perfectly will help us understand disease mechanisms and make drug actions safe.

We studied the cells extracted from an adult male patient with an autosomal recessive congenital ichthyosis caused by a homozygous nonsense mutation in *TGM1* and the cells from a late female infant patient with severe photosensitive Trichothiodystrophy (TTD) with a homozygous mutation in *ERCC2*. Here we present a simple and fast protocol to differentiate human feeder-cultured iPSC into melanin expressing pure cultures of melanocytes. We used only one commercially available medium slightly modified and supplemented with two low-cost additional components after initial neuro-ectodermal induction with BMP4 and retinoic acid for the first days. Cells were passaged using typical melanocyte protocols with low concentrations of trypsin to enrich melanocytes from passage to passage. From roughly five iPSC colonies we have been able to cryo-preserve more than 40 M cells per line within the first six passages.

Melanocytes derived from iPSC underwent extensive characterization including immuno-histological staining, FACS analysis, melanin-assay, and extensive array-based qPCR analysis which showed that iPSC-derived melanocytes are highly comparable to those extracted from epidermal sheets.

To proof in-vitro functionality of the cells in regard to melanin-transfer and viability in a tissue environment, we have used iPSC-derived melanocytes for patient-specific 3D skin modelling. With success we showed iPSC-derived melanocyte integration into the basal layers of the epidermal sheets as well as expression of melanin and darkening of 3D skin models after exposure to UV light. To our knowledge this protocol is currently to most efficient to generate iPSC-derived melanocytes from iPS cells in a very short time at low cost, and with only minor efforts for splitting, sorting or other cell culture duties. iPSC-derived melanocytes gained can be frozen and re-cultured and used for downstream applications including 3D skin modelling and drug-screening.

P-Therap-264

Genomic editing in iPS cells- an important tool to identify patient specific cellular phenotypes in neurons

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Opitz BBB/G Syndrome (OS) is a monogenic disorder caused by mutations in the MID1 gene. OS affects the development of the ventral midline and is characterized by hypertelorism, laryngo-tracheo-esophageal abnormalities, and neurological deficits including intellectual disability (ID). We have shown previously that OS is caused by mutations in the MID1 gene and that the MID1 protein is a ubiquitin ligase regulating the ubiquitin specific degradation of microtubule-associated PP2A thereby upregulating mTOR activity. mTOR signaling is a key player in the formation of learning and memory and a common mechanism in patients with ID. For example has mTOR dysregulation been found in fragile X syndrome, in tuberous sclerosis, in RETT syndrome and in Down syndrome. Because MID1 is directly linked to PP2A and mTOR OS is an important model for ID with mTOR dysfunction.

In order to study ID with mTOR dysfunction in a human model and to possibly set up assays for drug screening we have generated iPSCs from several OS patients with mutations in different domains of the MID1 gene. We are currently establishing genome editing in these iPSCs by using the CRISPR/Cas9 system. Genome editing is a process of generating genetically modified cell lines or animals using engineered, sequence-specific nucleases. Nucleases are introduced into cells and create double-strand breaks (DSB) in the DNA at desired locations in order to produce indel-mutations or to facilitate homologous recombination. We are using the system to “repair” patients mutations, generate isogenic controls and to introduce patients’ mutations into control iPS cells with unrelated genetic background. We will then differentiate iPSCs into neurons and generate 2D and 3D neuron cultures. Comparison of patients’ cells and isogenic controls with wildtyp and mutation carrying control cells will allow us to differentiate between cellular and molecular phenotypes caused by specific mutations in the MID1 gene from effects caused by genetic background.

P-Therap-265

Activation of AMPK-induced autophagy ameliorates Huntington disease pathology

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The expansion of a polyglutamine repeat in huntingtin (HTT) causes Huntington disease (HD). Although the exact pathogenesis is not entirely understood, mutant huntingtin (mHTT) causes disruption of various cellular functions, formation of aggregates and ultimately cell death. The process of autophagy is the main degradation pathway for mHTT, and various studies have demonstrated that the induction of autophagy leads to an amelioration of aggregate formation and an increase in cell viability. Commonly, this is achieved by inhibition of the mammalian target of rapamycin (mTOR), a prominent regulator of cell metabolism. Alternatively, non-canonical AMPK or mTOR-independent autophagy regulation has been recognized. Given mTOR’s involvement in major cellular pathways besides autophagy, its inhibition may come with potentially detrimental effects in the course of treating HD. Here, we asked if AMPK activation may provide us with a target opportunity to induce autophagy in an mTOR-independent manner in HD. We demonstrate here that activation of AMPK by A769662 and overexpression of a constitutively active form of AMPKα in STHdh cells and mouse embryonic fibroblasts (MEFs), led to increased expression of the autophagosomal markers LC3 and p62, suggesting an efficient induction of autophagy. Induction of autophagy was independent of mTOR, and accompanied by a decrease of mHTT-containing aggregates and improved cell viability.

Therefore, we validated AMPK as a promising therapeutic target to treat HD, and identified A769662 as a potential therapeutic compound to facilitate the clearance of mHTT.

P-Therap-266

Old friends on new paths: metformin as an early phase treatment in Huntington's Disease?

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that is caused by an unstable glutamine (CAG) trinucleotide repeat expansion within exon 1 of the Huntingtin gene and leads to cognitive decline and affects motor abilities. In the prodromal phase of the disease patients develop mood swings, personality changes and subtle cognitive impairment. Close understanding of clinical signs and molecular mechanisms behind this early stage of HD is an important requisite for the development of a causal therapy. We have analysed a knock-in mouse model that carries 150 CAG repeats and the human exon 1 in the 5' end of the murine huntingtin gene. By using a novel object recognition test with a 24 h interval between sample and test phase we have found a profound deficiency of hippocampus dependent long-term memory in heterozygous transgenics. This phenotype was detected as early as 12 weeks of age and is complementary to deficits that we have identified in the HdhCAG111 mouse model previously. Motor deficits as well as intranuclear aggregates are described at much later stages in both of these models. This early phenotype makes the two models ideal for drug screening. Metformin is a biguanide, an AMPK antagonist and mTOR agonist. It is commonly used as an anti-diabetic drug. Side effects of metformin in glucose normal people have not been described. We have shown previously that by interfering with the mTOR kinase and its opposing phosphatase, PP2A, metformin regulates local protein synthesis in the brain and is able to suppress the production of disease making protein in HD. In addition, beneficial effects of metformin in HD e.g. prolongation of survival in males (Thong et al. 2006) and delay of HD-associated symptoms by improving mitochondrial functions (Adhihetty et al. 2010) have been found. By using the described test battery we are now testing hippocampus based long-term memory in heterozygous and WT HdhCAG150 males at 12 weeks with and without application of metformin. In preliminary experiments we have seen an improvement in long-term memory in treated heterozygous mice compared to non-treated. If we can confirm these data metformin is a very promising candidate for early phase treatment of HD patients.

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